REVIEW

Phosphodiesterases in endocrine physiology and disease

Delphine Vezzosi^{1,2,3} and Jérôme Bertherat^{1,2,4}

¹Inserm U1016, CNRS UMR 8104, Institut Cochin, 75014 Paris, France, ²Faculté de Médecine Paris 5, Université Paris Descartes, 75005 Paris, France, ³Department of Endocrinology, Hôpital Larrey, 31480 Toulouse, France and ⁴Department of Endocrinology, Reference Center for Rare Adrenal Diseases, Assistance Publique Hôpitaux de Paris, Hôpital Cochin, APHP, 75014 Paris, France

(Correspondence should be addressed to D Vezzosi; Email: delphine.vezzosi@gmail.com)

Abstract

The cAMP–protein kinase A 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 PDEs.

European Journal of Endocrinology 165 177–188

Introduction

The 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 (TSHR) in thyroid toxic adenomas (2), for the LH receptor in Leydig cell tumors (3), for the calcium sensing receptor in familial hypocalcaemia (4), and aberrant expression of G-protein-coupled receptors in bilateral macronodular adrenal hyperplasia (5–7). Secondly, somatic activating mutations of the stimulatory subunit α of the Gs protein have been found in McCune–Albright syndrome or in sporadic pituitary GH adenomas (8, 9). Finally, germ line 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) (10–12).

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 (Fig. 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 PDE superfamily

Adenylyl and guanylyl cyclases 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 into 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 (PKA) form

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 G_{α} subunit is released from the complex and binds to adenylyl cyclase, which then catalyzes 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 hydrolyze 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.

complexes that act to create these simultaneous, multiple cAMP gradients. A cell without these 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 11 PDE gene families (PDE 1-11; Fig. 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 versus 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 ~300 amino acids located near the C-terminal regions and a variable regulatory domain located in the N-terminal regions. In mammals, three of the 11 PDE families selectively hydrolyze cAMP (PDEs 4, 7, and 8), three families are selective for cGMP (PDEs 5, 6, and 9), and five 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 PDE 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 localization of PDEs are summarized 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 assignment 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 PDE isoforms are subjected to different regulations or are targeted to different subcellular compartments that account 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.

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 cGMP-regulated cyclic nucleotide phosphodiesterase, adenylyl cyclase, and the bacterial transcription factor FhlA. 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.

Table 1 Major properties and localization of the phosphodiesterases.

PDE family	Isoforms	Substrate preference	Major localization	Major known functions	References
<i>PDE1</i>	<i>PDE1A</i> <i>PDE1B</i> <i>PDE1C</i>	cAMP < cGMP for <i>PDE1A</i> and <i>PDE1B</i> cAMP = cGMP for <i>PDE1C</i>	Testis, thyroid, pituitary, kidney, liver, pancreas, brain, heart, aorta, bladder, thymus, vascular smooth muscle cells, and vascular endothelial cells	Regulate vascular smooth muscle contraction Play a role in sperm function Contribute to neuronal functions	(26, 78–80)
<i>PDE2</i>	<i>PDE2A</i>	cAMP = cGMP	Brain, heart, liver, lung, kidney, platelets, adrenal cortex and glomerulosa, pituitary, endothelial cells	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	(26, 29, 36, 81–83)
<i>PDE3</i>	<i>PDE3A</i> <i>PDE3B</i>	cAMP > cGMP	Platelets, smooth muscle cells, cardiac myocytes, oocytes, kid- ney, hepatocytes, brain, devel- oping spermatocytes, thymus, colon	Regulate cardiac contractility, pla- telet aggregation, vascular smooth muscle contraction, oocyte maturation and regulation of renin release Have an important impact on lipo- lysis, glycogenolysis, insulin secretion, and cardiac function Play a role in brain function, monocyte and macrophage activation, neutrophil infiltration, vascular smooth muscle proliferation, fertility	(84–89)
<i>PDE4</i>	<i>PDE4B</i> <i>PDE4D</i>	cAMP	Brain (frontal cortex and hypo- thalamus), skeletal muscle, spleen, liver, white and brown adipose tissue, pancreatic β -cells, cardiac myocytes, smooth muscles, inflammatory cells, ovary, pituitary	Regulate β -adrenergic signaling and excitation–contrac- tion coupling in the heart and thus play a role in vasodilatation and cardiac contractility Modulate NO/cGMP effects in vascular smooth muscles, pla- telets, and lower urinary tract organs	(26, 37, 84, 90–92)
<i>PDE5</i>	<i>PDE5A</i>	cGMP > > cAMP	Corpus cavernosum, aorta, pla- centa, colon, bladder, platelets	Regulate cardiac stress responses Primary effector enzyme in the phototransduction cascade. Regulate cGMP concentration in rod and cone photoreceptors	(26, 93–96)
<i>PDE6</i>	<i>PDE6A</i> <i>PDE6B</i> <i>PDE6C</i>	cGMP > > cAMP	Photoreceptors cells, pancreas, liver, prostate, fallopian tubes, aorta, ovary, and skeletal muscle	Play a critical role in the regulation of the human T cells function	(26, 97)
<i>PDE7</i>	<i>PDE7A</i> <i>PDE7B</i>	cAMP	Heart, skeletal muscle, spleen, thyroid, placenta, ovary, bladder, thymus, T cell, brain		(26, 98)
<i>PDE8</i>	<i>PDE8A</i> <i>PDE8B</i>	cAMP	Brain, thyroid, testis, adrenal cortex, colon, liver, thymus, placenta, vena cava, aorta, fallopian tube	Play a role in T cell activation Regulate adrenal steroidogenesis Regulate TSH levels Control of LH signaling and steroidogenesis in Leydig cells	(26, 28, 34, 79)
<i>PDE9</i>	<i>PDE9A</i>	cGMP > > cAMP	Colon, prostate, kidney, brain, small intestine, lung, thymus, spleen, lymph nodes, T cells	Not well known. Candidate gene in regulation of energy balance	(26, 99–101)
<i>PDE10</i>	<i>PDE10A</i>	cAMP > cGMP	Stomach, skeletal muscle, small intestine, testis, kidney, heart, and brain	Play a role in striatal activation and behavioral activity	(26, 102)
<i>PDE11</i>	<i>PDE11A</i>	cAMP = cGMP	Prostate, heart, liver, skeletal muscle, salivary gland, thyroid, adrenal cortex, testis, pituitary, thymus	Play a role in sperm production	(24, 26, 35, 44, 103, 104)

PDE, Phosphodiesterases

PDEs in endocrine physiology

Adrenals

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

PDE2A *PDE2A* is the predominant PDE 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* knockout 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 PDEs most highly expressed in the thyroid (25, 26).

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 (thyroxine (T_4) and triiodothyronine (T_3) 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 signaling. *PDE8B* acts in the thyroid to catalyze the hydrolysis and inactivation of cAMP after TSH signaling. 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 T_4 and T_3 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 PDEs most highly expressed in the pituitary (35–40).

The physiological role of these PDEs 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). Recently, it was demonstrated that *PDE1* and *PDE4* are key cAMP metabolizing 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 PDEs 1A, 1C, 3B, 5A, 8A, 10A, 11A are those most highly expressed in the testis (26, 44, 45).

The role of two of these PDEs has 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 with 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 in 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 signaling 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 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 signaling and steroidogenesis in Leydig cells. Using *PDE8A*-deficient mice, it was shown that LH-induced testosterone basal release was increased compared with 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 PDEs, *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, s.c. 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*.

PDEs and endocrine diseases

Table 2 summarizes the major implications of PDEs in endocrine diseases.

Adrenals

Primary 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 *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 PDEs, *PDE11A* and *PDE8B*, have been identified in such patients. Figure 3 summarizes the implications of these PDEs 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

Table 2 Major implications of the phosphodiesterases in endocrine disease.

PDE family	Organs	Implication	References
<i>PDE2A</i>	Pituitary	Could have a role in the pathogenesis of GH-secreting adenomas because of protein interaction between AIP and <i>PDE2A</i>	(72)
<i>PDE4</i>	Thyroid	Could have a role in the autonomous thyroid adenomas bearing a TSHR or Gs α mutation	(65)
	Pituitary	Could have a role in the pathogenesis of GH-secreting adenomas because of protein interaction between AIP and <i>PDE4A5</i>	(37, 67, 69–71)
<i>PDE8B</i>	Adrenals	Predisposition gene to PPNAD (inactivating point mutations)	(62, 63)
	Thyroid	Association of the <i>PDE8B</i> 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	(34, 64)
<i>PDE11A</i>	Pituitary	Altered expression in GH-secreting adenomas	(37)
	Adrenals	Predisposition gene to PPNAD (inactivating point mutations, leading mainly to premature stop codon). Missense variants of <i>PDE11A</i> were found with increased frequency among patients with macronodular adrenocortical hyperplasia, adrenocortical adenomas, and adrenal cancer compared with control patients. <i>PDE11A</i> is a modifier of the phenotype in patients with CNC due to <i>PRKAR1A</i> mutations	(24, 59–61)
	Testis	Association between non-synonymous substitutions of <i>PDE11A</i> and familial testicular germ cell tumors	(74)

PDE, Phosphodiesterases

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 missense 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 enzyme levels in the affected tissue.

In addition, in patients with CNC and *PRKAR1A* 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 *PRKAR1A* mutation (61). *In vitro* studies have shown that the simultaneous inactivation of *PRKAR1A* 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 *PRKAR1A* 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

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 seven-transmembrane receptor, leading to activation of adenylyl cyclase (AC) and cAMP synthesis. cAMP activates the protein kinase A made of two regulatory subunits (R) and two 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 feedback of cortisol on the hypothalamic and pituitary adrenal axis lead to reduced ACTH circulating levels.

(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 significantly showed 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 TSHR genes (34).

PDE8B could also have a role in the regulation of thyroid function during pregnancy (64). Serum TSH, Free T₄, and Free T₃ 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 signaling can be constitutively activated by mutations affecting two elements, the TSHR and the Gsα protein. Although somatic mutations in the TSHR gene are a frequent finding in autonomous thyroid adenomas, somatic mutations of the Gsα gene are rare. It was also shown that some PDEs could have a role in the pathogenesis of these adenomas. A study demonstrated a *PDE4* induction in the autonomous thyroid adenomas bearing a *TSHR* or Gsα mutation (65). In these tumors, a two to threefold increase in total PDE activity was observed. The authors demonstrated that this increase was due to the increase of *PDE4* activity, with a tenfold 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 upregulation 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 GH-secreting

adenomas (9, 66). As PDEs catalyze 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 previously been 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 *PDE4*, but also involved *PDE8B*. Indeed, it was shown that expression of *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 Gsα gene (37).

PDE11A Recently, one study screened 78 acromegalic patients and 110 controls for the presence of variants of the *PDE11A* gene (73). The frequency of *PDE11A* missense variants in acromegalic patients was found to be only slightly increased compared with 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.

Gonads

Testis PDE11A. The *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 *PDE11A* have been detected with a frequency significantly higher in patients with familial and bilateral testicular germ cell tumors compared with controls. Moreover, functional studies showed that these mutations reduce PDE activity and increase cAMP levels. Thus, *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 signaling 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 signaling 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. As 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.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

Our work is supported in part by the Agence Nationale de la Recherche (ANR08-GENOPAT-002; and ANR-10-Blan-1136). D Vezzosi is the recipient of a fellowship from the Institut National du Cancer (INCa).

References

- Rosenberg D, Groussin L, Bertagna X & Bertherat J. cAMP pathway alterations from the cell surface to the nucleus in adrenocortical tumors. *Endocrine Research* 2002 **28** 765–775. (doi:10.1081/ERC-120017071)
- Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J & Vassart G. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* 1993 **365** 649–651. (doi:10.1038/365649a0)
- Liu G, Duranteau L, Carel JC, Monroe J, Doyle DA & Shenker A. Leydig-cell tumors caused by an activating mutation of the gene encoding the luteinizing hormone receptor. *New England Journal of Medicine* 1999 **341** 1731–1736. (doi:10.1056/NEJM19991203412304)
- Mancilla EE, De Luca F & Baron J. Activating mutations of the Ca^{2+} -sensing receptor. *Molecular Genetics and Metabolism* 1998 **64** 198–204. (doi:10.1006/mgme.1998.2716)
- Lacroix A, N'Diaye N, Tremblay J & Hamet P. Ectopic and abnormal hormone receptors in adrenal Cushing's syndrome. *Endocrine Reviews* 2001 **22** 75–110. (doi:10.1210/er.22.1.75)
- Vezzosi D, Cartier D, Regnier C, Otal P, Bennet A, Parmentier F, Plantavid M, Lacroix A, Lefebvre H & Caron P. Familial adrenocorticotropin-independent macronodular adrenal hyperplasia with aberrant serotonin and vasopressin adrenal receptors. *European Journal of Endocrinology* 2007 **156** 21–31. (doi:10.1530/eje.1.02324)
- Groussin L, Perlemoine K, Contesse V, Lefebvre J, Tabarin A, Thieblot P, Schlienger JL, Luton JP, Bertagna X & Bertherat J. The ectopic expression of the gastric inhibitory polypeptide receptor is frequent in adrenocorticotropin-independent bilateral macronodular adrenal hyperplasia, but rare in unilateral tumors. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 1980–1985. (doi:10.1210/jc.87.5.1980)
- Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E & Spiegel AM. Activating mutations of the stimulatory G protein in the McCune–Albright syndrome. *New England Journal of Medicine* 1991 **325** 1688–1695. (doi:10.1056/NEJM199112123252403)
- Vallar L, Spada A & Giannattasio G. Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. *Nature* 1987 **330** 566–568. (doi:10.1038/330566a0)
- Kirschner LS, Sandrini F, Monbo J, Lin JP, Carney JA & Stratakis CA. Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the Carney complex. *Human Molecular Genetics* 2000 **9** 3037–3046. (doi:10.1093/hmg/9.20.3037)
- Veugelers M, Wilkes D, Burton K, McDermott DA, Song Y, Goldstein MM, La Perle K, Vaughan CJ, O'Hagan A, Bennett KR, Meyer BJ, Legius E, Karttunen M, Norio R, Kaariainen H, Lavyne M, Neau JP, Richter G, Kirali K, Farnsworth A, Stapleton K, Morelli P, Takanashi Y, Bamforth JS, Eitelberger F, Noszian I, Manfroi W, Powers J, Mochizuki Y, Imai T, Ko GT, Driscoll DA, Goldmuntz E, Edelberg JM, Collins A, Eccles D, Irvine AD, McKnight GS & Basson CT. Comparative PRKAR1A genotype–phenotype analyses in humans with Carney complex and prkar1a haploinsufficient mice. *PNAS* 2004 **101** 14222–14227. (doi:10.1073/pnas.040535101)
- Groussin L, Kirschner LS, Vincent-Dejean C, Perlemoine K, Jullian E, Delemer B, Zacharieva S, Pignatelli D, Carney JA, Luton JP, Bertagna X, Stratakis CA & Bertherat J. Molecular analysis of the cyclic AMP-dependent protein kinase A (PKA) regulatory subunit 1A (PRKAR1A) gene in patients with Carney complex and primary pigmented nodular adrenocortical disease (PPNAD) reveals novel mutations and clues for pathophysiology: augmented PKA signaling is associated with adrenal tumorigenesis in PPNAD. *American Journal of Human Genetics* 2002 **71** 1433–1442. (doi:10.1086/344579)
- Hansen G, Jin S, Umetsu DT & Conti M. Absence of muscarinic cholinergic airway responses in mice deficient in the cyclic nucleotide phosphodiesterase PDE4D. *PNAS* 2000 **97** 6751–6756. (doi:10.1073/pnas.97.12.6751)
- Zhang HT, Huang Y, Jin SL, Frith SA, Suvana N, Conti M & O'Donnell JM. Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. *Neuropsychopharmacology* 2002 **27** 587–595. (doi:10.1016/S0893-133X(02)00344-5)
- Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, Malloy MP, Chubb JE, Huston E, Baillie GS, Thomson PA, Hill EV, Brandon NJ, Rain JC, Camargo LM, Whiting PJ, Houslay MD, Blackwood DH, Muir WJ & Porteous DJ.

- DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* 2005 **310** 1187–1191. (doi:10.1126/science.1112915)
- 16 Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM, Hawkins M, Gudmundsson G, Gudmundsdottir H, Andrasen H, Gudmundsdottir AS, Sigurdardottir M, Chou TT, Nahmias J, Goss S, Sveinbjornsdottir S, Valdimarsson EM, Jakobsson F, Agnarsson U, Gudnason V, Thorgeirsson G, Fingerle J, Gurney M, Gudbjartsson D, Frigge ML, Kong A, Stefansson K & Gulcher JR. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nature Genetics* 2003 **35** 131–138. (doi:10.1038/ng1245)
 - 17 McVary KT. Clinical practice. Erectile dysfunction. *New England Journal of Medicine* 2007 **357** 2472–2481. (doi:10.1056/NEJMc067261)
 - 18 Kumar P, Francis GS & Tang WH. Phosphodiesterase 5 inhibition in heart failure: mechanisms and clinical implications. *Nature Reviews. Cardiology* 2009 **6** 349–355. (doi:10.1038/nrcardio.2009.32)
 - 19 De LaVega AJ & Derk CT. Phosphodiesterase-5 inhibitors for the treatment of Raynaud's: a novel indication. *Expert Opinion on Investigational Drugs* 2009 **18** 23–29. (doi:10.1517/13543780802525100)
 - 20 Archer SL & Michelakis ED. Phosphodiesterase type 5 inhibitors for pulmonary arterial hypertension. *New England Journal of Medicine* 2009 **361** 1864–1871. (doi:10.1056/NEJMc0904473)
 - 21 Lipworth BJ. Phosphodiesterase-4 inhibitors for asthma and chronic obstructive pulmonary disease. *Lancet* 2005 **365** 167–175. (doi:10.1016/S0140-6736(05)17708-3)
 - 22 Hertz AL, Bender AT, Smith KC, Gilchrist M, Amieux PS, Aderem A & Beavo JA. Elevated cyclic AMP and PDE4 inhibition induce chemokine expression in human monocyte-derived macrophages. *PNAS* 2009 **106** 21978–21983. (doi:10.1073/pnas.0911684106)
 - 23 Zaccolo M. Phosphodiesterases and compartmentalized cAMP signalling in the heart. *European Journal of Cell Biology* 2006 **85** 693–697. (doi:10.1016/j.ejcb.2006.01.002)
 - 24 Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, Stein E, Levine E, Delimpasi G, Hsiao HP, Keil M, Heyerdahl S, Matyakhina L, Libe R, Fratticci A, Kirschner LS, Cramer K, Gaillard RC, Bertagna X, Carney JA, Bertherat J, Bossis I & Stratakis CA. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. *Nature Genetics* 2006 **38** 794–800. (doi:10.1038/ng1809)
 - 25 Lakics V, Karran EH & Boess FG. Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 2010 **59** 367–374. (doi:10.1016/j.neuropharm.2010.05.004)
 - 26 Bingham J, Sudarsanam S & Srinivasan S. Profiling human phosphodiesterase genes and splice isoforms. *Biochemical and Biophysical Research Communications* 2006 **350** 25–32. (doi:10.1016/j.bbrc.2006.08.180)
 - 27 Sonnenburg WK, Mullaney PJ & Beavo JA. Molecular cloning of a cyclic GMP-stimulated cyclic nucleotide phosphodiesterase cDNA. Identification and distribution of isozyme variants. *Journal of Biological Chemistry* 1991 **266** 17655–17661.
 - 28 Tsai LC, Shimizu-Albergine M & Beavo JA. The high affinity cAMP-specific phosphodiesterase 8B (PDE8B) controls steroidogenesis in the mouse adrenal gland. *Molecular Pharmacology* 2011 **79** 639–648. (doi:10.1124/mol.110.069104)
 - 29 Cote M, Payet MD, Rousseau E, Guillon G & Gallo-Payet N. Comparative involvement of cyclic nucleotide phosphodiesterases and adenylyl cyclase on adrenocorticotropin-induced increase of cyclic adenosine monophosphate in rat and human glomerulosa cells. *Endocrinology* 1999 **140** 3594–3601. (doi:10.1210/en.140.8.3594)
 - 30 Wayman C, Phillips S, Lunny C, Webb T, Fawcett L, Baxendale R & Burgess G. Phosphodiesterase 11 (PDE11) regulation of spermatozoa physiology. *International Journal of Impotence Research* 2005 **17** 216–223. (doi:10.1038/sj.ijir.3901307)
 - 31 Di Luigi L, Baldari C, Sgro P, Emerenziani GP, Gallotta MC, Bianchini S, Romanelli F, Pigozzi F, Lenzi A & Guidetti L. The type 5 phosphodiesterase inhibitor tadalafil influences salivary cortisol, testosterone, and dehydroepiandrosterone sulphate responses to maximal exercise in healthy men. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 3510–3514. (doi:10.1210/jc.2008-0847)
 - 32 Hansen PS, Brix TH, Sorensen TI, Kyvik KO & Hegedus L. Major genetic influence on the regulation of the pituitary–thyroid axis: a study of healthy Danish twins. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1181–1187. (doi:10.1210/jc.2003-031641)
 - 33 Panicker V, Wilson SG, Spector TD, Brown SJ, Falchi M, Richards JB, Surdulescu GL, Lim EM, Fletcher SJ & Walsh JP. Heritability of serum TSH, free T₄ and free T₃ concentrations: a study of a large UK twin cohort. *Clinical Endocrinology* 2008 **68** 652–659. (doi:10.1111/j.1365-2265.2007.03079.x)
 - 34 Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, Sestu N, Maschio A, Busonero F, Albai G, Dei M, Lai S, Mulas A, Crisponi L, Tanaka T, Bandinelli S, Guralnik JM, Loi A, Balaci L, Sole G, Prinzi A, Mariotti S, Shuldiner AR, Cao A, Schlessinger D, Uda M, Abecasis GR, Nagaraja R, Sanna S & Naitza S. Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *American Journal of Human Genetics* 2008 **82** 1270–1280. (doi:10.1016/j.ajhg.2008.04.019)
 - 35 Loughney K, Taylor J & Florio VA. 3',5'-cyclic nucleotide phosphodiesterase 11A: localization in human tissues. *International Journal of Impotence Research* 2005 **17** 320–325. (doi:10.1038/sj.ijir.3901317)
 - 36 Stephenson DT, Coskran TM, Wilhelms MB, Adamowicz WO, O'Donnell MM, Muravnick KB, Menniti FS, Kleiman RJ & Morton D. Immunohistochemical localization of phosphodiesterase 2A in multiple mammalian species. *Journal of Histochemistry and Cytochemistry* 2009 **57** 933–949. (doi:10.1369/jhc.2009.953471)
 - 37 Persani L, Borgato S, Lania A, Filopanti M, Mantovani G, Conti M & Spada A. Relevant cAMP-specific phosphodiesterase isoforms in human pituitary: effect of Gs(alpha) mutations. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3795–3800. (doi:10.1210/jc.86.8.3795)
 - 38 Ang KL & Antoni FA. Functional plasticity of cyclic AMP hydrolysis in rat adenohypophyseal corticotroph cells. *Cell Signalling* 2002 **14** 445–452. (doi:10.1016/S0898-6568(01)00267-4)
 - 39 Azhar S & Menon KM. Cyclic nucleotide phosphodiesterases from rat anterior pituitary. Characterization of multiple forms and regulation by protein activator and Ca²⁺. *European Journal of Biochemistry* 1977 **73** 73–82. (doi:10.1111/j.1432-1033.1977.tb11292.x)
 - 40 Nagasaka A, Ohkubo S & Hidaka H. 3':5'-cyclic-nucleotide phosphodiesterase in the bovine pituitary gland. *Biochimica et Biophysica Acta* 1983 **755** 481–487.
 - 41 Gonzalez-Iglesias AE, Jiang Y, Tomic M, Kretschmannova K, Andric SA, Zemkova H & Stojilkovic SS. Dependence of electrical activity and calcium influx-controlled prolactin release on adenylyl cyclase signaling pathway in pituitary lactotrophs. *Molecular Endocrinology* 2006 **20** 2231–2246. (doi:10.1210/me.2005-0363)
 - 42 Kumari M, Cover PO, Poyser RH & Buckingham JC. Stimulation of the hypothalamo-pituitary–adrenal axis in the rat by three selective type-4 phosphodiesterase inhibitors: *in vitro* and *in vivo* studies. *British Journal of Pharmacology* 1997 **121** 459–468. (doi:10.1038/sj.bjp.0701158)
 - 43 Hadley AJ, Kumari M, Cover PO, Osborne J, Poyser R, Flack JD & Buckingham JC. Stimulation of the hypothalamo-pituitary–

- adrenal axis in the rat by the type 4 phosphodiesterase (PDE-4) inhibitor, denbufylline. *British Journal of Pharmacology* 1996 **119** 463–470.
- 44 D'Andrea MR, Qiu Y, Haynes-Johnson D, Bhattacharjee S, Kraft P & Lundeen S. Expression of PDE11A in normal and malignant human tissues. *Journal of Histochemistry and Cytochemistry* 2005 **53** 895–903. (doi:10.1369/jhc.5A6625.2005)
 - 45 Wang P, Wu P, Egan RW & Billah MM. Human phosphodiesterase 8A splice variants: cloning, gene organization, and tissue distribution. *Gene* 2001 **280** 183–194. (doi:10.1016/S0378-1119(01)00783-1)
 - 46 Hellstrom WJ, Overstreet JW, Yu A, Saikali K, Shen W, Beasley CM Jr & Watkins VS. Tadalafil has no detrimental effect on human spermatogenesis or reproductive hormones. *Journal of Urology* 2003 **170** 887–891. (doi:10.1097/01.ju.0000081053.97792.da)
 - 47 Pomara G, Morelli G, Canale D, Turchi P, Caglieresi C, Moschini C, Liguori G, Selli C, Macchia E, Martino E & Francesca F. Alterations in sperm motility after acute oral administration of sildenafil or tadalafil in young, infertile men. *Fertility and Sterility* 2007 **88** 860–865. (doi:10.1016/j.fertnstert.2006.12.019)
 - 48 Bischoff E. Potency, selectivity, and consequences of nonselectivity of PDE inhibition. *International Journal of Impotence Research* 2004 **16** (Suppl 1) S11–S14. (doi:10.1038/sj.ijir.3901208)
 - 49 Saez JM. Leydig cells: endocrine, paracrine, and autocrine regulation. *Endocrine Reviews* 1994 **15** 574–626. (doi:10.1210/edrv-15-5-574)
 - 50 Vasta V, Shimizu-Albergine M & Beavo JA. Modulation of Leydig cell function by cyclic nucleotide phosphodiesterase 8A. *PNAS* 2006 **103** 19925–19930. (doi:10.1073/pnas.0609483103)
 - 51 Sasseville M, Albuz FK, Cote N, Guillemette C, Gilchrist RB & Richard FJ. Characterization of novel phosphodiesterases in the bovine ovarian follicle. *Biology of Reproduction* 2009 **81** 415–425. (doi:10.1095/biolreprod.108.074450)
 - 52 Nogueira D, Albano C, Adriaenssens T, Cortvrindt R, Bourgain C, Devroey P & Smits J. Human oocytes reversibly arrested in prophase I by phosphodiesterase type 3 inhibitor *in vitro*. *Biology of Reproduction* 2003 **69** 1042–1052. (doi:10.1095/biolreprod.103.015982)
 - 53 Conti M, Andersen CB, Richard F, Mehats C, Chun SY, Horner K, Jin C & Tsafiriri A. Role of cyclic nucleotide signaling in oocyte maturation. *Molecular and Cellular Endocrinology* 2002 **187** 153–159. (doi:10.1016/S0303-7207(01)00686-4)
 - 54 Masciarelli S, Horner K, Liu C, Park SH, Hinckley M, Hockman S, Nedachi T, Jin C, Conti M & Manganiello V. Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. *Journal of Clinical Investigation* 2004 **114** 196–205. (doi:10.1172/JCI21804)
 - 55 Tsafiriri A, Chun SY, Zhang R, Hsueh AJ & Conti M. Oocyte maturation involves compartmentalization and opposing changes of cAMP levels in follicular somatic and germ cells: studies using selective phosphodiesterase inhibitors. *Developmental Biology* 1996 **178** 393–402. (doi:10.1006/dbio.1996.0226)
 - 56 McKenna SD, Pietropaolo M, Tos EG, Clark A, Fischer D, Kagan D, Bao B, Chedrese PJ & Palmer S. Pharmacological inhibition of phosphodiesterase 4 triggers ovulation in follicle-stimulating hormone-primed rats. *Endocrinology* 2005 **146** 208–214. (doi:10.1210/en.2004-0562)
 - 57 Jin SL, Richard FJ, Kuo WP, D'Ercole AJ & Conti C. Impaired growth and fertility of cAMP-specific phosphodiesterase PDE4D-deficient mice. *PNAS* 1999 **96** 11998–12003. (doi:10.1073/pnas.96.21.11998)
 - 58 Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS & Stratakis CA. Mutations of the gene encoding the protein kinase A type I- α regulatory subunit in patients with the Carney complex. *Nature Genetics* 2000 **26** 89–92. (doi:10.1038/79238)
 - 59 Horvath A, Giatzakis C, Robinson-White A, Boikos S, Levine E, Griffin K, Stein E, Kamvissi V, Soni P, Bossis I, de Herder W, Carney JA, Bertherat J, Gregersen PK, Remmers EF & Stratakis CA. Adrenal hyperplasia and adenomas are associated with inhibition of phosphodiesterase 11A in carriers of PDE11A sequence variants that are frequent in the population. *Cancer Research* 2006 **66** 11571–11575. (doi:10.1158/0008-5472.CAN-06-2914)
 - 60 Libe R, Fratticci A, Coste J, Tissier F, Horvath A, Ragazzon B, Rene-Corail F, Groussin L, Bertagna X, Raffin-Sanson ML, Stratakis CA & Bertherat J. Phosphodiesterase 11A (PDE11A) and genetic predisposition to adrenocortical tumors. *Clinical Cancer Research* 2008 **14** 4016–4024. (doi:10.1158/1078-0432.CCR-08-0106)
 - 61 Libe R, Horvath A, Vezzosi D, Fratticci A, Coste J, Perlemoine K, Ragazzon B, Guillaud-Bataille M, Groussin L, Clauser E, Raffin-Sanson ML, Siegel J, Moran J, Drori-Herishanu L, Faucz FR, Lodish M, Nesterova M, Bertagna X, Bertherat J & Stratakis CA. Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E208–E214. (doi:10.1210/jc.2010-1704)
 - 62 Horvath A, Giatzakis C, Tsang K, Greene E, Osorio P, Boikos S, Libe R, Patronas Y, Robinson-White A, Remmers E, Bertherat J, Nesterova M & Stratakis CA. A cAMP-specific phosphodiesterase (PDE8B) that is mutated in adrenal hyperplasia is expressed widely in human and mouse tissues: a novel PDE8B isoform in human adrenal cortex. *European Journal of Human Genetics* 2008 **16** 1245–1253. (doi:10.1038/ejhg.2008.85)
 - 63 Horvath A, Mericq V & Stratakis CA. Mutation in PDE8B, a cyclic AMP-specific phosphodiesterase in adrenal hyperplasia. *New England Journal of Medicine* 2008 **358** 750–752. (doi:10.1056/NEJMc0706182)
 - 64 Shields BM, Freathy RM, Knight BA, Hill A, Weedon MN, Frayling TM, Hattersley AT & Vaidya B. Phosphodiesterase 8B gene polymorphism is associated with subclinical hypothyroidism in pregnancy. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 4608–4612. (doi:10.1210/jc.2009-1298)
 - 65 Persani L, Lania A, Alberti L, Romoli R, Mantovani G, Filetti S, Spada A & Conti M. Induction of specific phosphodiesterase isoforms by constitutive activation of the cAMP pathway in autonomous thyroid adenomas. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2872–2878. (doi:10.1210/jc.85.8.2872)
 - 66 Lania AG, Mantovani G, Ferrero S, Pellegrini C, Bondioni S, Peverelli E, Braidotti P, Locatelli M, Zavanone ML, Ferrante E, Bosari S, Beck-Peccoz P & Spada A. Proliferation of transformed somatotroph cells related to low or absent expression of protein kinase a regulatory subunit 1A protein. *Cancer Research* 2004 **64** 9193–9198. (doi:10.1158/0008-5472.CAN-04-1847)
 - 67 Lania A, Persani L, Ballare E, Mantovani S, Loma M & Spada A. Constitutively active Gs α is associated with an increased phosphodiesterase activity in human growth hormone-secreting adenomas. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1624–1628. (doi:10.1210/jc.83.5.1624)
 - 68 Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gundogdu S, De Menis E, Makinen MJ, Launonen V, Karhu A & Aaltonen LA. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science* 2006 **312** 1228–1230. (doi:10.1126/science.1126100)
 - 69 Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, Stolbrink M, Christian HC, Wray J, Bishop-Bailey D, Berney DM, Wass JA, Popovic V, Ribeiro-Oliveira A Jr, Gadelha MR, Monson JP, Akker SA, Davis JR, Clayton RN, Yoshimoto K, Iwata T, Matsuno A, Eguchi K, Musat M, Flanagan D, Peters G, Bolger GB, Chapple JP, Frohman LA, Grossman AB & Korbonits M. The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2390–2401. (doi:10.1210/jc.2007-2611)

- 70 Bolger GB, Peden AH, Steele MR, MacKenzie C, McEwan DG, Wallace DA, Huston E, Baillie GS & Houslay MD. Attenuation of the activity of the cAMP-specific phosphodiesterase PDE4A5 by interaction with the immunophilin XAP2. *Journal of Biological Chemistry* 2003 **278** 33351–33363. (doi:10.1074/jbc.M303269200)
- 71 Igreja S, Chahal HS, King P, Bolger GB, Srirangalingam U, Guasti L, Chapple JP, Trivellin G, Gueorguiev M, Guegan K, Stals K, Khoo B, Kumar AV, Ellard S, Grossman AB & Korbonits M. Characterization of aryl hydrocarbon receptor interacting protein (AIP) mutations in familial isolated pituitary adenoma families. *Human Mutation* 2010 **31** 950–960. (doi:10.1002/humu.21292)
- 72 de Oliveira SK, Hoffmeister M, Gambaryan S, Muller-Esterl W, Guimaraes JA & Smolenski AP. Phosphodiesterase 2A forms a complex with the co-chaperone XAP2 and regulates nuclear translocation of the aryl hydrocarbon receptor. *Journal of Biological Chemistry* 2007 **282** 13656–13663. (doi:10.1074/jbc.M610942200)
- 73 Peverelli E, Ermetici F, Filopanti M, Elli FM, Ronchi CL, Mantovani G, Ferrero S, Bosari S, Beck-Peccoz P, Lania A & Spada A. Analysis of genetic variants of phosphodiesterase 11A in acromegalic patients. *European Journal of Endocrinology* 2009 **161** 687–694. (doi:10.1530/EJE-09-0677)
- 74 Horvath A, Korde L, Greene MH, Libe R, Osorio P, Faucz FR, Raffin-Sanson ML, Tsang KM, Drori-Herishanu L, Patronas Y, Remmers EE, Nikita ME, Moran J, Greene J, Nesterova M, Merino M, Bertherat J & Stratakis CA. Functional phosphodiesterase 11A mutations may modify the risk of familial and bilateral testicular germ cell tumors. *Cancer Research* 2009 **69** 5301–5306. (doi:10.1158/0008-5472.CAN-09-0884)
- 75 Chen C, Wickenheisser J, Ewens KG, Ankener W, Legro RS, Dunaif A, McAllister JM, Spielman RS & Strauss J III. PDE8A genetic variation, polycystic ovary syndrome and androgen levels in women. *Molecular Human Reproduction* 2009 **15** 459–469. (doi:10.1093/molehr/gap035)
- 76 Omori K & Kotera J. Overview of PDEs and their regulation. *Circulation Research* 2007 **100** 309–327. (doi:10.1161/01.RES.0000256354.95791.f1)
- 77 Conti M & Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annual Review of Biochemistry* 2007 **76** 481–511. (doi:10.1146/annurev.biochem.76.060305.150444)
- 78 Michibata H, Yanaka N, Kanoh Y, Okumura K & Omori K. Human Ca²⁺/calmodulin-dependent phosphodiesterase PDE1A: novel splice variants, their specific expression, genomic organization, and chromosomal localization. *Biochimica et Biophysica Acta* 2001 **1517** 278–287. (doi:10.1016/S0167-4781(00)00293-1)
- 79 Bender AT & Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacological Reviews* 2006 **58** 488–520. (doi:10.1124/pr.58.3.5)
- 80 Jeon KI, Jono H, Miller CL, Cai Y, Lim S, Liu X, Gao P, Abe J, Li JD & Yan C. Ca²⁺/calmodulin-stimulated PDE1 regulates the beta-catenin/TCF signaling through PP2A B56 gamma subunit in proliferating vascular smooth muscle cells. *FEBS Journal* 2010 **277** 5026–5039. (doi:10.1111/j.1742-4658.2010.07908.x)
- 81 Sadhu K, Hensley K, Florio VA & Wolda SL. Differential expression of the cyclic GMP-stimulated phosphodiesterase PDE2A in human venous and capillary endothelial cells. *Journal of Histochemistry and Cytochemistry* 1999 **47** 895–906. (doi:10.1177/002215549904700707)
- 82 Mongillo M, Tocchetti CG, Terrin A, Lissandron V, Cheung YF, Dostmann WR, Pozzan T, Kass DA, Paolucci N, Houslay MD & Zaccolo M. Compartmentalized phosphodiesterase-2 activity blunts beta-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. *Circulation Research* 2006 **98** 226–234. (doi:10.1161/01.RES.0000200178.34179.93)
- 83 MacFarland RT, Zelus BD & Beavo JA. High concentrations of a cGMP-stimulated phosphodiesterase mediate ANP-induced decreases in cAMP and steroidogenesis in adrenal glomerulosa cells. *Journal of Biological Chemistry* 1991 **266** 136–142.
- 84 Pyne NJ & Furman BL. Cyclic nucleotide phosphodiesterases in pancreatic islets. *Diabetologia* 2003 **46** 1179–1189. (doi:10.1007/s00125-003-1176-7)
- 85 Degerman E & Manganiello VC. Phosphodiesterase 3B: an important regulator of energy homeostasis. In *Cyclic Phosphodiesterases in Health and Disease*, pp 79–99. Eds JA Beavo, MD Houslay & SH Frances, Broca Raton, FL, USA: CRC Press, 2007.
- 86 Waddleton D, Wu W, Feng Y, Thompson C, Wu M, Zhou YP, Howard A, Thornberry N, Li J & Mancini JA. Phosphodiesterase 3 and 4 comprise the major cAMP metabolizing enzymes responsible for insulin secretion in INS-1 (832/13) cells and rat islets. *Biochemical Pharmacology* 2008 **76** 884–893. (doi:10.1016/j.bcp.2008.07.025)
- 87 Shakur Y, Holst LS, Landstrom TR, Movsesian M, Degerman E & Manganiello V. Regulation and function of the cyclic nucleotide phosphodiesterase (PDE3) gene family. *Progress in Nucleic Acid Research and Molecular Biology* 2001 **66** 241–277. (doi:10.1016/S0079-6603(00)66031-2)
- 88 Reinhardt RR, Chin E, Zhou J, Taira M, Murata T, Manganiello VC & Bondy CA. Distinctive anatomical patterns of gene expression for cGMP-inhibited cyclic nucleotide phosphodiesterases. *Journal of Clinical Investigation* 1995 **95** 1528–1538. (doi:10.1172/JCI117825)
- 89 Degerman E, Belfrage P & Manganiello VC. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). *Journal of Biological Chemistry* 1997 **272** 6823–6826. (doi:10.1074/jbc.272.11.6823)
- 90 Zhang R, Maratos-Flier E & Flier JS. Reduced adiposity and high-fat diet-induced adipose inflammation in mice deficient for phosphodiesterase 4B. *Endocrinology* 2009 **150** 3076–3082. (doi:10.1210/en.2009-0108)
- 91 Bolger G, Michaeli T, Martins T, St John T, Steiner B, Rodgers L, Riggs M, Wigler M & Ferguson K. A family of human phosphodiesterases homologous to the dunce learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. *Molecular and Cellular Biology* 1993 **13** 6558–6571.
- 92 Houslay MD & Adams DR. PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochemical Journal* 2003 **370** 1–18. (doi:10.1042/BJ20021698)
- 93 Wang P, Wu P, Myers JG, Stamford A, Egan RW & Billah MM. Characterization of human, dog and rabbit corpus cavernosum type 5 phosphodiesterases. *Life Sciences* 2001 **68** 1977–1987. (doi:10.1016/S0024-3205(01)00989-4)
- 94 Qiu Y, Kraft P, Lombardi E & Clancy J. Rabbit corpus cavernosum smooth muscle shows a different phosphodiesterase profile than human corpus cavernosum. *Journal of Urology* 2000 **164** 882–886. (doi:10.1016/S0022-5347(05)67331-4)
- 95 Ito M, Nishikawa M, Fujioka M, Miyahara M, Isaka N, Shiku H & Nakano T. Characterization of the isoenzymes of cyclic nucleotide phosphodiesterase in human platelets and the effects of E4021. *Cellular Signalling* 1996 **8** 575–581. (doi:10.1016/S0898-6568(96)00112-X)
- 96 Kass DA, Champion HC & Beavo JA. Phosphodiesterase type 5: expanding roles in cardiovascular regulation. *Circulation Research* 2007 **101** 1084–1095. (doi:10.1161/CIRCRESAHA.107.162511)
- 97 Itona MA & Pittler SJ. Focus on molecules: rod cGMP phosphodiesterase type 6. *Experimental Eye Research* 2007 **84** 1–2. (doi:10.1016/j.exer.2005.12.012)
- 98 Glembycz MA & Smith SJ. Phosphodiesterase 7A: a new therapeutic target for alleviating chronic inflammation? *Current Pharmaceutical Design* 2006 **12** 3207–3220. (doi:10.2174/138161206778194123)

- 99 Rentero C, Monfort A & Puigdomenech P. Identification and distribution of different mRNA variants produced by differential splicing in the human phosphodiesterase 9A gene. *Biochemical and Biophysical Research Communications* 2003 **301** 686–692. (doi:10.1016/S0006-291X(03)00021-4)
- 100 Wang P, Wu P, Egan RW & Billah MM. Identification and characterization of a new human type 9 cGMP-specific phosphodiesterase splice variant (PDE9A5). Differential tissue distribution and subcellular localization of PDE9A variants. *Gene* 2003 **314** 15–27. (doi:10.1016/S0378-1119(03)00733-9)
- 101 Kumar KG, DiCarlo LM, Volaufova J, Zuberi AR & Richards BK. Increased physical activity cosegregates with higher intake of carbohydrate and total calories in a subcongenic mouse strain. *Mammalian Genome* 2010 **21** 52–63. (doi:10.1007/s00335-009-9243-0)
- 102 Kleiman RJ, Kimmel LH, Bove SE, Lanz TA, Harms JF, Romegialli A, Miller KS, Willis A, des Etages S, Kuhn M & Schmidt CJ. Chronic suppression of phosphodiesterase 10A alters striatal expression of genes responsible for neurotransmitter synthesis, neurotransmission, and signaling pathways implicated in Huntington's disease. *Journal of Pharmacology and Experimental Therapeutics* 2011 **336** 64–76. (doi:10.1124/jpet.110.173294)
- 103 Yuasa K, Kotera J, Fujishige K, Michibata H, Sasaki T & Omori K. Isolation and characterization of two novel phosphodiesterase PDE11A variants showing unique structure and tissue-specific expression. *Journal of Biological Chemistry* 2000 **275** 31469–31479. (doi:10.1074/jbc.M003041200)
- 104 Fawcett L, Baxendale R, Stacey P, McGrouther C, Harrow I, Soderling S, Hetman J, Beavo JA & Phillips SC. Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. *PNAS* 2000 **97** 3702–3707. (doi:10.1073/pnas.050585197)

Received 20 April 2011

Accepted 16 May 2011