CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) ENZYME FAMILY

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Siklik Nükleotid Fosfodiesteraz (PDE) Enzim Ailesi

ÖZET

Siklik nükleotid fosfodiesterazlar (PDE), hücre içinde ikincil habercilerden siklik AMP (cAMP= siklik 3',5'-adenozin monofosfat) ve cyclic GMP (cGMP= siklik 3',5'-guanozin monofosfat)'lerin hidrolizini katalize ederler. Siklik AMP, çeşitli hormonlara (insülin ve adrenalin, gibi) aracılık yaparak karbohidrat ve yağların yıkımlanmasında önemli bir rol oynar. cGMP, retinol rod hücrelerinin cGMP-bağımlı Na^+ kanallarının açık kalmasını, bazı PDE activitelerinin ve düz kas kontraksiyonlarının düzenlenmesinde (penis ereksiyonunu, gibi) görev yapar. Bu ikincil habercilerin yıkımlanmasını sağlayan tek enzim fosfodisterazlardır. PDE üst ailesi on alt aile grubunu içerir ve bunlardan çoğu birden fazla izoenzime sahiptir. cAMP ve cGMP'nin yıkımlanması, bunların aracı olduğu uyarı sistemlerinin durdurulmasına sebep olur.

Anahtar Kelimeler: Fosfodiesteraz, Enzim, cAMP, cGMP

SUMMARY

Cyclic nucleotide phosphodiesterase enzymes catalyse the hydrolysis of second messengers, cAMP and cGMP. cAMP plays an important role in the carbohydrate and lipid breakdown in response to certain type of hormones (e.g. insulin, adrenaline). cGMP provides the cGMP-dependent Na^+ channels to keep open of the retinal rod photoreceptor cells, regulating some of the PDE's activity and also has a role in mediating smooth muscle contractions (e.g. penile erection). The only hydrolysing enzymes of these second messengers are phosphodiesterases. PDE superfamily consists of ten phosphodiesterase subfamilies and most of them have more than one isoform. Breakdown of cAMP and cGMP play a pivotal role in terminating cAMP and cGMP signal transduction.

Key words: Phosphodiesterase, Enzyme, cAMP, Cgmp

INTRODUCTION

Many hormones, growth factors, cytokines and neurotransmitters alter cellular growth by binding to receptors that activate membrane bound adenylate cyclase (EC 4.6.1.1). This enzyme synthesizes the second messenger cAMP (cyclic 3',5'-adenosine monophosphate) from intracellular ATP (28) (Figure 1). cAMP mediates many important physiological responses and metabolic processes such as exocytosis, platelet aggregation and neurotransmission as well as having long term effects on key processes such as cell growth and differentiation (14). The mechanisms by which cAMP modulates cell function are not completely understood. However, they appear to depend on the activation of PKA (protein kinase A) and subsequent phosphorylation of hydroxy-amino acid residues or regulatory subunit-dependent transport of cAMP to the cytoplasm and nucleus (14).

Intracellular cAMP homeostasis is maintained not only by regulating its synthesis by adenylate cyclase (13), but also by control of its degradation through the action of the cyclic nucleotide phosphodiesterases, PDEs (4, 13) (Figure 1).

Phosphodiesterases (EC 3.1.4.17) catalyze the hydrolysis of the 3′-phosphodiester bond of cAMP to form 5′-nucleoside monophosphate product (5'-AMP) (Figure 1) which is unable to activate PKA. Therefore, breakdown of cAMP plays a pivotal role in terminating cAMP signal transduction.

PDE family isoforms represents a family of enzymes with a wide range of properties that are exemplified by their different sensitivities to specific inhibitors, cofactor requirements, tissue and subcellular distributions, hormonal regulation, phosphorylation by kinases and interaction with other proteins.

PDEs can be divided into ten identified classes (Table 1) that are categorised on the basis of their primary amino acid sequence.

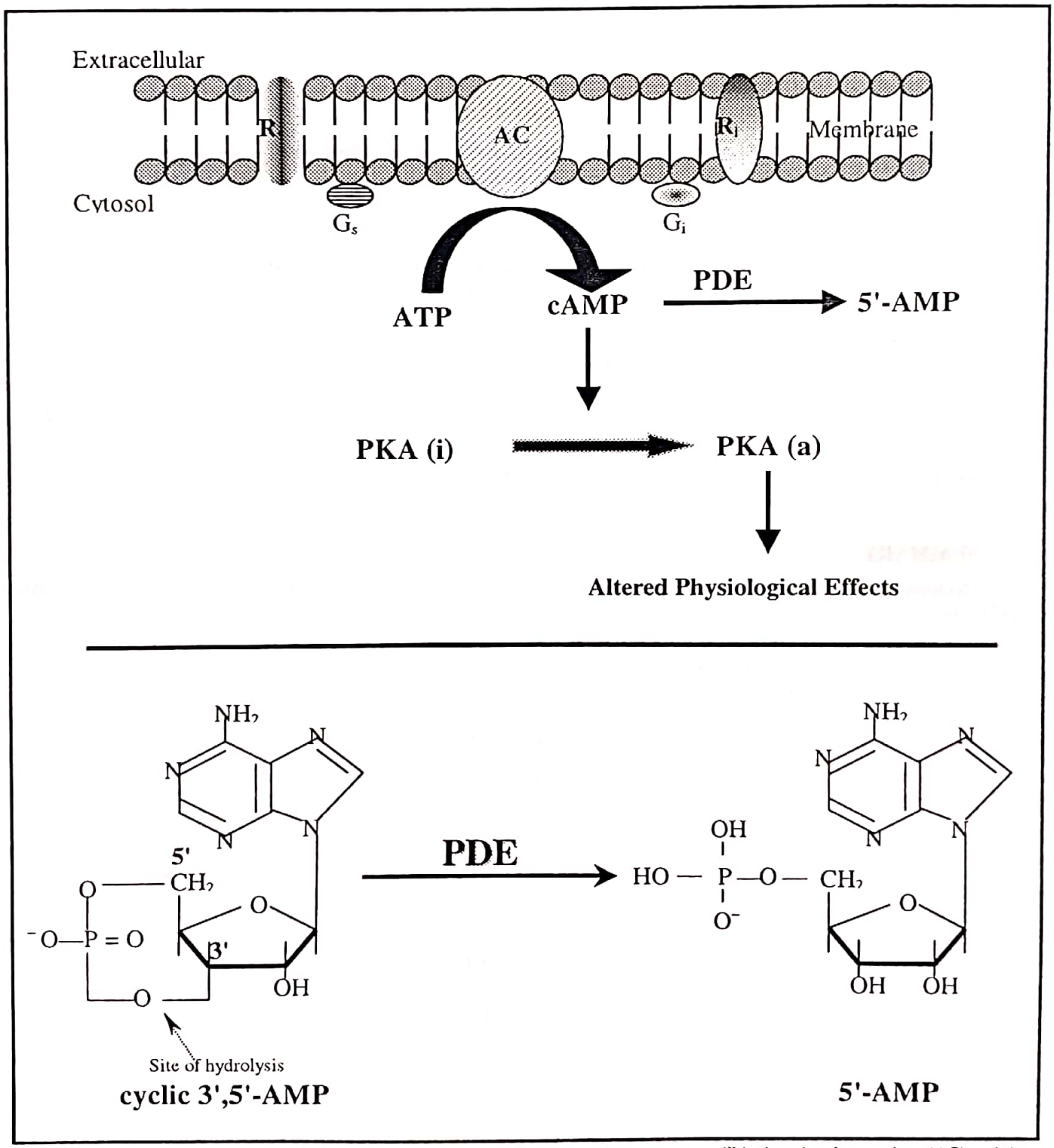

Figure 1. Upper figure shows the relation of the stimulatory (R_s), the inhibitory receptors (R_i), the adenylate cyclase (AC) and the PDE. [PKA(i) inactive, PKA(a) active, G_s stimulatory G-protein, G_i inhibitory G-protein]. Lower shows the breakdown of cAMP to 5'-AMP by phosphodiesterase (PDE) (modified, 11).

PDE1: Ca^{2+}/calmodulin-stimulated PDEs

PDE1 hydrolyzes both cAMP and cGMP and its activity is stimulated by Ca^{2+} in a manner that required a small protein-activator factor, calmodulin (Table 1). PDE1 subfamily possesses a great diversity in apparent molecular weight, substrate specificity, tissue distribution, kinetic properties and affinity for calmodulin (4, 21). Different isoforms were distinguished by direct protein sequencing and cDNA cloning studies. To date, three different genes have been identified in this family: PDE1A (59-61kDa), PDE1B (63kDa) and PDE1C (75kDa) (4, 22).

PDE2: cyclic GMP-stimulated PDEs

PDE2 efficiently hydrolyzes both cAMP and cGMP (cyclic 3',5'-guanosine monophosphate) with positively co-operative kinetics for cGMP as an effector (Table 1). cGMP, however, is the preferred substrate and also serves at low concentrations (μM) as an activator of cAMP hydrolysis (4) where it can stimulate the hydrolysis of cAMP by ~50-fold (2). Only one PDE2 specific inhibitor has recently been found, EHNA (erythro-9-(2-hydroxyl-3-nonyl)-adenine) which was originally called MEP-1 (19).

PDE3: cyclic GMP-inhibited PDEs

PDE3 isoforms are found in a large array of tissues, including bovine and rat adipose tissues, human platelets and T-cells, rat liver and epididymal tissues, human and bovine heart (8, 9, 12, 15). PDE3 activity is encoded by two genes, PDE3A and PDE3B (18, 29). The subcellular distribution of PDE3 species varies, with the enzyme present in the cytosol as well as membrane-associated. Some variation has been found in the analysis of the molecular masses of PDE3 isoforms ~125 kDa (Table 1). Cilostimide which augments myocardial contractility and relaxes smooth muscle, inhibits PDE3s with low IC_{50} (0.005μM) values (3).

PDE4: cyclic AMP-specific PDEs

The PDE4 enzymes are a large multi-gene family consisting over 13 different isoforms seen in both humans and rodents (6,7). These are the predominant cAMP hydrolysing enzymes family found in most, if not all, immune and inflammatory cells (9, 17, 32).

Four different genes encode mRNAs for PDE4 isoforms in mammals with a great similarity between species (Table 1). These are called PDE4A, PDE4B, PDE4C and PDE4D. Each of these four genes appears to produce multiple mRNA transcripts involving 5'-domain swopping, yielding isoforms with distinct N-terminal domains (5). In contrast, the C-terminal domain of each particular PDE4 gene family is unique and is thus common to all active isoforms produced from a particular PDE4 gene. The four rat and four human genes show a one to one homology, in that each of the four human PDE4 genes is more closely related to one specific rat gene than to any other human gene (5).

The enzymes of the PDE4 multigene family are characterised by their high and specific affinity for cAMP, selective and specific inhibition by the anti-depressant drug rolipram, and insensitivity to the cGMP and Ca^{2+}/CaM (4, 7).

Differential regulation of PDE4 enzymes can be achieved by regulation of the level of cAMP. This may form part of a long-term adaptation process for PDE4 isoforms which is mediated via a cAMP-dependent mechanism. Such a process may result from hormonal or direct activation of adenylate cyclase and can also be achieved with cAMP analogues (9, 32).

Table 1. Some of the enzymatic properties of phosphodiesterases (PDE).

PDE Family	Number of Genes	Regulatory properties	Inhibited by:	SDS-PAGE (kDa)
1	3	Ca^{2+} calmodulin	Nicardipine, Vinpocetine	59-63
2	1	Stimulated by low cGMP	EHNA	240
3	2	Phosphorylation by PKA	Cilostimide, Milrinone	125
4	4	cAMP specific	Rolipram, Ro 20-1724	64-125
5	2	cGMP specific	Zaprinast	177
6	3	cGMP specific	Unknown	84-88
7	1	cAMP specific	Unknown	Unknown
8	1	cAMP specific	Dipyridamole	Unknown
9	1	cGMP specific	Unknown	62
10	1	cAMP specific	Unknown	Unknown

A large number of compounds which can serve as selective PDE4 inhibitors have been described (rolipram, Ro 20-1724, CDP840, RP73401) and these compounds inhibit PDE4 enzymes with IC_{50} values in the nM-μM range (1, 26). To date, however, there is no compound which can potently and selectively discriminate between the various PDE4 isoforms.

Investigations have revealed that many functions of the immune system and inflammatory responses are inhibited by agents that increase intracellular cAMP concentrations. Elevation of cAMP levels by rolipram can markedly inhibit the production of the pro-inflammatory mediator TNF-α but not other cytokines such as IL-1 and arachidonic acid metabolism in monocytes (27). PDE4 inhibitors rolipram and RP 73401 suppress the functioning of eosinophils by reducing their superoxide generation, infiltration and adhesion functions (31). Inhibition of PDE4s in T-lymphocytes causes a profound decrease in exocytosis, proliferation, cytokine production (IFN-α, IL2, IL6) and expression of CD7 and IL2 receptors (12). Due to the effect of selective PDE4 inhibitors on the reduction of pro-inflammatory cell functions, inhibitor development has focused on the central nervous system (CNS), with emphasis on depression in disorders of the immune and inflammatory systems, vascular intimal proliferation and relaxation of airway smooth muscles. However, although PDE4 inhibitors promise to be useful in the treatment of certain diseases, it appears that they also have some unwanted side-effects. A particular contra-indication of PDE4 inhibitors is gastro-intestinal discomfort, mainly indicated by nausea, vomiting and emetic side effects. Immunosuppression and metabolic disturbances (e.g. altered glucose metabolism) are also other side effects. (30) .

PDE5: cyclic GMP-specific PDEs

PDE5 enzymes were purified from rat lung and are present in a variety of tissues including platelets vascular smooth muscle, rat spleen and guinea pig lung. To date, only one gene isoform has been cloned for this family, BTPDE5A (4, 21).

PDE5 has a high affinity for cGMP and practically no hydrolysing activity for cAMP. It forms a homodimer of native mass ~177 kDa from two ~93 kDa subunits (10) (Table 1).

PDE6: photoreceptor PDEs

PDE6 enzymes play key roles in photoreceptor signal transduction. In retinal rod photoreceptor cells, visual signalling is triggered upon the absorption of photons by rhodopsin. Photoexcited rhodopsin leads to an activation of the retinal GTP-binding protein (transducin). Rapid hydrolysis of cGMP by activated PDE6 results in closure of the cGMP-dependent Na^+ channel in the plasma membrane which is normally kept open by cGMP (21).

Three distinct genes encode this PDE family; one cone and two rod PDE genes. Rod PDE6 protein consists of α (88 kDa) and β (84 kDa) and two inhibitory γ (11 kDa) subunits (16, 21) as indicated by sequence comparison and DNA hybridisation. IBMX inhibits the bovine rod PDE6 in a similar way to all other PDE enzymes (except PDE7).

PDE7: cAMP-specific rolipram-insensitive PDE

Only one member of the PDE7 gene family has been cloned to date. This was from a human glioblastoma cDNA library (20). Northern blotting and transcript analyses have shown that PDE7 is highly abundant in human skeletal muscle with much lower levels noted in a number of other human tissues including kidney, brain, heart and some, but not all, T lymphocyte forms (12, 20). PDE7 appears to be resistant to inhibition by the non-selective reversible PDE inhibitor, IBMX (20).

PDE8: This new PDE was first cloned from mouse testis and has specificity to hydrolyse cAMP with a K_m of 0.15μM. PDE8 shows highest expression in testis and low in eye, liver, skeletal muscle and brain (23)

PDE9: This novel phosphodiesterase (PDE9A1) shows high hydrolysing activity for cGMP (K_m of approximately 0.07μM for cGMP). Its mRNA is highly expresessed in kidney with lower levels in liver, lung, and brain. PDE9 activity is not inhibited well by nonselective PDE inhibitor, IBMX (24).

PDE10: This PDE, PDE10A, has recently been cloned from testis and brain which hydrolyses cAMP with a K_m of 0.05 μM and cGMP with a K_m of 3μM. (25).

CONCLUSION

Cyclic nucleotide phosphodiesterase enzymes hydrolyse the second messengers cAMP and cGMP which mediate phosphorylation of PKA and PKG (pro-

tein kinase A and G), respectively. These enzyme family have been studied intensively in various cell types and tissues, in order to demonstrate their enzymatic properties, cellular compartmentalization, regulation,

interaction to other proteins, chromosomal localization and inhibition by selective drugs. Due to their role in the suppression of asthma symptoms and the role of the breakdown of carbohydrate and lipid metabolizm, PDE enzymes are under focus. Researchers and some drug companies have been trying to develop izotype specific PDE inhibitors to avoid side effects of inhibitors which were developed previously.

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