

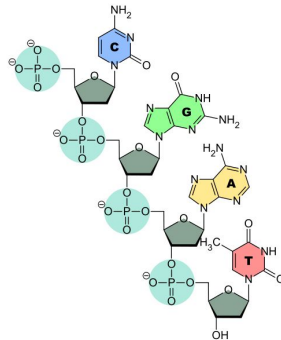
Phosphodiesterases as drug targets

Therapeutic targeting of 3', 5'-cyclic nucleotide phosphodiesterases: inhibition and beyond.

George S. Baillie, Gonzalo S. Tejeda and Michy P. Kelly
Nat Rev Drug Discov 18, 770–796 (2019)

Phosphodiesterases

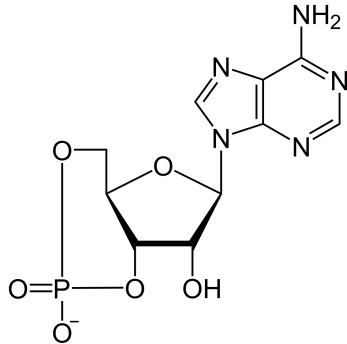
- A Phosphodiesterase is an enzyme that breaks phosphodiester bonds
- A cyclic nucleotide phosphodiesterase is therefore an enzyme that breaks the phosphodiester bond in a cyclic nucleotide



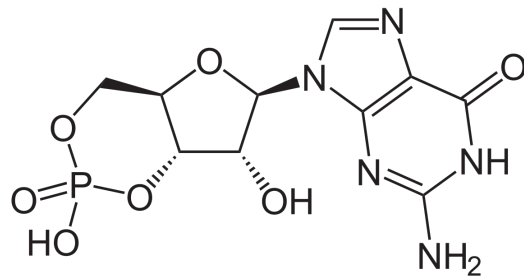
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- A Phosphodiesterase is an enzyme that breaks a phosphodiester bond
- A cyclic nucleotide is therefore an enzyme that breaks the phosphodiester bond in a cyclic nucleotide
- Here you can see, Adenylyl cyclase converts ATP to cyclic AMP and phosphodiesterase breaks the phosphodiester bond to convert it to AMP
- Similarly, guanylate cyclase converts GTP to cyclic GMP and phosphodiesterase converts it to GMP

Cyclic nucleotides



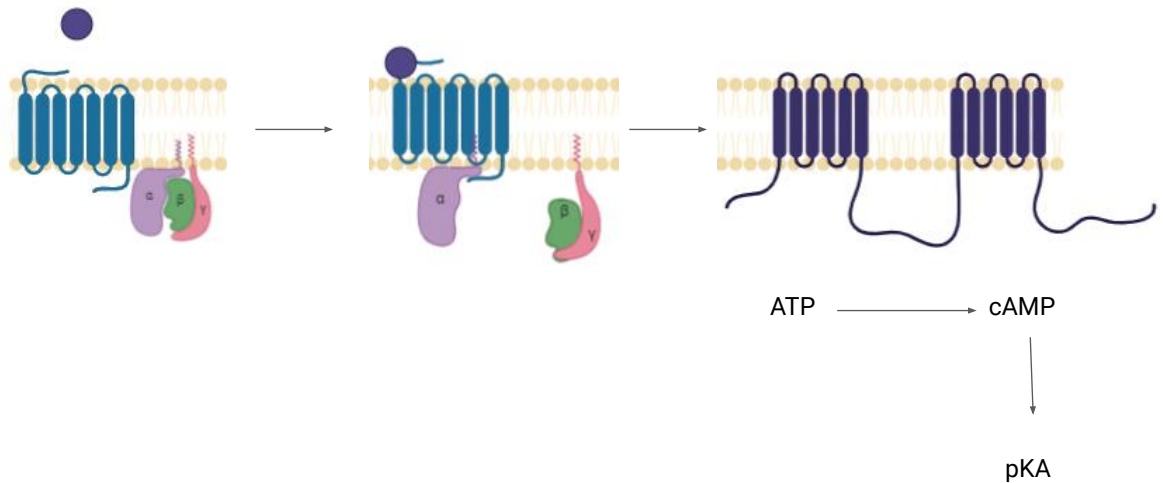
cAMP



cGMP

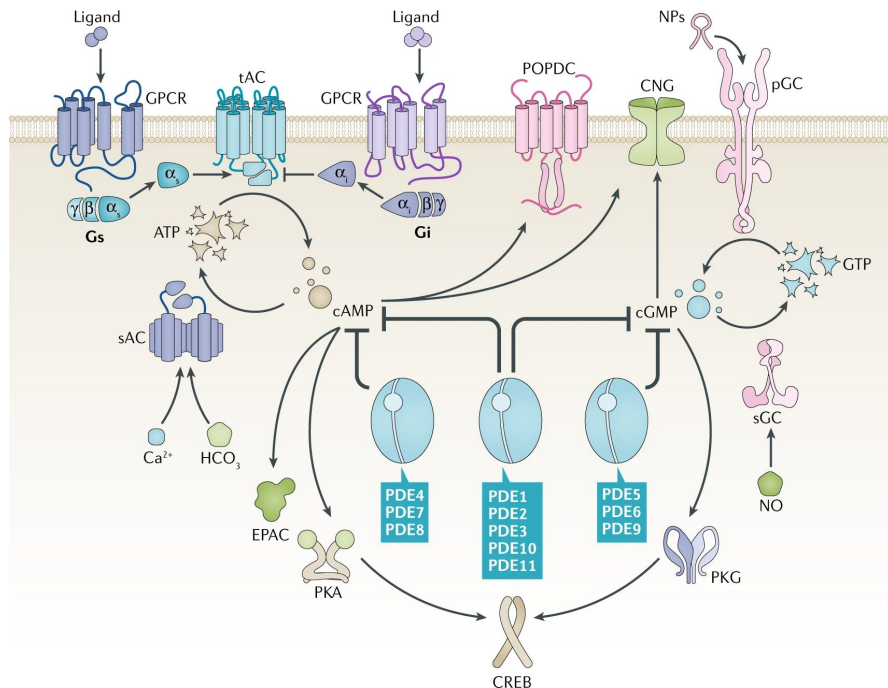
- Cyclic AMP and GMP are the most prominent secondary messengers and are involved in a large number of signalling processes. They are called the canonical secondary messengers and besides them there are non canonical cyclic nucleotides like cCMP, UMP, IMP and cyclic diGMP.
- Since these molecules are involved in a large variety of signalling processes, any issue in their intracellular concentration can have severe biological effects. Before we go into the disorders associated with these signalling molecules, I'd like to quickly take you through the signaling mechanism of cyclic AMP and GMP

Cyclic nucleotides



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- The G Protein coupled receptors are the largest known family of cell membrane receptors. Interestingly, Brian Kobilka and Robert Lefkowitz won the 2012 Nobel Prize in Chemistry for their work on GPCRs.
- Found only in eukaryotes, there are over 800 different types of GPCRs each one has a specific function - they can activate or inhibit other proteins. Since these GPCRs bind ligands like hormones, neurotransmitters, they can regulate the immune system, growth, vision, mood etc. Because of this they are also a very important drug target.
- A GPCR has 7 transmembrane alpha helices that are bound to G proteins. What is a G protein? As you can see here, a G protein is heterotrimeric and has three subunits- alpha, beta and gamma. When inactive, the G protein is bound to GDP via its alpha subunit.
- When a GPCR binds to its ligand, the G protein's alpha subunit exchanges the GDP for GTP causing the alpha subunit to dissociate from beta and gamma subunits. The alpha subunit then goes and binds another protein for example the adenylyl cyclase. The adenylyl cyclase in turn converts ATP to cyclic AMP which can then activate effector molecules like protein kinase A (others include Epac and CNGs) triggering a signalling cascade for example protein kinase A can activate transcription factors like CREB.
- To my understanding, the process is similar for cyclic GMP where nitric oxide or ANP (atrial natriuretic peptide) bind to a guanylyl cyclase, cyclic GMP is made which can activate protein kinase G.
- Phosphodiesterases are therefore key in regulating their concentration and maintaining homeostasis. Alterations in phosphodiesterases have been implicated in various diseases and cancer pathologies.



This is an image from the article showing the various signalling pathways that the cyclic nucleotides are involved in. As you can see, that the G protein coupled receptors can be membrane bound or soluble. Both cyclic AMP and cyclic GMP activate cyclic nucleotide gated channels (CNGs) which modulate the activity of phosphodiesterases.

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tAC: transmembrane adenylyl cyclase

sAC: soluble adenylyl cyclase - activated by bicarbonate and calcium

pGCs: particulate guanylyl cyclase - activated by natriuretic peptides

sGC: soluble guanylyl cyclase - activated by nitric oxide

POPDC: Popeye domain containing protein

EPAC: Exchange protein activated by cAMP

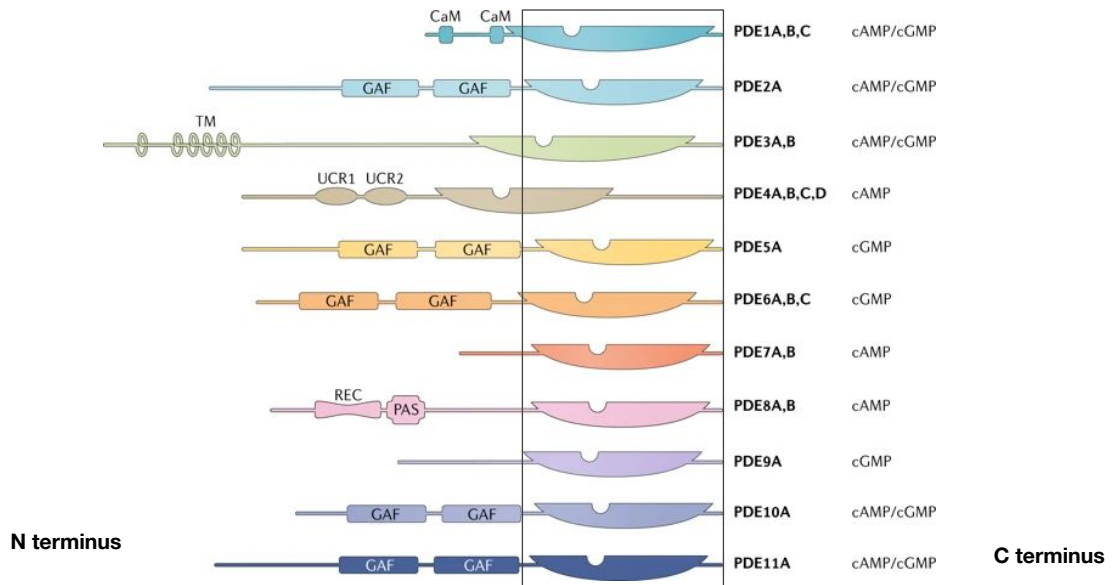
CREB: cAMP response element binding protein

PKA: protein kinase A

PKG: protein kinase G

CNG: A cyclic nucleotide gated channel is an ion channel that is activated when a cyclic nucleotide binds to it (usually Na⁺, K⁺, Ca²⁺ associated)

Types of PDEs



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Cyclic nucleotides vary a lot on where they are present, the ligand associated and functioning. Unsurprisingly,, multiple phosphodiesterases are needed to hydrolyse them.

There are 11 families of PDEs which have been categorised on the basis of catalytic activity. The semicircle structure you see are the catalytic domains in the C terminus. They can be either cytosolic or membrane bound.

In this figure you can see the different phosphodiesterase families. The numbers 1,2,3 refer to the family while the alphabets are for the genes they come from.

As you can see these enzymes differ more in their N terminus than their C terminus. But since these enzymes all have different affinities for cAMP and cGMP, that is how they're categorised. Obviously, it is also possible to group them according to where they're found in the body. Some families are cAMP specific, some cGMP specific. Five PDE families (1,2,3,10 and 11) hydrolyse both cAMP and cGMP. Due to alternate promoters and splicing, each PDE family has multiple isoforms that differ in terms of the length and complexity of their N-terminal regulatory domains.

Also, besides degrading cyclic nucleotides, phosphodiesterases are also involved in creating nanodomains or subcellular compartments in which the cyclic nucleotide signalling is localised.

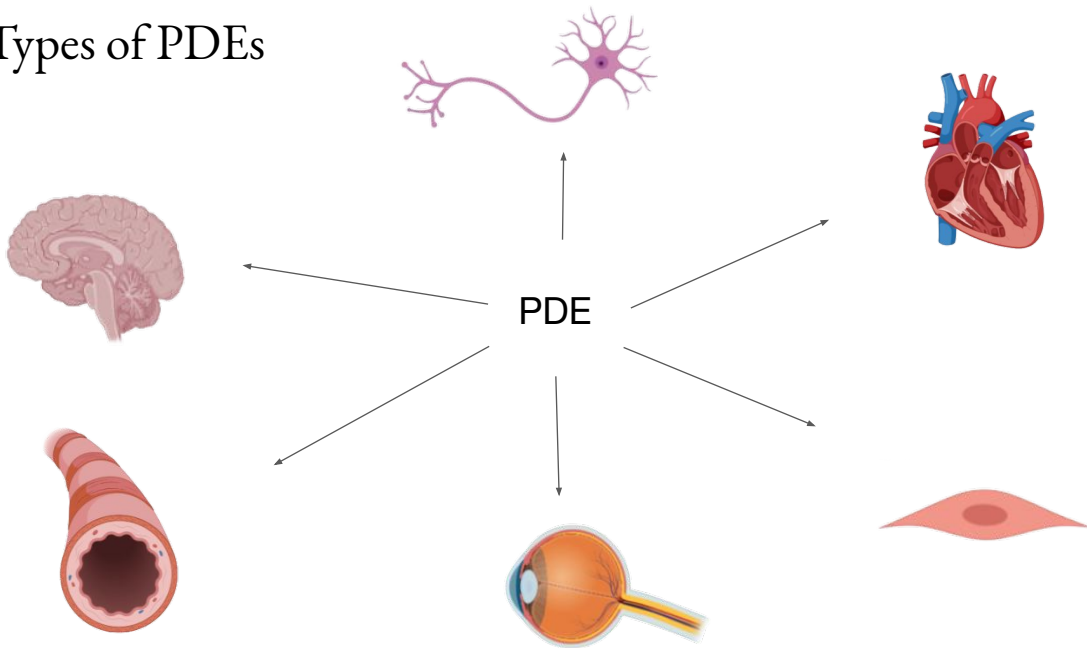
GAF:cGMP-binding PDEs Anabaena adenylyl cyclases and Escherichia coli FhlA

PAS: Per-Arnt-Sim domain

TM: transmembrane domain

UCR: upstream conserved region.

Types of PDEs



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As I said, phosphodiesterases can also be grouped according to where they are found. For example, PDE1, 4,7,9,10 are found in the brain

PDE 6 is found in retinal rods and cones and this is specific for cGMP since cyclic GMP is involved in phototransduction in the eye.

PDE 1,2,3,7 are found in the heart.

PDE9 is found in the small intestine

And finally many Phosphodiesterases like PDE1,4 are found in smooth muscles

Since they can influence muscle contraction/relaxation (inotropic effect) and also vasodilation, PDEs can also be called inotropic vasodilators though I am not sure if anyone really does.

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There is a huge list of PDEs in the article which you can look up but I have also mentioned a few at the end of this presentation which you can check once this gets uploaded.

Physiological Role of PDEs



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Phosphodiesterases are the only family of enzymes that degrade cyclic nucleotides. Therefore they play a very important role in health and disease.

Most phosphodiesterases are expressed in the nervous system where they regulate neurodevelopment, neuronal excitability, synaptic transmission. Every brain region expresses more than one PDE but no two PDEs have the exact same regional distribution.

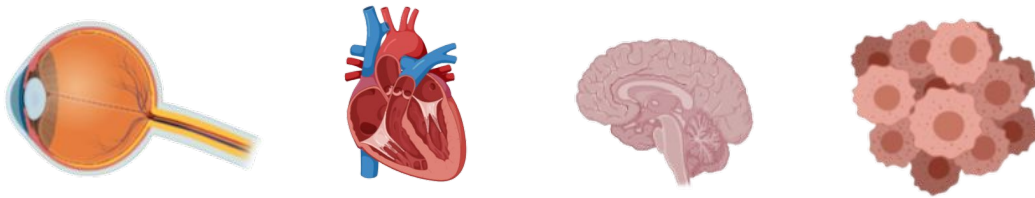
For example, PDE11A is the only phosphodiesterase to be present in the hippocampus where it regulates how a person respond to the mood stabiliser lithium. However it isn't involved in depression related behaviours and anxiety. That is regulated by PDE4D which is present in almost all regions of the brain.

Similarly, there are PDEs that are associated with motor functions, PDE6 mediates the light response in the eye. PDE2,3, and 4 isoforms regulate myocardial contraction and relaxation and the cell growth and survival of myocytes. Unsurprisingly, dysfunction of these PDEs can cause heart disease.

Many phosphodiesterases are involved in regulating sperm motility, or in the male excurrent duct and disorders can cause erectile dysfunction.

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Nanodomains and disease states

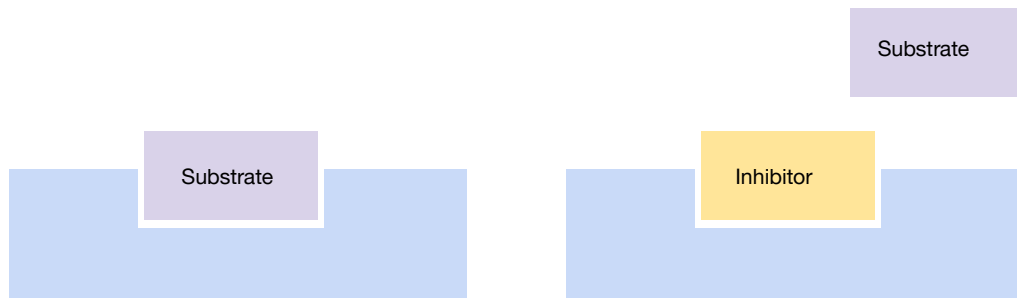


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Phosphodiesterases have multiple isoforms and no two isoforms will have the same substrate specificity, tissue expression and subcellular localisation. In addition, the cyclic nucleotides are also present in subcellular pockets and different pockets have have different roles. For example, particulate guanylyl cyclase is impaired in the brains of patients with Alzheimers this decreases the cyclic GMP concentration in the cytosol. But in colon cancer, membrane bound guanylyl cyclase is suppressed and membrane enriched PDE10A is overexpressed which decreases the concentration of GMP in membrane proximal pools. Other diseases which have compartment or pocket specific defects in cyclic nucleotide concentrations are hypertension, bipolar disorder.

This subcellular compartmentalisation might make therapeutic targeting easier since you can target a particular PDE isoform without affecting the concentrations of cyclic nucleotides anywhere else. .

PDE Inhibition



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When we are looking to target an enzyme, the so called easiest thing to do would be to inhibit it by competitive blocking of substrate and that's precisely what most of the marketed phosphodiesterase inhibitors are based on.

Marketed PDE Inhibitors



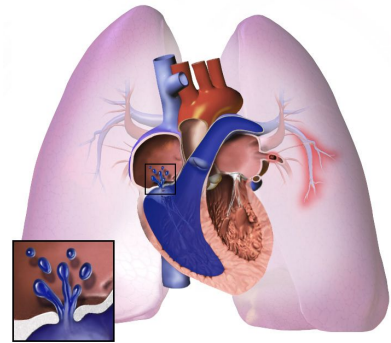
Theophylline

Asthma and bronchoconstriction



Apremilast

Plaque psoriasis and psoriatic arthritis



Sildenafil

Erectile dysfunction and pulmonary hypertension

There are currently many PDE inhibitors available in the market. These inhibitors can be PDE selective that is specific to a particular phosphodiesterase family or isoform or can act on multiple i.e. be non-selective.

Theophylline, an analog of caffeine, is a non-selective phosphodiesterase inhibitor that inhibits PDE3,4,7. It is given to patients suffering from asthma and bronchoconstriction.

Vinpocetine an over the counter drug is a non-selective inhibitor, which in combination with caffeine can improve memory and recovery from stroke.

Roflumilast is a PDE4 inhibitor and given to patients of chronic obstructive pulmonary disease.

Apremilast is also another PDE4 inhibitor given to patients suffering from psoriasis

Sildenafil, a PDE5 inhibitor, was originally developed for treatment of erectile dysfunction. However it has also been seen to aid in pulmonary hypertension.

Ibudilast, a non selective, PDE 2,4,10, and 11 inhibitor inhibits glial cell activation and is approved for use as a bronchodilator and is being studied for substance abuse/withdrawal

The ones that failed



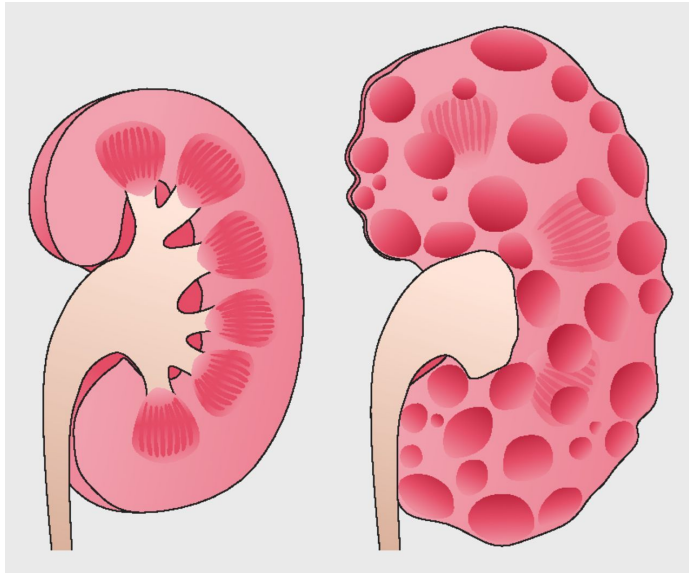
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There are many reasons why a drug fails to reach the market. Sometimes it's not safe, has low efficacy, sometimes the side effects are more than the benefits. The first generation of phosphodiesterase inhibitors like Cilostazol or Milrinone (both PDE3 inhibitors) are FDA approved drugs but not without issues. Cilostazol, a vasodilator, which was given for patients with limping issues has severe gastrointestinal side effects. Milrinone is given to treat severe congestive heart failure but it tends to get deposited in the kidneys causing renal failure so it is only given to end stage patients.

Sometimes a marketed drug can fail when tested for the treatment of another disorder. For example, sildenafil is marketed for erectile dysfunction and pulmonary hypertension but clinical trial for patients of schizophrenia didn't show promising results. And even though a lot of focus has been on developing phosphodiesterase inhibitors that target disorders of the brain, be it Alzheimer's, cognition disorders, depression or anxiety, a significant percentage of these have failed.

And this is why we need to look at newer drug target mechanisms besides competitive inhibition. There are many PDEs in development where researchers are looking at creating new chemical entities or repurposing existing ones. There are also ongoing efforts in looking at new modes of action. For example, I already spoke about Sildenafil (viagra) being used to treat pulmonary hypertension, Ibudilast a non selective inhibitor inhibits glial cell activation is used in Japan as a bronchodilator.

PDE Activation

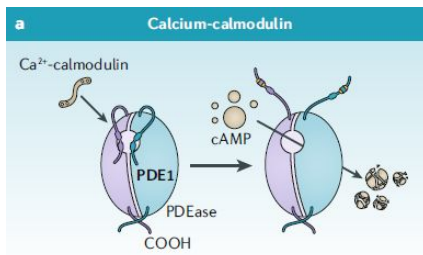


<https://adultpediatricuro.com/>

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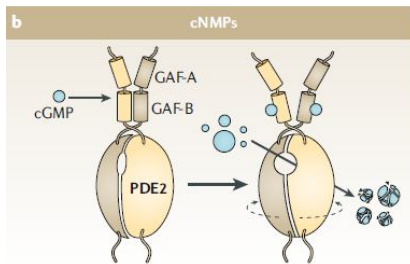
It has been seen that sometimes phosphodiesterases in these pools can be suppressed causing disease states like migraine, infertility, melanoma and basal cell carcinoma. A PDE activator will increase the level of phosphodiesterase restoring normal state. A PDE4 activator has been developed for treatment of polycystic kidney disease where increased levels of vasopressin receptors increased the cyclic AMP concentration leading to cyst formation.

Relieving auto-inhibition of catalytic activity

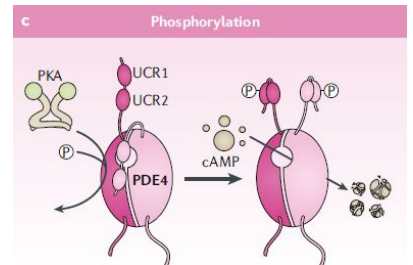


PDE Activation

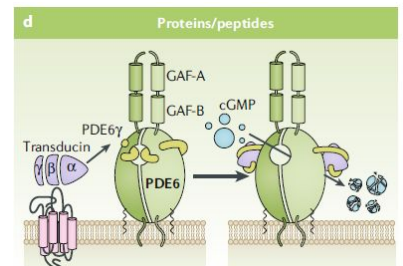
Targeting GAF domains



Preventing trans-capping



Manipulate protein-protein binding



Remember how I said that the cyclic nucleotides and phosphodiesterase isoforms are present in subcellular compartments? It has been seen that sometimes phosphodiesterases in these pools can be suppressed causing disease states like migraine, infertility, melanoma and basal cell carcinoma. A PDE activator will increase the level of phosphodiesterase restoring normal state. A PDE4 activator has been developed for treatment of polycystic kidney disease where increased levels of vasopressin receptors increased the cyclic AMP concentration leading to cyst formation.

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a | Calcium-calmodulin (CaM) binding to the CaM domains of PDE1 relieves N-terminal auto-inhibition of the catalytic site, thereby promoting enzymatic activity

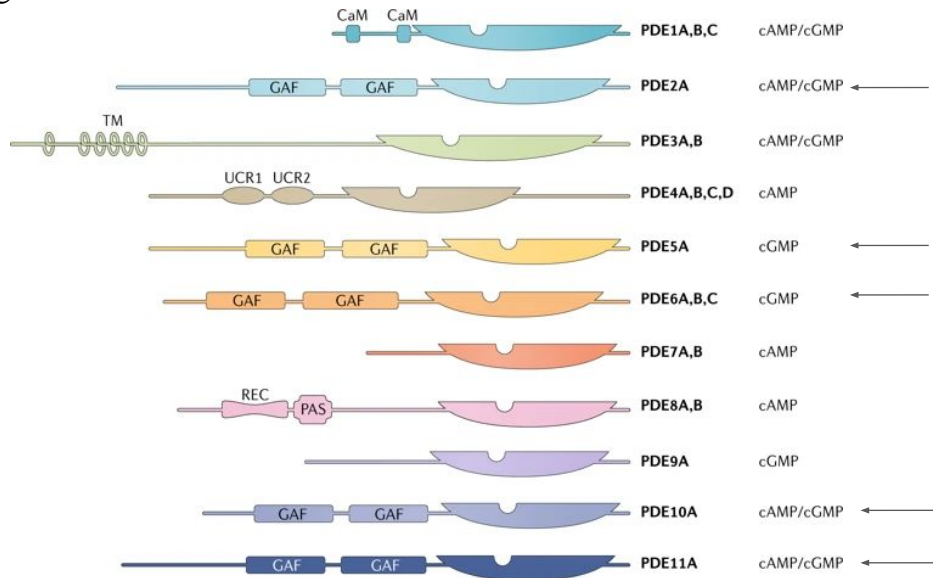
b | Cyclic nucleotides (cNMPs) binding to GAF domains of dimeric phosphodiesterases (PDEs) (shown here: cGMP binding the GAF-B domain of PDE2) are thought to promote catalytic activity by inducing an outward rotation of the catalytic domains and, thus, enabling access to substrates

c | Phosphorylation by protein kinase A (PKA) or protein kinase G (PKG) activates several PDEs. In the case of PDE4D, phosphorylation of the UCR1 domain by PKA

causes UCR1 to bind its own UCR2 domain instead of the catalytic site of the other monomer, thereby locking the enzyme in an active state.

d | PDE activity can also be modulated by protein–protein binding interactions. One such well-characterized example involves membrane-bound PDE6, where the rhodopsin-activated G-protein α -subunit transducin displaces the inhibitory PDE6 γ C-termini from the catalytic sites on PDE6 $\alpha\beta$, thus promoting cGMP hydrolysis³²⁵. GAF, cGMP-binding PDEs *Anabaena* adenylyl cyclases and *Escherichia coli* FhlA ; UCR , upstream conserved region.

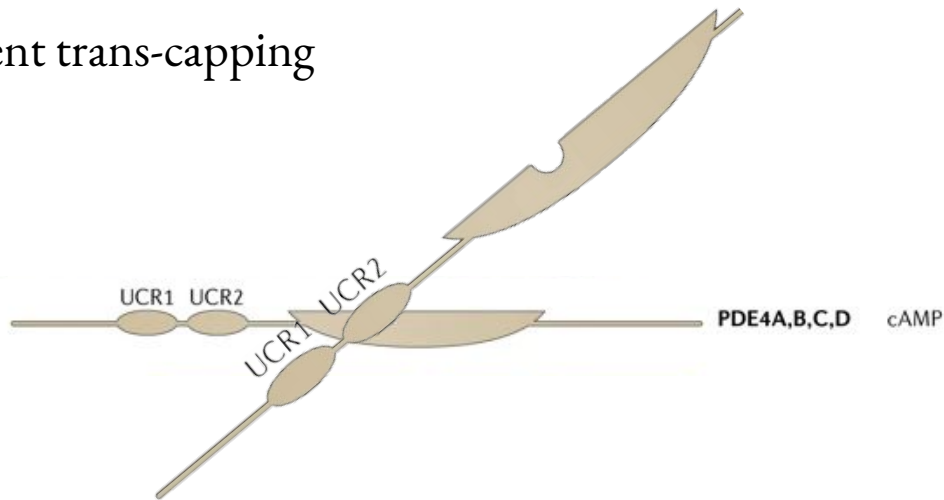
Targeting GAF Domains



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The **GAF domain** is named after some of the proteins it is found in, including cGMP-specific phosphodiesterases, *Anabaena* adenylyl cyclases and *E. coli* FhIA. In mammals it is found in PDE 2,5,6,10 and 11. Though the mechanism isn't really clear, these phosphodiesterases get activated when cyclic GMP binds to the GAF domains on them. It is believed that binding of cyclic GMP causes structural changes in the GAF domains which relieves autoinhibition. And this has been tested to try to activate these phosphodiesterases. It has been seen that these GAF domains bind a multitude of small molecules and cyclic nucleotide analogues and while some activate catalytic activity of the phosphodiesterase, others inhibit it.

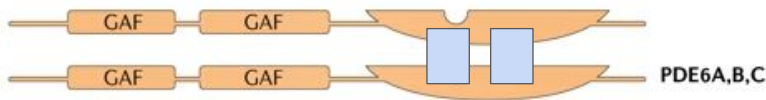
Prevent trans-capping



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When the crystal structure of phosphodiesterases was analysed, it was seen that they can exist in three conformational states. In the first is an open conformation where substrate or inhibitor can bind easily, the second is a closed conformation in which the C terminal helix caps the catalytic site blocking the substrate's access to it. A third one is when the UCR2 helix of another phosphodiesterase blocks the catalytic site. This is called trans capping. Data suggest that phosphorylation of UCR1 by protein kinase A can prevent this trans capping locking the phosphodiesterase in an active state. However, this "proof of principle" is only available for PDE4.

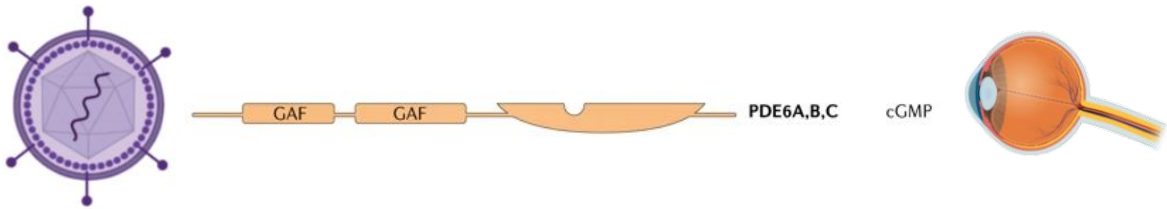
Manipulate protein-protein binding



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PDE6 is a heterodimer of alpha beta and its holoenzyme has two inhibitory subunits on the catalytic pockets of the dimer. This obviously prevents cyclic GMP from binding to it. Transducin is a G protein expressed in the retina's rods and cones. Its alpha subunit binds to the C terminal of PDE6 and relieves the inhibition. Since PDE6 dysfunction is related to vision loss, activating PDE6 by manipulating the protein-protein interaction is a good therapeutic idea.

Gene Therapy



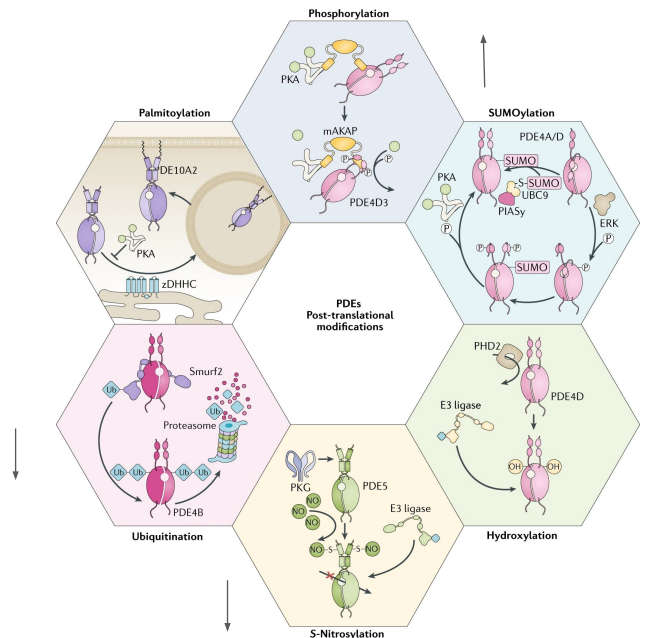
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Scientists are also looking at activating phosphodiesterases using viral transfer of phosphodiesterase genes, gene editing, microRNAs etc.

Transducin mediated activation of pDE6 results in high levels of cyclic GMP which causes the loss of primary rods and ultimately vision. To treat this, recombinant PDE6alpha has been expressed using adeno-associated virus and it was seen that the recombinant PDE6 preserved retinal structure and phototransduction in mice.

Gene therapy is also being tried for viral delivery of recombinant, light activated proteins in optogenetic medicine i.e. using light to control how the brain behaves.

Target Post Translational Modifications



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Finally, post translational modifications are a good way to influence trafficking of phosphodiesterases and their concentration in the subcellular compartments. For example, Palmitoylation promotes membrane association of phosphodiesterases. Phosphorylation of PDE4D3 causes the termination of signaling in the muscle compartment. Ubiquitination is known to target proteins for degradation and it has been seen that phosphodiesterases interact with E3 ligase to shorten the PDE half life. SUMO (small ubiquitin like modifier) alters the location, activity or protein-protein interaction of a protein and with respect to PDEs protects them against inhibitory phosphorylation. S-nitrosylation and proline hydroxylation both target PDE for degradation. So, these can also be looked into if we want to increase the concentration of cyclic nucleotides.

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Phosphorylation is a very common mechanism to control PDE activity as depicted by the action of protein kinase A (PKA) on PDE4D3. Both enzymatic activity and binding affinity of PDE4D3 for mAKAP are increased by PKA phosphorylation, allowing a faster signal termination in myocytes.

Palmitoylation of PDE10A2 in its N-terminal region translocates the enzyme to the plasma membrane, although its phosphorylation by PKA can prevent the action of the palmitoyl acyltransferase (zDHHC)¹⁵⁸.

Ubiquitylation can influence PDE function by controlling stability. For example, the E3 ubiquitin ligase Smurf2 targets PDE4B for degradation which leads to the attenuation of liver fibrosis²⁷⁰.

S-Nitrosylation can also tag PDEs for destruction. Thus, the covalent incorporation of nitric oxide (NO) to the GAF-A domain of a protein kinase G (PKG)-phosphorylated and active PDE5 directs the enzyme to the proteasome²⁷⁵. Hydroxylation of proline residues has emerged as another post-translational modification to stimulate turnover of PDEs. Prolyl hydroxylase domain protein 2 (PHD2) action on PDE4D increases its recognition by E3 ligase complexes in cardiomyocytes²⁷⁷.

Finally, SUMOylation can intensify the activity of PDE4A and PDE4D. The small ubiquitin-like modifier (SUMO) transfer from the E2 conjugase UBC9-E3 enzyme PIASy complex to the PDEs enhances their activation by PKA phosphorylation and represses their inhibition induced by extracellular signal-regulated kinase (ERK) activity²⁷².

Challenges

Which

Where

When

How

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Drug development is difficult because there is so much that is not known and proof of principle does not always guarantee success. Phosphodiesterase inhibitors might not necessarily be a new thing but before we develop new targets, we have to take several things into consideration

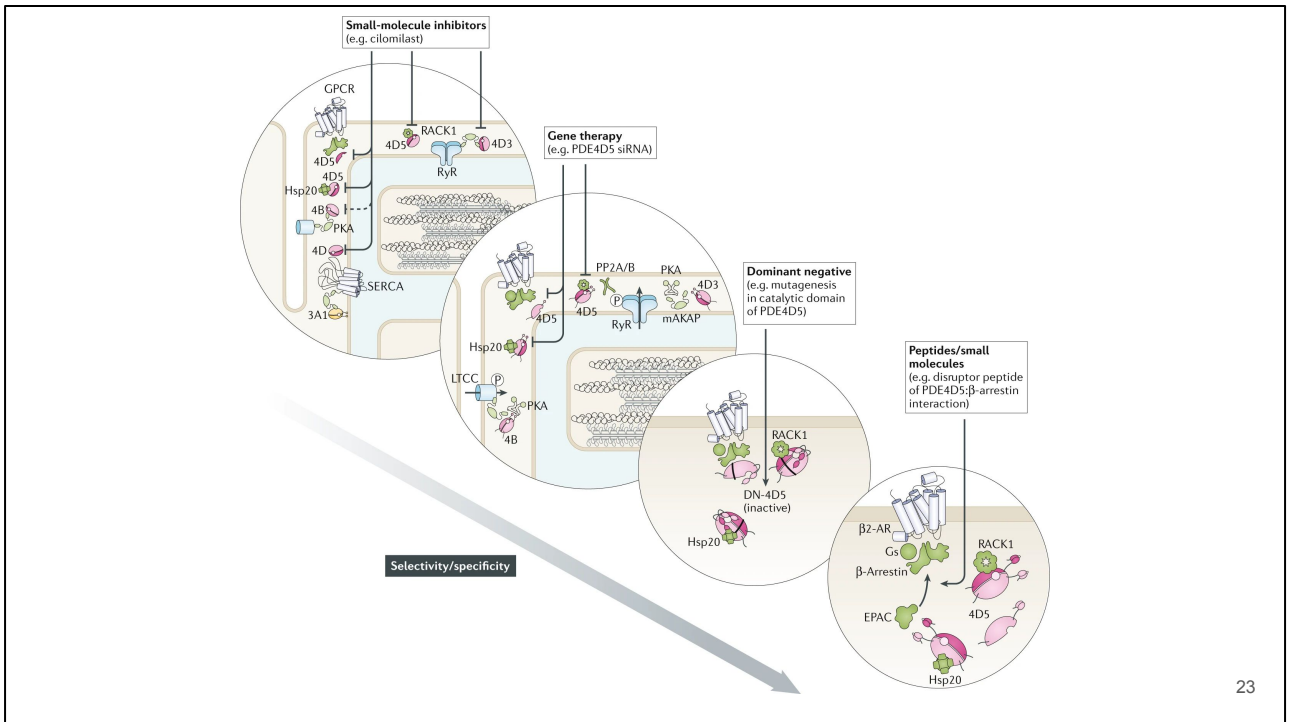
- Which PDE, which isoform of that phosphodiesterase
- Where is it located and can we target that particular subcellular compartment without affecting other compartments
- When is the PDE expressed or suppressed
- How do we target it.

This is difficult for any drug development process but it is especially difficult for phosphodiesterases since so much is still unknown. And obviously, everything needs to be done keeping safety and efficacy in mind.

References

1. Howell, L. L. Comparative effects of caffeine and selective phosphodiesterase inhibitors on respiration and behavior in rhesus monkeys. *J. Pharmacol. Exp. Ther.* 266, 894–903 (1993).
2. Ahmad, F. et al. Cyclic Nucleotide Phosphodiesterases: important signaling modulators and therapeutic targets. *Oral Diseases* 21, e25–e50 (2015).
3. Liras, S. & Bell, A. S. *Phosphodiesterases and Their Inhibitors*. (John Wiley & Sons, 2014).
4. Baillie, G. S., Tejeda, G. S. & Kelly, M. P. Therapeutic targeting of 3',5'-cyclic nucleotide phosphodiesterases: inhibition and beyond. *Nature Reviews Drug Discovery* 18, 770–796 (2019).

Thank you!



It is becoming clear that selective targeting of PDE function will be required to achieve efficacy while diminishing undesirable side effects.

Small-molecule inhibitors (for example, cilomilast) are readily developed with family-specific selectivity (for example, targeting PDE4 over PDE3); however, isoform specificity remains a challenge (for example, cilomilast inhibits PDE4D with only sevenfold selectivity versus PDE4B)³²⁶.

Conversely, gene therapy (that is, expressing a recombinant construct to knock down or restore expression of a given PDE isoform) and dominant-negative approaches (that is, expressing a catalytically inactive PDE4D5 that displaces the endogenous isoform from its interacting partners) can target isoform subtypes exclusively (for example, targeting PDE4D5 but not PDE4D3 nor PDE4B).

That said, dominant-negative approaches would influence signalling within all microdomains regulated by that isoform. The greatest specificity can be achieved with peptide/small-molecule binding disruptors or mutagenesis approaches (not shown) that are designed to prevent a specific PDE isoform from binding a specific partner, thus altering signalling only within one specific complex.

Phosphodiesterase: Location

Phosphodiesterase	Found in
PDE 1	Brain, smooth muscle, heart, lung
PDE 2	Platelet, macrophages, endothelial cells, heart
PDE 3	Heart, vascular smooth muscle, platelets
PDE 4	Smooth muscle, cardiovascular tissue, brain, inflammatory cells
PDE 5	Corpus cavernosum (erectile tissue of the penis)
PDE 6	Retinal rods and cones

Phosphodiesterase Inhibitors: Location

Phosphodiesterase	Found in
PDE 7	B lymphocytes, brain, heart
PDE 8	Testes
PDE 9	Spleen, small intestine, brain
PDE 10	Brain, thyroid, testes
PDE 11	Testes, prostate

PDE Inhibitors under development

Drug	PDE Inhibited	Used to treat (if repurposed)	Studied for
Ibudilast	PDE 3,4,10,11	Inhibits glial cell activation	Bronchodilator
ITI-214	PDE 1	-	Cognitive defects, heart failure
TAK915	PDE2	-	Cognitive impairment
Cilostazol	PDE3	Intermittent claudication (limping)	Type 2 diabetes related nephropathies, tinnitus
Roflumilast	PDE4	COPD	Weight loss in women with PCOS
Sildenafil	PDE5	Erectile dysfunction, pulmonary hypertension	Concussion-related reductions in cardiovascular reactivity

PDEs as disease markers

PDEs can be genotyped from blood samples to assess risk for specific diseases. This can be done ex vivo when the tissue has already been biopsied like in cancers or in vivo.

E.g. PDE4D7 expression is initially upregulated with the development of primary tumours but then is downregulated when the disease progresses to an androgen-independent state -m can be used to identify stage of prostate cancer patient is in.

PDE3A is greatly expressed in certain cancer cell types such as squamous carcinoma cell lines or gastrointestinal stromal tumour cells.

In vivo, PDE10A downregulation seen in patients with Huntington's and Parkinson's.

More about cGMP signalling

In the kidney and intestine, cyclic GMP triggers changes in **ion transport and water retention**. In the Cardiac muscle, it signals **relaxation**. In the brain it is involved in both development and brain function.

Heart: When the heart is stretched by increased blood volume, heart cells release Atrial natriuretic factor (ANF). ANF travels via the blood to the collecting ducts of the kidney and activates guanylyl cyclase. The activated GC then triggers excretion of sodium ions from the kidney which in turn changes osmotic pressure and causes water loss. This water loss then reduces blood volume and this loss acts as a feedback to stop the production of ANF.

Smooth Muscles: In vascular smooth muscles, ANF on binding to its receptor relaxes the blood vessel i.e. causes vasodilation which increases blood flow while decreasing blood pressure.

The cytosolic GC has a heme group and is activated by nitric oxide. The nitric oxide is produced from arginine, and on binding to the heme group triggers cGMP production. In the heart, cGMP induces relaxation of cardiac muscles and therefore nitroglycerin tablets and nitrovasodilators are given to relieve angina.

Retina: In the eye, cGMP causes ion specific channels to open in retinal rod and cone cells (think the ligand is rhodospin and the G protein is called transducin)

Books and videos that can help

GPCR and cAMP Signaling:

- <https://www.youtube.com/watch?v=FD3oksR-bhk&list=LLi7nugOnl1xNcxWSasAhkxw&index=2&t=0s>
- Lehninger 4th Edition Chapter 12 (Biosignaling)
- Molecular Biology of the Cell 6th Edition Chapter 15
- <https://www.youtube.com/watch?v=9Bq6qHJaSJs>

Phosphodiesterases:

- <https://www.ncbi.nlm.nih.gov/books/NBK27996/> (slightly dated)
- <https://www.youtube.com/watch?v=U4X8HplwEVk> (series)

Sildenafil: <https://www.youtube.com/watch?v=kRx7nZkk-V4> , <https://www.youtube.com/watch?v=D5XepaL7q8Y> , https://www.youtube.com/watch?v=jFukkwp_caA