

Review Article

Phosphodiesterase 4 cAMP phosphodiesterases as targets for novel anti-inflammatory therapeutics

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ABSTRACT

Cyclic nucleotides are powerful signaling molecules and their sole means of removal is through the action of cyclic nucleotide phosphodiesterases (PDEs). Elevating levels of cAMP is beneficial in many diseases, including neurological conditions, cancers, viral infections and most inflammatory disorders. There are 11 known PDE families, with PDE4 being the most highly expressed. Evidence of the clinical usefulness of PDE4 inhibition has come from the use of specific chemical inhibitors and the generation of knock-out mice models. There exists an extensive range of PDE4 family members, because there are four subfamilies, each encoded by their own gene and each capable of generating multiple isoforms. Several members of the PDE4 family are found to be expressed in every cell and tissue studied and evidence is now being uncovered indicating unique functions for each. Their actions are controlled by their expression patterns, subcellular location and interaction with other signaling pathways. The first generation of PDE4 inhibitors, although potent anti-inflammatory agents, failed as pharmaceuticals owing to their emetic and gastric side-effects. A new generation of chemical inhibitors is now nearing the market, which display greatly reduced side-effects. In the future lies the generation of more specific inhibitors that will focus upon particular diseases. This will be achieved by targeting specific PDE4 family members. Because the current target of chemical inhibition (the catalytic site) is virtually identical between isoforms, this specificity is likely to be

achieved by blocking the enzyme's interaction with other signaling cascades.

Key words: A-kinase anchoring protein (AKAP), β -arrestin, phosphodiesterase 4, phosphodiesterase inhibitors, Src tyrosine kinase.

INTRODUCTION

The potent anti-inflammatory effects of elevating cellular cAMP levels have been known for many years.¹ More recently, our understanding of the mechanisms controlling the concentration of this second messenger has allowed the identification of new targets for therapeutic intervention. As important regulators of cellular function, the synthesis and degradation of cAMP is tightly regulated, with the sole means of removing cAMP through the action of cyclic nucleotide phosphodiesterases (PDEs). The PDE4 family is the predominant cAMP-hydrolysing enzyme in cells of the immune system, making them a good target for drug therapy.²

The activation and recruitment of inflammatory cells and the unregulated release of excessive levels of cytokines is implicated in many allergic and autoimmune diseases, such as rheumatoid arthritis, irritable bowel disease, atopic dermatitis and psoriasis. Recruitment of immune cells and release of proinflammatory mediators are all blocked by the action of PDE4 inhibitors. The PDE4 inhibitors can also cause airway smooth muscle relaxation. Although they are not as potent as β -adrenergic agonists in relaxation, this dual role suggests great potential in the treatment of airway disease. Advanced clinical trials have proved PDE4 inhibitors to have benefit in chronic obstructive pulmonary disease (COPD), asthma, allergic rhinitis and rheumatoid arthritis.^{3,4}

Many PDE4 inhibitors have shown a high frequency of observed side-effects, such as excessive gastric acid

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secretion, nausea and vomiting.^{2,5} Therefore, the current challenge is to improve the separation between these side-effects and the efficacy of the drug. The presence of multiple isoforms in association with differential expression patterns and regulation among target tissues, provides many opportunities to develop a new generation of specific inhibitors lacking undesirable side-effects.

The PDE4 family consists of four subfamilies, each coded by its own gene and each capable of generating multiple splice variants.⁶ All members possess a common catalytic region conferring similar kinetic properties and ion requirements upon the isoforms. The N-terminal and C-terminal regions show variability, containing motifs involved in the regulation of catalytic activity and intracellular targeting of the enzyme. The value of targeting subfamilies is demonstrated using knock-out mice deficient in one of the PDE4 genes. Animals lacking the *PDE4B* gene have an impaired immune system,⁷ whereas those lacking the *PDE4D* gene showed effects in their airways.⁸ More focused inhibitors may also possess better efficacy because it may be possible to select against inhibiting the PDE4 causing the side-effects.

The cellular mechanisms controlling the location and activity of the PDE4 family are extremely complex. Much work has gone into identifying regions within the PDE enzyme responsible for this tight control.⁶ These controlling mechanisms and their unique sites on the enzyme represent future target areas that could allow targeted disruption of the cAMP signaling system in specific regions.

PHOSPHODIESTERASES

The PDE family is a large family of catalytically active enzymes.⁹ There are currently 11 gene families identified,^{9,10} differing in their substrate specificity and K_m , most encoded on multiple genes and with many able to generate multiple splice variants. Although the catalytic region is conserved throughout the families, the catalytic regions are sufficiently different to allow the generation of family specific inhibitors. All PDE enzymes possess sequence differences around their N- and C-terminal regions. These unique regions play important roles in controlling the function and location of these enzymes.

All PDE4 isoforms have the same general structure.¹⁰ Four genes, labeled A–D, encode this family, with each gene capable of generating a range of isoforms differing at their N-terminal regions. The catalytic region occupies the central portion of the enzyme and is conserved within

members of the same gene family (Fig. 1a). Regions N-terminal to the catalytic region show variability and contain motifs involved in the regulation of catalytic activity and intracellular targeting. Within this variable N-terminal region in the PDE4 family are found two sections of conservation termed the Upstream Conserved Regions (UCR). The C-terminus of UCR1 interacts with the N-terminus of UCR2 and this UCR1/2 domain is also suggested to then interact with the catalytic domain through UCR2.¹¹ These UCR regions play crucial roles in regulating the enzymatic activity of PDE4.

INHIBITORS OF PDE4 ENZYMES

Inhibitors of PDEs have been used as anti-inflammatory agents for many years. Compounds such as caffeine and theophylline possess weak PDE inhibitory abilities and have been used to treat asthma and COPD.^{2,5} Recently, interest has developed in the PDE4 family of cAMP-specific PDEs as anti-inflammatory agents. Multiple members of all four PDE4 subfamilies have been identified in almost all cell types studied and are highly expressed in cells of the immune system.^{2,5}

A great many inhibitors of PDE4 have demonstrated potent anti-inflammatory effects in a wide range of cell-based assay systems, many using human-derived material. Inhibition of PDE4 blocks the release of cytokines and prevents immune cell infiltration. A few of these compounds have advanced to clinical trials, extending the observation to human inflammatory conditions.^{2,3} More recently, advanced clinical trials have proved PDE4 inhibitors to have distinct benefits in asthma and COPD.^{2,3} Through cAMP elevation, not only do PDE4 inhibitors reduce the inflammatory response, but they can also relax airway smooth muscle. Therefore, they are of particular interest in the development of agents for chronic airway diseases, such as asthma and COPD, because they have the added advantage of being able to target the underlying inflammatory condition that causes the lung damage.¹²

The first generation of specific PDE4 inhibitors, such as rolipram and RO-20 1724, showed great promise as anti-inflammatory agents, being capable of potentially blocking many immune cell functions.^{2,5} However, their utility was greatly degraded by the observation of equally potent side-effects. These side-effects of emesis, nausea and increased gastric acid secretion eventually resulted in the failure of these compounds.

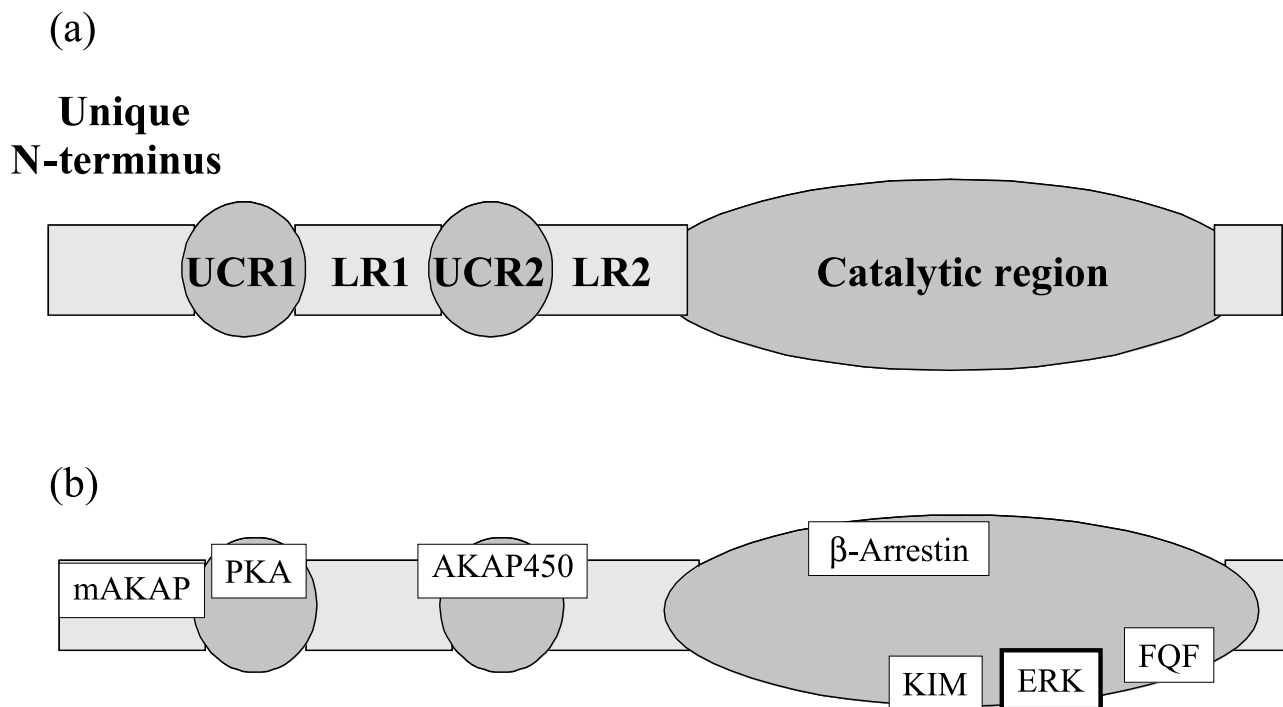


Fig. 1 (a) All phosphodiesterase (PDE) 4 isoforms have the same general structure.¹⁰ The catalytic region occupies the central portion of the enzyme and is conserved within members of the same gene family. Regions N-terminal to the catalytic region show variability and contain regulatory motifs. Within this variable N-terminal region in the PDE4 family are found two sections of conservation termed the Upstream Conserved Regions (UCR). These UCR regions play crucial roles in regulating the enzymatic activity of PDE4. (b) An increasing number of protein–protein interactions involving PDE4 enzymes have been identified. These interactions hold the PDE within a discrete location within the cell causing the cAMP concentration to be tightly regulated.¹⁹ Disrupting these binding interactions would specifically remove the PDE from these compartments, causing local cAMP concentrations to rise, resulting in controlled and potent effects upon a specific signaling pathway. Chemical disruption of these interactions could be used to target specific inflammatory processes, leading to potent and selective anti-inflammatory agents. This figure highlights some of the regions identified in the regulatory control of a long-form PDE4D. AKAP, A-kinase anchoring protein; mAKAP, muscle-selective AKAP; PKA, protein kinase A; KIM, kinase interaction domain; ERK, extracellular signal-regulated kinase.

SECOND-GENERATION PDE4 INHIBITORS

Using the information gained on the side-effects observed with the original PDE4 specific inhibitors, many companies undertook the development of a second generation of compounds with greatly reduced side-effects. The observed gastrointestinal discomfort is probably due to increased acid secretion from parietal cells. Emesis, the other major observed side-effect, is believed to be due to binding of the inhibitor to a high-affinity site in the brain. It is not yet known whether there is a common mechanism to these side-effects.

Pharmaceutical companies have used a high-affinity binding-site assay to select for compounds that preferentially bind to a low-affinity binding site and, thus, reduce the emetic effect.^{13,14} Compounds selecting against this

high-affinity site derived from rat brain are now being developed.¹⁵ The cause of these side-effects remains poorly understood. A *PDE4D*-deficient mouse anesthesia model has been used to suggest that a member of this subfamily is responsible for the emetic side-effects.¹⁶ However, compounds more selective for the PDE4D subfamily do not exhibit enhanced side-effects.

Mice deficient in PDE4D demonstrate further important roles for this subfamily in showing a greatly reduced ability for muscarinic cholinergic agonists, such as acetylcholine, to cause bronchoconstriction.¹⁷ The mice retain a functioning contraction mechanism because their airways can contract through alternative stimulants. It is thought that this effect is caused by a loss in the ability of the muscarinic receptor to decrease cAMP levels, presumably through the action of a PDE4D.

Phosphodiesterase 4D is the major PDE4 subfamily expressed in immune cells; however, a mouse deficient in this gene shows little compromise in its lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF)- α levels. In contrast, *PDE4B*-knock-out mice show an impaired immune response.⁷ This demonstrates that even though all PDE4 subfamilies are expressed in immune cells, there is specificity in their function. There appears little redundancy in their function and, thus, inhibition of specific subfamilies, or even isoforms, could have as potent anti-inflammatory effects as global inhibition of the entire PDE4 family.

Others have developed inhibitors more selective for specific PDE4 subfamilies. Although the catalytic regions within the PDE4 subfamilies possess very similar sequences, there are some subtle differences. It is now known that the unique N-terminal regions interact with the catalytic site, increasing the differences between subfamilies that can be used in the development of selective inhibitors.⁶

The development of PDE4D-selective compounds suggests that the catalytic region can exhibit usable differences. These PDE4D-selective inhibitors have shown good efficacy in cell-based anti-inflammatory models. Therefore, there has been some success in the development of subfamily selective inhibitors; however, it would be predicted that the potential for more selectivity is limited if the inhibitor compounds are targeted against the catalytic site of the enzyme. The development of PDE4 inhibitors targeted to the catalytic site has been reviewed extensively by Burnouf and Pruniaux.¹²

TARGETING NON-CATALYTIC SITES

So far, all inhibitors of the PDE4 family have been targeted against the catalytic site of the enzyme. Although this guarantees inhibition of the enzyme, it makes it very hard to be selective for particular PDE4 isoforms involved in controlling specific inflammatory processes.¹⁸ It may not be necessary to inhibit the activity of PDE4 enzymes through catalytic site inhibitors to provide an anti-inflammatory outcome. Instead, by disrupting the specific mechanisms used to control this large family of enzymes, compounds could be developed that would only block defined signaling processes.

The function and location of PDE4 enzymes are highly regulated and this is possibly the reason why so many isoforms are expressed in cells. Through interactions with

signaling pathways, certain PDE4 isoforms can play specific roles in controlling the local concentration of cAMP in defined areas within the cell. This tight regulation of cAMP allows for the differential regulation of signaling cascades depending upon how they are activated and where they lie within the cell.

Current research has started to identify the role of specific PDE4 isoforms in a multitude of signaling cascades. Sequence analysis of PDE4 isoforms identifies many potential phosphorylation sites, some of whose action has now been detailed.^{20,23,25} Phosphorylation at these sites leads to a rapid change in activity of the enzyme. Physical interactions of PDE4 enzymes with key components of cellular signaling pathways have also been identified. The specific binding of PDE4 isoforms to members of other signaling cascades allows these enzymes to exert controlling influences on many pathways involved in inflammatory processes.⁶

An increasing number of protein-protein interactions involving PDE4 enzymes have been identified.^{26,32,38,41,43} Most of these involve a single PDE4 isoform, the specificity of binding being controlled by sequences within the N-terminal region of the protein (Fig. 1b). It is believed that these interactions hold the PDE within a discrete location within the cell, causing the cAMP concentration to be tightly regulated.¹⁹ Disrupting these binding interactions would specifically remove the PDE from these compartments, causing local cAMP concentrations to rise, resulting in controlled and potent effects upon a specific signaling pathway. Chemical disruption of these interactions could be used to target specific inflammatory processes, leading to potent and selective anti-inflammatory agents. Alternatively, disruption of the phosphorylation-mediated control of these enzymes would result in the local deregulation of cAMP, with resultant anti-inflammatory action. As an added advantage of this approach, blocking these mechanisms would have little effect upon the catalytic activity of the enzyme so, where the targeted isoform was associated with alternative pathways, there would be no disruption. This approach has been proved to be a viable option through the following examples.

Phosphorylation as a means of control

Phosphodiesterase 4 isoforms possess many putative phosphorylation sites, providing the potential of many possible kinase-driven controlling mechanisms. A number

of these sites have been demonstrated to be true phosphorylation sites for specific kinases.^{20,23,25}

It is perhaps not surprising that protein kinase A (PKA), the protein kinase activated by cAMP, plays an important functional role for the PDE4 family. A PKA phosphorylation consensus sequence has been identified within the N-terminal UCR1 region of all long-form PDE4 enzymes²⁰ (Fig. 1b). Phosphorylation at this site causes marked activation of the enzyme, demonstrating a classic negative feedback mechanism. As cAMP levels increase, PKA is activated, leading to the increase in activity of the cAMP PDE, returning the levels of cAMP to basal and lowering PKA activity.^{21,22}

The discovery of a site for extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) phosphorylation within the catalytic domain of all PDE4 enzymes added a layer of complexity to the simple PKA controlling mechanism.²³ The effect of phosphorylation at this site is dependent upon how much of an N-terminal controlling region the PDE possesses²⁴ (Fig. 2). Long-form enzymes, possessing both UCR1 and UCR2, are rapidly inhibited by ERK phosphorylation, leading to a local increase in cAMP and activation of PKA. Protein kinase A then phosphorylates the PDE at the UCR1 site, causing recovery of PDE activity. This results in the lowering of cAMP back to basal concentrations and the switching off of PKA. This whole process takes minutes and gives the cell a means of a short, controlled increase in PKA.

In contrast, ERK phosphorylation of short-form PDE4 isoforms, lacking UCR1, causes a sustained activation of the enzyme and a lowering of the local cAMP concentration. In ultrashort PDE4 isoforms, lacking both UCR1 and the N-terminal region of UCR2, ERK phosphorylation results in a sustained inhibition of the enzyme.²⁴ Thus, phosphorylation at a single site can have dramatically different outcomes depending upon which isoform is being targeted.

Lipopolysaccharide stimulation of the immune system results in the activation of a number of signaling cascades. In the U937 monocytic cell line, the activation of PDE4A4 upon LPS treatment of cells was mediated through a phosphatidylinositol 3-kinase-controlled pathway.²⁵ Although PDE4A4 makes up a small proportion of the total cellular PDE4 activity in U937 cells, it has a powerful controlling influence upon the phosphorylation of cAMP response element-binding protein (CREB),²⁵ suggesting a key role in inflammatory signaling processes.

Protein–protein interactions as controlling mechanisms

In addition to regulation through protein kinases, the location of PDE4 enzymes within the cell is also carefully controlled through their interaction and regulation by association with other proteins. The PDE4A5 isoform provides a good example of this.²⁶ Mutation experiments where the N-terminus of this enzyme was progressively truncated identified multiple regions controlling its intracellular distribution. Two sites were found in the unique N-terminal region, which mediated localization within the cell margins. A third membrane association region was identified in the N-terminal portion of the UCR2 region and this allowed PDE4A5 to localize in the perinuclear region.

One of these unique N-terminal sites has been studied extensively. This is a proline-rich region that was found to specifically control the interaction of this PDE with the SH3 domain of Src tyrosine kinases.²⁶ Endogenous PDE4A5 immunoprecipitated from rat brains was found to be associated with Src tyrosine kinases, demonstrating this interaction occurs in real cellular systems. The human form of PDE4A5, PDE4A4, also forms complexes with Src tyrosine kinases, although this enzyme has an additional proline-rich region within the LR2 region.²⁷ Binding Src to this region was found to alter the sensitivity of the enzyme to inhibition by rolipram,²⁷ suggesting that this interaction causes an alteration within the catalytic site.

Interestingly, although PDE4A4/A5 is the only member of the PDE4A subfamily capable of existing in a complex with Src tyrosine kinases, it is not the only PDE4 using this controlling mechanism. Phosphodiesterase 4D4 also contains an N-terminal proline-rich region and has been shown to associate with Src kinases through their SH3 domains.²⁸

The large family of Src tyrosine kinases is known to be involved in many cellular pathways, a number of which are sensitive to PKA action and, thus, local cAMP concentrations. By holding a PDE4 in close proximity, the cAMP concentration around the Src kinase can be carefully controlled. The tyrosine kinase Csk (C-terminal Src kinase) controls signaling through the T cell receptor.²⁹ In the resting state, Csk is located in a membrane domain, from where it is released upon T cell receptor activation, leading to tyrosine phosphorylation of the receptor. Increased levels of cAMP reduce the levels of Csk dissociation from the membrane domains, reducing T cell signaling. It could be envisaged that association of a PDE

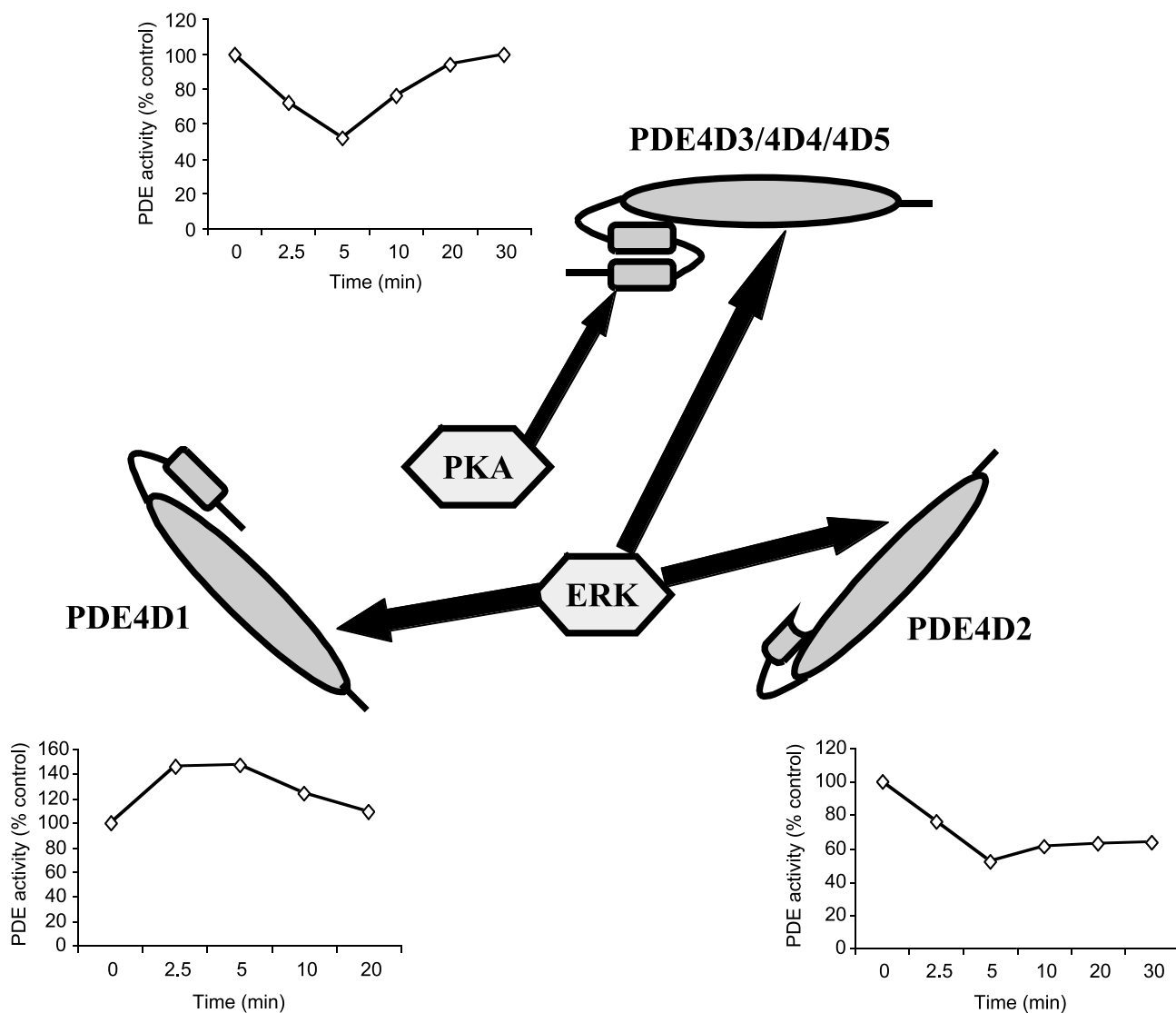


Fig. 2 The effect of phosphodiesterase (PDE) 4 phosphorylation by extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase is dependent upon how much of an N-terminal controlling region the PDE possesses.²⁴ Long-form enzymes such as PDE4D3, possessing both Upstream Conserved Regions (UCR) 1 and UCR2, are rapidly inhibited by ERK phosphorylation, leading to a local increase in cAMP and activation of protein kinase A (PKA). Protein kinase A then phosphorylates the PDE at the UCR1 site, causing recovery of PDE activity. This results in the lowering of cAMP back to basal concentrations and the switching off of PKA. This whole process takes minutes and gives the cell a means of a short, controlled increase in PKA. In contrast, ERK phosphorylation of short-form PDE4 isoforms such as PDE4D1, lacking UCR1, causes a sustained activation of the enzyme and a lowering of the local cAMP concentration. In ultrashort PDE4 isoforms like PDE4D2, lacking both UCR1 and the N-terminal region of UCR2, ERK phosphorylation results in a sustained inhibition of the enzyme. Thus, phosphorylation at a single site can have markedly different outcomes depending upon which isoform is being targeted.

with Csk would control cAMP levels, allowing activation of the receptor.

Src is itself regulated through phosphorylation and it has been found that prostaglandin (PG) E₂-activated PKA inhibits this tyrosine kinase.³⁰ This inhibitory effect is dependent upon Csk being located in membrane

domains. A mechanism is proposed where Src is inhibited through the action of Csk in a cAMP-PKA-mediated manner, controlled by G-protein-coupled receptors.

Proof that the interaction with Src kinases plays a role in the location of the PDE was shown using PDE4A5, which contains a caspase-3 cleavage site within its

N-terminus.³¹ Cleavage at this site results in the loss of the N-terminal portion containing the proline-rich, SH3-binding domain. Rat1 fibroblasts undergoing apoptosis demonstrate this reaction, which results in a redistribution of the enzyme away from the perinuclear region. This also provides an insight into the action a compound disrupting this interaction would have on the target cell. By blocking the binding to Src, the PDE4 would no longer exist in association with the particular pathway, allowing local cAMP concentrations to rise.

Just as PKA is involved in the control of PDE4 activity, the components of its signaling pathway also control PDE4 location. Protein kinase A is regulated through the distribution of the enzyme to specific intracellular regions. A-Kinase anchoring proteins (AKAPs) are the mechanism by which the cellular location of PKA is controlled. The AKAPs are a large family of proteins that bind to the dimerization site of the RII regulatory subunit of PKA; they also act as scaffold molecules for a number of signaling molecules.³² The constitutively active catalytic PKA subunit is held by the regulatory PKA subunit. Upon increases in cAMP levels, the catalytic unit is released, allowing it to phosphorylate its targets.

It is perhaps not surprising then that the PDE4 isoforms are also found to associate with AKAPs, allowing PKA activity to be tightly regulated. In sertoli cells, PDE4D3 is held with PKA-RII α in an enzyme complex through the association with AKAP450. Through a site within the unique N-terminus of PDE4D3, muscle-selective AKAP (mAKAP) is found to be associated.³² In AKAP450, a separate site found within the UCR2 region of PDE4D3 mediates the interaction.³³

In human airway smooth muscle, increasing cAMP concentrations either by stimulation of β_2 -adrenergic receptors or through treatment with PDE4 inhibitors, causes a reduction in the release of the eosinophil-activating cytokines granulocyte-macrophage colony stimulating factor (GM-CSF), RANTES and eotaxin by TNF- α or interleukin (IL)-1b.³⁴ Interleukin-1b causes the release of these cytokines through the action of MAPKs, which have been shown to be regulated by PDE4-controlled cAMP levels.³⁵ Unlike steroids, cAMP elevation by β -adrenergic receptor agonists or PDE4 inhibitors has been found to have no direct effect upon TNF- α -mediated IL-8 release from airway smooth muscle. However, cAMP elevation greatly enhances the steroid-mediated inhibition of IL-8.³⁵

Stimulation of β_2 -adrenergic receptors using selective β_2 -adrenergic receptor agonists causes a decrease in M2

muscarinic receptors in human embryonic lung cells.³⁶ This lowering of receptor number is mimicked by forskolin and non-hydrolysable analogs of cAMP, demonstrating a cAMP-dependent mechanism. Prolonged treatment with β_2 -adrenergic receptor agonists causes both a reduction in M2 receptor number at the cell surface and a reduction in muscarinic receptor mRNA, both believed to occur through the action of PKA.³⁷ Binding of muscarinic agonists, such as acetylcholine, induces contraction of airway smooth muscle. By reducing M2 receptor numbers, β_2 -adrenergic receptor agonists decrease the contractile ability of muscarinic agonists. Therefore, inhibition of PDE4 and the resultant increase in PKA activity would have a comparable pharmacologic effect of reducing airway hyperresponsiveness, a similar outcome to that seen in the PDE4D-deficient mouse.

β -Adrenergic receptor agonists provide broncho-protection in asthma through the previously described mechanism.³⁶ Signaling through this receptor leads to the production of cAMP through activation of adenylate cyclase by G_s. Phosphorylation of the receptor by PKA, activated by the rise in cAMP, leads to a switch to signaling through G_i, blocking cAMP production. β -Arrestin acts as a scaffolding protein, playing a role in the desensitization of G-protein-coupled receptors, such as the β_2 -adrenergic receptors.³⁸ Upon receptor binding and G_s activation, the kinase G-protein-coupled receptor kinase (GRK) is activated. This phosphorylates the receptor, leading to the recruitment of β -arrestin, resulting in a switching of signaling to G_i. β -Arrestin has been shown to associate with PDE4D3 and PDE4D5 isoforms.³⁹ In addition, it is believed that β -arrestin controls the desensitization of the receptor through the recruitment of the PDE4, reducing cAMP synthesis through G-protein switching and enhancing its degradation through the presence of the PDE.³⁹ Stimulation of HEK293 cells overexpressing the β_2 -adrenergic receptor results in PKA activation and recruitment of β -arrestin and PDE4D isoforms to the receptor.³⁸

Two sites of interaction have been identified on PDE4, one in the common catalytic region and one in the unique N-terminal region, controlling specificity of binding.⁴⁰ There are two corresponding sites of interaction on β -arrestin, one in the extreme N-terminus and one in the C-terminal region, allowing preferential interaction with PDE4D5 over other PDE4 isoforms. Breaking this regulation by either inhibition of PDE4 or transfection of cells with a catalytically inactive PDE4 enzyme leads to the more rapid desensitization of the β_2 -adrenergic receptor,

resulting in enhanced ERK activation.⁴⁰ This is consistent with enhanced switching from G_s to G_i and a reduction in M2 muscarinic receptor downregulation.

Not only does ERK phosphorylation cause isoform specific alterations in activity, but these kinases also physically bind PDE4 enzymes.⁴¹ This interaction occurs through two distinct binding sites within the catalytic region of PDE4, one termed the FQF region after its amino acid sequence and the other, the KIM (kinase interaction domain). The KIM sequence is a binding motif common for all members of the MAPK family; however, the presence of the additional FQF sequence confers specificity of binding towards ERK.

Phosphodiesterase 4D5 has been found associated with receptor for activated C kinase 1 (RACK1) in many cell types.⁴² It is suggested that RACK1 acts as a scaffolding protein, recruiting and holding PDE4D5 within a specific region of the cell, thus controlling local cAMP concentrations. RACK1 is also found to interact with Src, integrin β -subunits and the common β -chain of the IL-3 and IL-5 receptors.⁴³ It consequently forms a multienzyme complex in which PDE4 is also present, allowing the control of cAMP and PKA levels.

RACK1 binds PDE4D5 in a region within its unique N-terminus called RAID1⁴³ (RACK1 interaction domain). This is a helical domain consisting of hydrophobic amino acids. The amino acids involved in the binding of RACK1 to PDE4D5 have been carefully mapped, allowing the generation of a binding site model. This information could be used in the generation of specific compounds capable of disrupting this interaction.

CONCLUSIONS

Inhibitors of the PDE4 family have been shown to be potent anti-inflammatory agents. Their clinical use has been severely limited due to the potent side-effects observed. The latest inhibitors emerging show greatly reduced side-effects; however, the potential still exists.

Specific PDE4 isoforms are involved in the control of specific cellular signaling pathways, through protein phosphorylation or physical interaction with key components. These interactions demonstrate unique functional roles for a number of PDE4 isoforms. It must be presumed that there are many more of these interactions remaining to be identified. It also provides a reason for the potential expression of many PDE4 isoforms within a single cell, all of which have one enzymatic function, that of degrading cAMP. By selective expression of members

of the PDE4 family, cells are able to tightly control cAMP regulation of their cellular signaling pathways.

Targeted disruption of these interactions and phosphorylation events provides a means of blocking inflammatory pathways while allowing PDE4 isoforms to function as normal in other pathways. This strategy would allow the development of novel anti-inflammatory agents lacking any side-effects.

Crystallization of full-length PDE4 has so far proved impossible due to the great flexibility of the N-terminal controlling region. Thus, although the shape of the catalytic domain is known and available for the development of more specific inhibitors, only small regions of the N-terminus in isolation have been modeled. Compounds targeting the enzymes regulation within the N-terminal controlling regions will require this information to lead to enhanced binding potency.

The future of PDE4 inhibitors seems bright, with the arrival of a new generation of potent, low side-effect drugs on the market. The next goal will be the targeting of selected PDE4 enzymes in order to block specific cellular processes. Potentially, targeting the non-catalytic controlling regions of the enzyme would allow this.

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