Phosphodiesterases in endocrine physiology and diseases

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Abstract

The cyclic AMP-Protein Kinase A (cAMP-PKA) pathway plays a central role in the development and physiology of endocrine tissues. cAMP mediates the intracellular effects of numerous peptide hormones. Various cellular and molecular alterations of the cAMP-signaling pathway have been observed in endocrine diseases.

Phosphodiesterases (PDEs) are key regulatory enzymes of intracellular cAMP levels. Indeed, PDEs are the only known mechanism for inactivation of cAMP by catalysis to 5’-AMP. It has been suggested that disruption of PDEs could also have a role in the pathogenesis of many endocrine diseases. This review summarizes the most recent advances concerning the role of the PDEs in the physiopathology of endocrine diseases. The potential significance of this knowledge can be easily envisaged by the development of drugs targeting specific phosphodiesterases.
Introduction

The cyclic AMP (cAMP) pathway plays an important role in the development and function of endocrine tissues. It is also altered in various endocrine disorders. Indeed, numerous genetic alterations of the cAMP-signaling pathway have been observed (1). First, abnormalities of the hormone receptor of a given endocrine pathway have been reported, for example: activating mutations of the gene encoding for the TSH receptor in thyroid toxic adenomas (2), for the LH receptor in Leydig-cell tumors (3), for the calcium sensing receptor in familial hypocalcemia (4), and aberrant expression of G-protein-coupled receptors in bilateral macronodular adrenal hyperplasia (5; 6; 7). Secondly, somatic activating mutations of the stimulatory subunit alpha of the Gs protein have been found in McCune-Albright syndrome or in sporadic pituitary GH adenomas (8; 9). Finally, germline inactivating mutations of the protein kinase A regulatory subunit type 1 (PRKAR1A) have been demonstrated in primary pigmented nodular adrenocortical diseases (PPNAD) and Carney complex (CNC) (10; 11; 12).

Phosphodiesterases (PDEs) have a key regulatory role in the cAMP pathway, as they are the only known mechanism for inactivating cAMP by its catalysis to 5’-AMP (figure 1). During the last several years, various attempts have been made to identify diseases associated with disruption of PDEs. Gene knockout, gene inactivation and genetic association studies have implicated PDEs in numerous diseases such as asthma (13), depression (14), schizophrenia (15) and stroke (16). Moreover, in daily clinical practice PDE inhibitors are able to effectively and safely treat diseases such as erectile dysfunction (17), heart failure (18), severe forms of Raynaud’s phenomenon (19), pulmonary arterial hypertension (20), and chronic obstructive pulmonary disease or asthma (21; 22).
In this review a brief overview of the PDE family will be presented, followed by a
description of their role in endocrine physiology. Finally, the known and potential roles of
PDEs in endocrine diseases will be discussed.

**The phosphodiesterase superfamily**

Adenylyl and guanylyl cyclase are the effector enzymes accounting for cyclic
nucleotide production by converting ATP to cAMP or GTP to cGMP respectively. By
contrast, PDEs cause the cyclic nucleotide degradation by hydrolyzing cAMP into 5’-AMP
or, depending on the type of PDE, cGMP to 5’-GMP. Thus PDEs play a critical role in the
intracellular cAMP and cGMP homeostasis. Moreover, in combination with A-kinase
anchoring proteins (AKAPs), PDEs contribute to compartmentalizing the cyclic nucleotides.
It has been shown that cAMP does not have a uniform intracellular distribution, but
accumulates at specific sites within a cell. AKAPs, PDEs and protein kinase A form
complexes that act to create these simultaneous, multiple cAMP gradients. A cell without
theses complexes and PDEs would be swamped with cAMP after activation of adenylyl
cyclase. Thus, PDEs have a major role in ensuring the proper intensity and spatio-temporal
distribution of cyclic nucleotides (14; 23).

Human PDEs comprise a complex superfamily of enzymes derived from 21 genes
separated into eleven PDEs gene families (*PDE 1-11*) (figure 2). Transcription from different
initiation sites in these genes and differential splicing of their mRNAs results in the
generation of about 100 isoforms of PDE proteins found in all cells and in almost all
subcellular compartments. These isoforms can have different substrate selectivity (cAMP vs
cGMP), kinetics, allosteric regulation, tissue distribution and susceptibility to
pharmacological inhibition. Although PDEs are structurally, biochemically and
pharmacologically different, they share some common structural features. They contain a
conserved catalytic domain with approximately 300 amino acids located near the C-terminal regions, and a variable regulatory domain located in the N-terminal regions. In mammals, 3 of the 11 PDE families selectively hydrolyze cAMP (PDEs 4, 7 and 8), 3 families are selective for cGMP (PDEs 5, 6 and 9) and 5 families present a dual specificity for both cyclic nucleotides but with variable efficiency (PDEs 1, 2, 3, 10 and 11).

Nowadays, it has been demonstrated that six phosphodiesterase families could have a role in endocrine physiology and endocrine diseases: PDE1, PDE2 (PDE2A), PDE3 (PDE3A), PDE4 (PDE4B and PDE4D), PDE8 (PDE8A and PDE8B), and PDE11 (PDE11A). The major properties and localisation of phosphodiesterases are summarised in table 1. However, it is important to underline that understanding PDE functions can be difficult due to the lack of selective pharmacological inhibitors and to the fact that the catalytic properties of many PDEs overlap, so that assignation of a specific role to a particular PDE family or to variants within a family is typically challenging.

Moreover, expression pattern of PDEs isoforms frequently varies with the developmental, proliferative status and with the hormonal stimuli of the cell. In addition, these PDEs isoforms are subjected to different regulations or are targeted to different subcellular compartments that accounts at least in part for creation of microdomains that spatially restrict cyclic nucleotides diffusion. This phenomenon could take part in a tissue specificity of the consequences of PDE mutations.

**Phosphodiesterases in endocrine physiology**

- **Adrenals**

  PDE2A, PDE8A, PDE8B and PDE11A are the major phosphodiesterases that are expressed in the adrenal cortex (24; 25; 26).
Recently it has been demonstrated that PDE2A, PDE8B and PDE11A have a role in the adrenal physiology.

- **PDE2A**

  *PDE2A* is the predominant phosphodiesterase in the adrenal cortex and may be implicated in adrenal physiology (25; 27; 28). To date, the regulation of intracellular cAMP accumulation after ACTH stimulation is not fully elucidated. *PDE2A* may be implicated in the regulation of the ACTH-induced increase in intracellular cAMP. In rat and human cell models, three sequential phases of the effect of ACTH leading to cAMP stimulation have been described. First a rapid and sustained activation of adenylyl cyclase was shown followed with a biphasic effect of ACTH on *PDE2* activity with an initial and rapid inhibition of *PDE2* activity followed with a delayed activation of *PDE2A* (29). Thus *PDE2A* seems to be strongly involved in the regulation of cAMP levels as its activity controls the production of cAMP induced by ACTH.

- **PDE8B**

  Recently, it was demonstrated that PDE8B is a major regulator of adrenal steroidogenesis by both acute and chronic mechanisms (28). Indeed, PDE8B knock-out mouse have elevated levels of corticosterone due to adrenal hypersensitivity to ACTH. In cell culture models pharmacological inhibition of PDE8 has the same effects. This suggests that in normal adrenal cortex PDE8B acts as a negative regulator of adrenal steroidogenesis.

- **PDE11A4**

  The role of *PDE11A4* in adrenal physiology has been poorly investigated. Mice deficient in *PDE11A4* have been generated but no effects on the adrenal have been reported (30). Even if selective PDE11 inhibitors have not yet been discovered, inhibitors that can
partially inhibit PDE11 have been used in murine models and in humans. Tadalafil, a PDE5 inhibitor that can partially inhibit PDE11, is widely used without reports of adrenal dysfunction. Tadalafil was used to study glucocorticoid secretion after a maximal-exercise-related stress in healthy humans (31). Conversely, it was shown that tadalafil administration is able, compared to placebo, to amplify mean salivary cortisol. The sites of action and the mechanisms involved in the observed effect of tadalafil on the hypothalamus-pituitary-adrenal axis response to physical stress are not known. However, these results suggest that PDE5 and/or PDE11A4 could have a role in the regulation of the hypothalamus-pituitary-adrenal axis.

- **Thyroid**

  PDE1A, PDE7A and PDE8B are the phosphodiesterases most highly expressed in the thyroid (25; 26).

  Thyroid-stimulating hormone (TSH) regulates thyroid function through stimulation of the cAMP pathway. Its serum level is a sensitive indicator of thyroid function even within the normal range. It has been suggested that up to 65% of baseline TSH levels are genetically determined, and could vary within the limit of the normal range in healthy people (32; 33).

  Recently, a genome-wide association study was performed to identify genes associated with TSH levels in normal subjects (34). The strongest association was shown at the PDE8B locus. Indeed, an association between a single nucleotide polymorphism rs4704397 in the PDE8B gene and circulating TSH levels was observed. This suggests that PDE8B is one of the major genetic determinants of TSH levels. Each additional copy of the minor PDE8B A allele was associated with a TSH increase of 0.13 mUI/L (T4 and T3 levels were not assessed in this study). This finding in the Sardinian population was confirmed by a meta-analysis that included multiple populations (34). The proposed mechanism by which PDE8B may

influence TSH levels is through control of cAMP signalling. *PDE8B* acts in the thyroid to catalyse the hydrolysis and inactivation of cAMP after TSH signalling. Thus *PDE8B* polymorphisms may reduce cAMP in the thyroid, leading to a decreased thyroid stimulatory response to TSH and hence lower subsequent thyroid hormone production. This is likely to result via feedback in a higher TSH levels required to maintain T4 and T3 in the normal range. However the consequence of this *PDE8B* polymorphism has not yet been shown in vitro.

Thus it is possible that *PDE8B* genetic variants might be involved in regulation of TSH levels.

- **Pituitary**

*PDE1, PDE2, PDE4* and *PDE11A* are the phosphodiesterases most highly expressed in the pituitary (35; 36; 37; 38; 39; 40).

The physiological role of these phosphodiesterases in human pituitary has not been extensively investigated. Nonetheless few studies conducted in rodents’ adenohypophysis demonstrated that PDEs have a role in the release of prolactin and ACTH.

First it was shown that the addition of 3-isobutyl-1-methylxanthine (IBMX), a common inhibitor of PDE subtypes, increased cyclic nucleotides in primary culture of rats anterior pituitary cells (41). Moreover, application of IBMX caused a dose-dependent increase in basal prolactin release. In addition, PDE4 inhibitors are known to activate the hypothalamic-pituitary-adrenocortical system at the pituitary level, implicating PDE4 in the control of corticotroph cell function (42; 43). More recently it was demonstrated that PDE1 and PDE4 are key cAMP metabolising enzymes in rat corticotrophs cells (38). In the normal human pituitary gland and similarly to that reported in rodent pituitary, PDE activity was almost completely inhibited by IBMX treatment with major and similar contributions of the PDE1 and the PDE4 (37).
Gonads

Testis

Phosphodiesterases 1A, 1C, 3B, 5A, 8A, 10A, 11A are those most highly expressed in the testis (26; 44; 45).

The role of two of these phosphodiesterases have been investigated and demonstrated in the testis: PDE11A in spermatozoa physiology and PDE8A in the regulation of hormone production.

- PDE11A

PDE11A plays a role in spermatozoa physiology. However, data are conflicting. PDE11A deficient mice present with alterations of spermatogenesis and sperm function. A significant reduction in sperm progression rate, sperm concentration and percentage of alive ejaculated sperm compared to wild type mice were shown in pde11a-/- mice (30). However, despite these alterations, the fertility of PDE11-null mice and the viability, histological features and anatomical abnormalities of offspring were comparable to that of wild-type mice.

Another way to demonstrate the role of PDE11A in spermatozoa physiology is to use PDE11A inhibitors. Tadalafil, a PDE5 inhibitor, has been tested as it is the most effective available PDE11A inhibitor. There are conflicting reports about the inhibition of PDE11A using this medication. In a clinical trial, daily intake of tadalafil at doses of 10 and 20 mg for 6 months produced no change in spermatogenesis or reproductive hormones in men (46). Another study demonstrated that tadalafil causes a statistically significant decrease sperm motility (47). In mice it was shown that chronic overexposure to tadalafil causes testicular tissue alterations with decreased testis weight, degeneration and atrophy of the seminiferous epithelium and decrease in sperm production.
As tadalafil inhibits PDE11 activity with a 40-fold weaker potency than for PDE5 activity, these effects should be carefully assessed before concluding that they are simply attributable to PDE11A inhibition in the testis (48). These findings could be consistent with the involvement of the cAMP signalling pathway, and particularly PDE11A, in testis functions. However studies with a potent and selective PDE11A inhibitor and more extensive clinical studies should be pursued to investigate the effects of PDE11A inhibition in testicular and sperm function.

- **PDE8A**

  Leydig cells are interstitial cells located adjacent to the seminiferous tubules in the testis, and produce testosterone under the control of luteinizing hormone (LH). cAMP is the major intracellular messenger for LH action on steroidogenesis, as stimulation of testosterone production by LH in Leydig cells is known to be mediated by an increase in the levels of cAMP (49).

  PDE8A plays a key role in the control of LH signalling and steroidogenesis in Leydig cells. Using PDE8A deficient mice, it was shown that LH-induced testosterone basal release was increased compared to controls (50). Leydig cells from deficient mice are sensitized to the effects of LH on testosterone synthesis. Thus, pharmacological manipulation of PDE8A could be used to modulate testosterone synthesis and represents a potential pharmacological target for modulation of testosterone synthesis.

- **Ovary**

  The phosphodiesterases PDE3A and PDE4D are highly expressed in human ovary (26; 51) (52).

  - **PDE3A**
PDE3 is the predominant functional PDE family expressed in human oocytes (52). In addition, it has been demonstrated that inhibition of PDE3A blocks oocyte maturation in vitro and in vivo (53). Similarly, PDE3A deficient mice are viable and ovulate a normal number of oocytes, but are completely infertile as their oocytes contain higher levels of cAMP and fail to undergo spontaneous maturation (54).

- **PDE4D**

PDE4D also plays a critical role in the ovarian follicle. It was shown in follicle culture that PDE4 inhibitors cause oocyte maturation in the absence of gonadotropin stimulation (55). In addition, subcutaneous injection of PDE4 inhibitors alone or in combination with low doses of human chorionic gonadotropin could induce ovulation in rats (56). Moreover, mice deficient in PDE4D exhibit impaired ovulation with reduced female fertility (57). This decrease in fertility is caused by impaired follicular function and development. Although inactivation of PDE4D does not cause a complete arrest of follicular development, the reduced viability of the oocytes in pde4d-/- mice resulted in a consequent reduced number of ovulated oocytes. Furthermore, a diminished sensitivity of the granulosa cells to gonadotropins at the level of receptor-G-protein coupling might also take part in the decreased fertility of these mice. Gonadotropin responses at the level of cAMP accumulation in granulosa cells, estrogen production and ovulation rate were decreased, suggesting that granulosa cell differentiation is disrupted after the inactivation of PDE4D.

**Phosphodiesterases and endocrine diseases**

Table 2 summarises the major implications of phosphodiesterases in endocrine diseases.

- **Adrenals**
Primary pigmented nodular adrenocortical disease (PPNAD) is a bilateral form of micronodular adrenal hyperplasia causing ACTH-independent Cushing syndrome. Most patients with PPNAD suffer from CNC. This is an autosomal dominant multiple neoplasia syndrome responsible for skin pigmented lesions, cardiac myxomas and other endocrine and non-endocrine tumors. The CNC gene 1 encodes the regulatory subunit type 1A of the protein kinase A (PRKAR1A) (58). However, over the last several years it has become apparent that PPNAD, when isolated without other clinical signs of CNC, was less frequently explained by PRKAR1A mutations. Germ-line mutations in two phosphodiesterases, PDE11A and PDE8B, have been identified in such patients. Figure 3 summarises the implications of these phosphodiesterases in adrenal Cushing syndrome.

- **PDE11A**

  A genome-wide scan associated with the study of allelic losses in the adrenal tumors of PPNAD patients using DNA chips have identified a region in 2q31-2q35 that encompasses the PDE11A gene (24). Further sequencing of the PDE11A gene in 16 patients with PPNAD but with no PRKAR1A mutation has uncovered five germ-line sequence variations, two producing a frameshift mutation disrupting the PDE11A4 adrenal-specific isoform protein, two missenses substitutions and one substitution that led to a premature stop codon. A decreased expression of PDE11A4 within the adrenal tumors of these patients was observed, together with increased cAMP and cGMP levels (24; 59). Subsequently, missense variants of PDE11A that are rare in the general population were found with increased frequency among patients with macronodular adrenocortical hyperplasia, adrenocortical adenomas and adrenal cancer (60). This association of PDE11A4 variants and adrenocortical tumors suggests a role in the susceptibility to develop these tumors. Moreover, consistent with the hypothesis that PDE11A may play a role as a tumor suppressor gene, it has been reported that adrenal tumors
expressing PDE11A variants present a loss of the wild-type allele, thus resulting in a significant reduction of enzymes levels in the affected tissue.

In addition, in patients with CNC and PRKARIA inactivating mutations, an association of these PDE11A variants with the development of PPNAD and testicular tumors has been demonstrated. This suggests that PDE11A could be a modifier gene of the phenotype in patients with CNC due to PRKARIA mutation (61). In vitro studies have shown that the simultaneous inactivation of PRKARIA and PDE11A by siRNA leads to a stimulation of the PKA-dependent transcription (61). These observations are compatible with the hypothesis of a synergistic effect of PRKARIA mutations and PDE11A4 variants in the tissues expressing PDE11A4, and in which tumorigenesis is sensitive to the dysregulation of the cAMP/PKA pathway.

- **PDE8B**

During the genome wide search for genes conferring a predisposition to PPNAD, a second chromosomal locus located at 5q13 was identified (62; 63). This locus contains the gene encoding PDE8B. The PDE8B coding region was then sequenced in 22 patients with isolated micronodular adrenal disease and Cushing syndrome and a single base substitution (c.914A→C, p.His305Pro) was found in one patient. This substitution was not found in any of the 1030 unrelated control subjects studied (62).

In vitro studies performed in HEK 293 cells showed significantly higher cAMP levels after transfection with the mutant PDE8B, indicating an impaired ability of the protein to degrade cAMP (62; 63).

- **Thyroid**

  - **PDE8B**
As previously described, *PDE8B* genetic variants may be involved in regulation of TSH levels, and could be responsible for the increased serum TSH occasionally observed in individuals with no evidence of thyroid autoimmunity or loss of function mutations in the thyroid hormone or TSH-receptor-genes (34).

*PDE8B* could also have a role in the regulation of thyroid function during pregnancy (64). Serum TSH, FT4 and FT3 were measured in 970 pregnant women at 28 weeks of gestation. The single nucleotide polymorphism rs4704397 genotype was available in 877 subjects. It was shown that TSH varied with genotype and was highest in patients with the AA genotype. These results suggested that a single nucleotide polymorphism in *PDE8B* leads to serum TSH concentration in the upper limit of the reference range, and could even be associated with subclinical hypothyroidism during pregnancy.

- **PDE4**

In autonomous thyroid adenomas, cAMP signalling can be constitutively activated by mutations affecting two elements, the TSH receptor (TSHR) and the Gsα protein. Although somatic mutations in the TSH receptor gene are a frequent finding in autonomous thyroid adenomas, somatic mutations of the Gsα gene are rare. It was also shown that some phosphodiesterases could have a role in the pathogenesis of these adenomas. One study demonstrated a *PDE4* induction in the autonomous thyroid adenomas bearing a TSHR or Gsα mutation (65). In these tumors, a 2- to 3-fold increase in total PDE activity was observed. The authors demonstrated that this increase was due to the increase of *PDE4* activity, with a 10-fold higher activity than measured in the surrounding normal tissue. These results indicate that the constitutive activation of the cAMP pathway in autonomous thyroid adenomas is associated with the up-regulation of *PDE4*. This suggests that in these adenomas the induction of specific PDE expression constitutes a mechanism opposing the chronic cAMP increase.
- Pituitary

It is well established that somatotroph cells represent a cell type in which the activation of the cAMP-dependant pathway leads to cell proliferation and differentiation. As a consequence, alterations of the cAMP pathway appear to be molecular hallmarks of most growth hormone (GH)-secreting adenomas (9; 66). As phosphodiesterases catalyse cAMP, some authors hypothesize the role of these enzymes in the pathogenesis of GH-secreting adenoma.

- PDE4

It was initially shown that PDE activity was dramatically increased in human GH-secreting adenomas with activating Gsα mutations compared with wild type Gsα adenomas and normal pituitary (37; 67). This high PDE activity was caused largely by PDE4. Indeed a selective PDE4 inhibitor, rolipram, is nearly as effective as IBMX, a non-selective PDE inhibitor, in stimulating cAMP accumulation in intact cells and blocking the enzyme activity in membrane preparations.

The aryl hydrocarbon receptor-interacting protein (AIP) is a co-chaperon protein involved in the functional maturation of aryl hydrocarbon receptor. It has been previously shown that germ-line mutations in the gene encoding AIP cause pituitary adenomas predisposition (68). Close interactions were demonstrated between AIP and PDE4A5 (69). Indeed AIP mutations disrupt protein interaction between AIP and PDE4A5 (70; 71). Another PDE, PDE2A is also a known partner of AIP (72).

- PDE8B
The induction of PDE isoforms by gsp mutations was not limited to \textit{PDE4}, but also involved \textit{PDE8B}. Indeed it was shown that expression of \textit{PDE8B} was absent in the normal pituitary, but was detectable in almost all GH-secreting adenomas and higher in adenomas with activating mutations of the \textit{G\alpha} gene (37).

- \textbf{PDE11A}

More recently, one study screened 78 acromegalic patients and 110 controls for the presence of variants of the \textit{PDE11A} gene (73). The frequency of \textit{PDE11A} missense variants in acromegalic patients was found to be only slightly increased compared to controls. In addition, the presence of the wild-type allele resulting in the normal expression of the enzyme in the majority of tumor tissues together with the lack of significant clinical phenotype suggests that these variants might only marginally contribute to the development of GH-secreting adenomas.

- \textbf{Gonads}

\textbf{Testis}

- \textbf{PDE11A}

The \textit{PDE11A}-gene coding region was sequenced in 95 patients with testicular germ cell tumors from 64 unrelated multiple-case kindreds (74). Non-synonymous substitutions of \textit{PDE11A} have been detected with a frequency significantly higher in patients with familial and bilateral testicular germ cell tumors compared to controls. Moreover, functional studies showed that these mutations reduce phosphodiesterase activity and increase cAMP levels. Thus, \textit{PDE11A}-inactivating sequence variants may contribute to inherited testicular germ line tumor susceptibility.
Ovary

As it was shown that PDE8A is a key regulator of LH signalling and testosterone production in Leydig cells, one study evaluated the human PDE8A gene as a polycystic ovary syndrome (PCOS) candidate gene (75). This was based on the hypothesis that reduced PDE8 activity or expression would contribute to excessive ovarian androgen production. However, these authors showed that the more common of these PDE8A variants were not associated with PCOS, excluding a significant role of PDE8A as PCOS candidate gene.

Conclusion

Significant progress has been made toward the elucidation of the role of PDE in endocrine physiology. This is not surprising, considering the important role of the cAMP signalling pathway in endocrine glands. However, as is often seen in the progress of cell biology, the mechanisms that inactivate a cellular function are studied later than the activating mechanisms. Over the last decade, several observations have shown a dysregulation of PDEs in endocrine diseases. Since the development of specific drugs to target PDEs have been successful in non-endocrine diseases, and the development of new drugs is conceivable, this could lead to new therapeutic approaches in endocrine disorders. However, despite the recent progress summarized in this review, it is clear that the field needs to be investigated more deeply. There is no doubt that future studies of PDEs will reveal new aspects of the endocrine physiology and pathophysiology helping to progress toward new treatments.

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Legends of tables and figures

Table 1: Major properties and localisation of the phosphodiesterases.

Table 2: Major implications of the phosphodiesterases in endocrine diseases.

Figure 1: Phosphodiesterases in the cAMP pathway
The cAMP pathway mediates key cellular processes. When the G-protein-coupled receptor is activated by an extracellular ligand, a conformational change occurs. The Gs alpha subunit is released from the complex and binds to adenylyl cyclase, which then catalyses the conversion of ATP into cAMP. Elevation in intra-cellular cAMP levels leads to dissociation of the catalytic subunit from the regulatory subunit of the protein kinase A. The activated protein kinase A can then phosphorylate a series of targets that regulate downstream effector enzymes, ion channels, and activates the transcription of specific genes mediating cell growth and differentiation. Phosphodiesterases are key regulators of the cAMP pathway, as they are able to hydrolyse the cAMP to inactive 5’-AMP leading to inactivation cAMP pathway.

Abbreviations used:

- CREB: cAMP response element-binding protein
- cAMP: cyclic adenosine monophosphate
- 5’AMP: 5’ adenosine monophosphate

Figure 2: Schematic representation of the human phosphodiesterases.
Adapted from (76; 77)

Phosphodiesterases are isoenzymes encoded by at least 21 different genes and organized into 11 families. Transcription from different initiation sites in these genes and differential splicing of their mRNAs results in the generation of about 100 isoforms of PDE proteins.
GAF domains are one of the largest families of small molecule binding units. They regulate the catalytic activity of phosphodiesterases by allosteric binding of cyclic nucleotides. The GAF acronym arises from the names of the first three different classes of proteins identified to contain them: cGMP-specific and -regulated cyclic nucleotide phosphodiesterase, Adenylyl cyclase, and the bacterial transcription factor FhIA.

PAS domains, named for Per, ARNT and Sim are a ubiquitous class of transduction domains. There is a considerable overlap between the function of GAF and PAS domains. Of the 11 PDE families, PDEs 2, 5, 6, 10 and 11 contain GAF domains in their N-terminal regulatory regions and one, PDE8, contains a PAS domain.

The PDE4 family contains a unique structural feature, two domains in the N-terminal region called upstream conserved region 1 and 2 (UCR1 and UCR2). These domains have been shown to form a module necessary for the activation of PDE4 upon phosphorylation by protein kinase A.

**Figure 3: Phosphodiesterases in adrenal Cushing syndrome.**

A: The left part of the figure shows the control of the cAMP pathway in the normal adrenal cortex. The pituitary hormone ACTH (adrenocorticotropin) stimulates its specific 7-transmembrane receptor, leading to activation of adenylyl cyclase (AC) and cAMP synthesis. cAMP activate the Protein Kinase A made of 2 regulatory subunits (R) and 2 catalytic subunits (C). Activation of the phosphodiesterases (PDEs) stimulates degradation of cAMP to 5'AMP leading to cAMP levels decrease. ACTH stimulates steroid synthesis and secretion and is required for adrenocortical cells survival.

B: The right part of the figure illustrates the dysregulation caused by PDE8B or PDE11A inactivating mutations. The reduced PDE activity leads to increased cAMP levels and PKA
stimulation. This potentiates or mimics the effects of ACTH and takes part in the steroid oversecretion and/or adrenal nodular hyperplasia observed in animal models and/or human diseases. In adrenal Cushing syndrome, the negative feed-back of cortisol on the hypothalamic and pituitary adrenal axis lead to reduced ACTH circulating levels.
Figure 1

Protein kinase A

G Protein

β

α

γ

CREB

5'AMP

cAMP

PDE

Phosphodiesterase

5'AMP → PDE → cAMP → Protein kinase A → CREB

Adenylyl cyclase

ATP

Protein kinase A

CREB
Figure 2

PDE

1
2
3
4
5
6
7
8
9
10
11

- GAF domain
- PAS domain
- Conserved catalytic domain
- UCRs
Figure 3

A

Steroidogenesis
Adrenocortical cell survival

B

Steroidogenesis
Adrenocortical cell survival
### Table 1:

<table>
<thead>
<tr>
<th>Phosphodiesterases (PDE) family</th>
<th>Isoforms</th>
<th>Substrate preference</th>
<th>Major localization</th>
<th>Major known functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE1</td>
<td>PDE1A</td>
<td>cAMP&gt;cGMP for PDE1A and PDE1B</td>
<td>Testis, thyroid, pituitary, kidney, liver, pancreas, brain, heart, aorta, bladder, thymus, vascular smooth muscle cells and vascular endothelial cells</td>
<td>Regulate vascular smooth muscle contraction  Play a role in sperm function  Contribute to neuronal functions</td>
<td>(26; 78; 79; 80)</td>
</tr>
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<td></td>
<td>PDE1B</td>
<td>cAMP=cGMP for PDE1C</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>PDE1C</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PDE2</td>
<td>PDE2A</td>
<td>cAMP = cGMP</td>
<td>Brain, heart, liver, lung, kidney, platelets, adrenal cortex and glomerulosa, pituitary, endothelial cells</td>
<td>Regulate aldosterone secretion  Regulate ACTH secretion  Mediate the cAMP signal in cardiac myocytes  Play a role in long term memory and in barrier function of endothelial cells under inflammatory conditions</td>
<td>(26; 29; 36; 81; 82; 83)</td>
</tr>
<tr>
<td>PDE3</td>
<td>PDE3A</td>
<td>PDE3B</td>
<td>cAMP&gt;cGMP</td>
<td>Platelets, smooth muscle cells, cardiac myocytes, oocytes, kidney, hepatocytes, brain, developing spermatocytes, thymus, colon</td>
<td>Regulate cardiac contractility, platelet aggregation, vascular smooth muscle contraction, oocyte maturation and regulation of renin release Have an important impact on lipolysis, glycogenolysis, insulin secretion and cardiac function</td>
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<tr>
<td>PDE4</td>
<td>PDE4B</td>
<td>PDE4D</td>
<td>cAMP</td>
<td>Brain (frontal cortex and hypothalamus), skeletal muscle, spleen, liver, white and brown adipose tissue, pancreatic β-cells, cardiac myocytes, smooth muscles, inflammatory cells, ovary, pituitary</td>
<td>Play a role in brain function, monocyte and macrophage activation, neutrophil infiltration, vascular smooth muscle proliferation, fertility, Regulate beta-adrenergic signalling and excitation-contraction coupling in the heart and play thus a role in vasodilatation and cardiac contractility</td>
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<td>PDE5</td>
<td>PDE5A</td>
<td>PDE5A</td>
<td>cGMP &gt;&gt;cAMP</td>
<td>Corpus cavernosum, aorta, placenta, colon, bladder, platelets</td>
<td>Modulate NO/cGMP effects in vascular smooth muscles, platelets and lower urinary tract organs Regulate cardiac stress responses</td>
</tr>
<tr>
<td>PDE6</td>
<td>PDE6A</td>
<td>PDE6B</td>
<td>cGMP &gt;&gt;cAMP</td>
<td>Photoreceptors cells, pancreas, liver, prostate, fallopian tubes, aorta, ovary and skeletal muscle</td>
<td>Primary effector enzyme in the phototransduction cascade. Regulate cGMP concentration in rod and cone photoreceptors</td>
</tr>
<tr>
<td>PDE7</td>
<td>PDE7A</td>
<td>PDE7B</td>
<td>cAMP</td>
<td>Heart, skeletal muscle, spleen, thyroid, placenta, ovary, bladder, thymus, T cell, brain</td>
<td>Play a critical role in the regulation of the human T cells function</td>
</tr>
<tr>
<td>PDE8</td>
<td>PDE8A</td>
<td>PDE8B</td>
<td>cAMP</td>
<td>Brain, thyroid, testis, adrenal cortex, colon, liver, thymus, placenta, vena cava, aorta, Fallopian tube</td>
<td>Play a role in T cell activation \nRegulate adrenal steroidogenesis \nRegulate TSH levels \nControl of LH signalling and steroidogenesis in Leydig cells</td>
</tr>
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</tr>
<tr>
<td>PDE9</td>
<td>PDE9A</td>
<td></td>
<td>cGMP&gt;&gt;cAMP</td>
<td>Colon, prostate, kidney, brain, small intestine, lung, thymus, spleen, lymph nodes, T cells</td>
<td>Not well known. Candidate gene in regulation of energy balance</td>
</tr>
<tr>
<td>PDE10</td>
<td>PDE10A</td>
<td>cAMP&gt;cGMP</td>
<td>Stomach, skeletal muscle, small intestine, testis, kidney, heart and brain</td>
<td>Play a role in striatal activation and behavioural activity</td>
<td>(26; 102)</td>
</tr>
<tr>
<td>PDE11</td>
<td>PDE11A</td>
<td>cAMP = cGMP</td>
<td>Prostate, heart, liver, skeletal muscle, salivary gland, thyroid, adrenal cortex, testis, pituitary, thymus</td>
<td>Play a role in sperm production</td>
<td>(24; 26; 35; 44; 103; 104)</td>
</tr>
</tbody>
</table>
Table 2

<table>
<thead>
<tr>
<th>Adenylate cyclase family</th>
<th>organs</th>
<th>Implication</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PDE2A</strong></td>
<td>pituitary</td>
<td>Could have a role in the pathogenesis of GH-secreting adenomas because of protein interaction between AIP and PDE2A</td>
<td>(72)</td>
</tr>
<tr>
<td><strong>PDE4</strong></td>
<td>thyroid</td>
<td>Could have a role in the autonomous thyroid adenomas bearing a TSHR or Gsα mutation</td>
<td>(65)</td>
</tr>
<tr>
<td></td>
<td>pituitary</td>
<td>Could have a role in the pathogenesis of GH-secreting adenomas because of protein interaction between AIP and PDE4A5</td>
<td>(37; 67; 69; 70; 71)</td>
</tr>
<tr>
<td><strong>PDE8B</strong></td>
<td>adrenals</td>
<td>Predisposition gene to PPNAD (inactivating point mutations)</td>
<td>(62; 63)</td>
</tr>
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<td></td>
<td>thyroid</td>
<td>Association of the PDE8B rs4704397 snp and circulating TSH levels. This association may be responsible for the increased TSH in patients with no evidence of thyroid autoimmunity and for subclinical hypothyroidism during pregnancy.</td>
<td>(34; 64)</td>
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<tr>
<td></td>
<td>pituitary</td>
<td>Altered expression in GH-secreting adenomas</td>
<td>(37)</td>
</tr>
<tr>
<td>Gene</td>
<td>Tissue</td>
<td>Description</td>
<td>Reference</td>
</tr>
<tr>
<td>------</td>
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</tr>
<tr>
<td>PDE11A</td>
<td>adrenals</td>
<td>Predisposition gene to PPNAD (inactivating point mutations, leading mainly to premature stop codon) Missense variants of <em>PDE11A</em> were found with increased frequency among patients with macronodular adrenocortical hyperplasia, adrenocortical adenomas and adrenal cancer compared to controls patients. <em>PDE11A</em> is a modifier of the phenotype in patients with CNC due to <em>PRKAR1A</em> mutations.</td>
<td>(24; 59; 60; 61)</td>
</tr>
<tr>
<td></td>
<td>testis</td>
<td>Association between non-synonymous substitutions of <em>PDE11A</em> and familial testicular germ cell tumors.</td>
<td>(74)</td>
</tr>
</tbody>
</table>