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REVIEW

Cyclic nucleotide phosphodiesterase (PDE) isozymes as targets of the intracellular signalling network: benefits of PDE inhibitors in various diseases and perspectives for future therapeutic developments

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Cyclic nucleotide phosphodiesterases (PDEs) that specifically inactivate the intracellular messengers cAMP and cGMP in a compartmentalized manner represent an important enzyme class constituted by 11 gene-related families of isozymes (PDE1 to PDE11). Downstream receptors, PDEs play a major role in controlling the signalosome at various levels of phosphorylations and protein/protein interactions. Due to the multiplicity of isozymes, their various intracellular regulations and their different cellular and subcellular distributions, PDEs represent interesting targets in intracellular pathways. Therefore, the investigation of PDE isozyme alterations related to various pathologies and the design of specific PDE inhibitors might lead to the development of new specific therapeutic strategies in numerous pathologies.

This manuscript (i) overviews the different PDEs including their endogenous regulations and their specific inhibitors; (ii) analyses the intracellular implications of PDEs in regulating signalling cascades in pathogenesis, exemplified by two diseases affecting cell cycle and proliferation; and (iii) discusses perspectives for future therapeutic developments.

Abbreviations

AKAP, A-kinase anchoring protein; ALL, acute lymphoblastic leukaemia; ANP, atrial natriuretic peptide; BRAF, B-RAF; CaM-PDE, calmodulin-stimulated PDE; CaM, calmodulin; CAM, chorioallantoic membrane; cAMP-GEFS, cAMP-regulated guanine nucleotide exchange factors; cGI-PDE, cGMP-inhibited PDE; cGS-PDE, cGMP-stimulated PDE; CLL, chronic lymphocytic leukaemia; CNGC, cyclic nucleotide gated channel; COPD, chronic obstructive pulmonary disease; CREB, cAMP response element-binding; CREM, cAMP response element modulator; EPAC, exchange protein activated by cAMP; FDA, Food and Drug Administration; GAF domain, cGMP-activated PDE, adenylyl cyclase- and Fh1A-binding domain; HUVEC, human umbilical vein endothelial cell; ICER, inducible cAMP early repressor; IKK, IκB kinase; IPAH, idiopathic pulmonary arterial hypertension; i.t., intratracheal; KO, knock out; NHRs, possess N-terminal hydrophobic membrane association regions; PAS, **P**er–**A**rnt–**S**im; PASMC, pulmonary arterial smooth muscle cell; PBMC, peripheral blood mononuclear cell; PDE, cyclic nucleotide phosphodiesterase; PHT, pulmonary hypertension; PI3K, PI3 kinase; Rap1, Ras-related protein 1; REC, Response Regulator Receiver; RT-PCR, real-time PCR; SMC, smooth muscle cell; SPH, secondary pulmonary hypertension; TAPAS, tryptophan anchoring phosphatidic acid selective-binding domain; UCR, upstream conserved region; UHRF1, ubiquitin-like, containing PHD and RING finger domains 1

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Cyclic nucleotide phosphodiesterases (PDEs)

Cyclic nucleotide phosphodiesterases play a critical role in intracellular signalling by selectively hydrolysing the second messengers cAMP and cGMP that control cAMP- and cGMP-regulated proteins and transcription factors. In the 1970s, 'the cyclic nucleotide system' concept was developed as providing a powerful tool to enhance the opportunity for drug discovery (Amer, 1977). This system encompassed adenylyl and guanylyl cyclases, PDEs and cAMP-dependent and cGMP-dependent kinases. Very early, the cAMP- and cGMP-PDEs have been considered as targets for drug development (Pang, 1988). At that time, only three types of PDE (PDEI, PDEII and PDEIII) were described in cardiovascular tissues on the basis of their chromatographic elution order (Lugnier *et al*., 1986; Weishaar *et al*., 1987; Pang, 1988). Nowadays, advances in PDE methodology and technology (see book: Lugnier, 2005) have favoured the development of knowledge concerning PDE families (Beavo and Brunton, 2002; and see book: Beavo *et al*., 2006). Presently, PDEs represent an important enzyme class constituted by 11 gene-related families of isozymes (PDE1 to PDE11), giving rise to a multiplicity of isozymes. Each PDE family encompasses one to four distinct genes, giving rise to more than 20 genes in mammals that encode more than 50 different PDE proteins that are all probably expressed in mammalian cells (Lugnier, 2006; Conti and Beavo, 2007; Francis *et al*., 2011). PDE isozymes generally exist as dimers. Their monomeric structure has common features with three distinct domains. The N-terminal regulatory domain characterizes each family and their variants. The catalytic domain is quite conserved, sharing 25% to 52% homology with other mammalian PDE catalytic domains, and contains a Zn^{2+} binding site. The C-terminal domain can be prenylated (Anant *et al*., 1992) or phosphorylated by MAPK (Baillie *et al*., 2000). New mechanisms for cAMP action have been highlighted with the findings of GAF domains (cGMP-activated PDE, adenylyl cyclase- and Fh1A-binding domain) (Heikaus *et al*., 2009; Schultz, 2009), exchange protein activated by cAMP (EPAC), cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFS), and cyclic nucleotide gated channels (CNGC; Cukkemane *et al*., 2011). This has resulted in expanding and diversifying cyclic-nucleotide-related pathways (for review, Borland *et al*., 2009; Gloerich and Bos, 2010).

In many pathologies including inflammation, cardiovascular diseases, neurodegenerescence and cancer, alterations of intracellular signalling related to PDE isozyme deregulation might contribute in explaining the difficulties observed in the prevention and treatment of these pathologies. The multiplicity of biochemical and structural properties of PDEs, their specific subcellular compartments, their transcriptional and posttranscriptional regulation make possible to envision to target only the altered PDE isozymes, thus avoiding and/or decreasing the adverse effects induced by non-specific treatments.

Below are described the 11 PDE families, their contribution in physiological functions and the therapeutical use of some specific PDE inhibitors (Table 1).

PDE1

Previously named calmodulin-stimulated PDE (CaM-PDE), PDE1 is the unique PDE family that is Ca^{2+}-dependently regulated via calmodulin (CaM, a 16 kDa Ca^{2+}-binding protein) complexed with four Ca^{2+} (for review, Stoclet *et al*., 1988; Kakkar *et al*., 1999; Bender, 2006). Thus, this family represents an interesting regulatory link between cyclic nucleotides and intracellular Ca^{2+}, as shown in olfactory mucosa in which Ca^{2+} stimulation of PDE1 might be necessary for termination of olfactory signals (Borisy *et al*., 1992). The PDE1 family is encoded by three genes: *PDE1A* (mapped on human chromosome 2q32), *PDE1B* (human chromosome location, hcl: 12q13) and *PDE1C* (hcl: 7p14.3). They have alternative promoters and give rise to a multitude of proteins by alternative splicing. More than 10 human isoforms are identified (Bolger, 2006). Their molecular weights vary from 58 to 86 kDa per monomer. The N-terminal regulatory domain that contains two Ca^{2+}/CaM binding domains and two phosphorylation sites differentiate their corresponding proteins and modulate their biochemical functions. PDE1A and PDE1B preferentially hydrolyse cGMP, whereas PDE1C hydrolyses cAMP and cGMP with similar K_m values (see Table 1). The association of Ca^{2+}/CaM complex changes PDE1 isozyme conformation, increasing their V_{max} for cyclic nucleotide hydrolysis without modifying their K_m. The sensitivity of PDE1 to Ca^{2+} activation, expressed in EC_{50}, varies from 0.27 μM for PDE1A1 to 3.01 μM for PDE1C1 (Yan *et al*., 1996). Phosphorylation of PDE1A1 (59 kDa) and PDE1A2 (61 kDa) by PKA and phosphorylation of PDE1B1 by CaM kinase II decrease their calmodulin and Ca^{2+} sensitivities, resulting in a decrease of PDE1 activity. Interestingly, PDE1A2 isozyme is sensitive to endogenous proteolysis by calpain that activates PDE1. This could be an alternative mechanism for the permanent activation of PDE1A2 (for review, see Sharma *et al*., 2006). PDE1 is mainly present in brain, cardiac tissues and smooth muscles. Its presence in isolated cardiomyocytes was recently reported (Keravis *et al*., 2007).

Initially, PDE1 activity has been described as cytosolic. It now appears that PDE1A is not restricted to the cytosol but is also present in the nucleus where it contributes to the regulation of transcription factors (Nagel *et al*., 2006). This opens a new field of research in transcriptional regulation. For example, we recently showed that PDE1A controls the expression of the epigenetic integrator UHRF1 (Abusnina *et al*., 2011). Changes in PDE1 location associated with cell differentiation might contribute to compartmental signalling (Nagel *et al*., 2006).

Until now, very few inhibitors of PDE1 were available for evaluating the contribution of PDE1 in tissue and cell function. Vinpocetine (Ahn *et al*., 1989) and 8-methoxymethyl-IBMX (Ahn *et al*., 1997) are common PDE1 inhibitors. However, these compounds are not specific for PDE1; they also inhibit PDE5 at lower concentrations and vinpocetine inhibits PDE7B with an IC_{50} of 59 μM (Sasaki *et al*., 2004). Furthermore, vinpocetine directly inhibits BK_{Ca} channels (Wu *et al*., 2001), and recently vinpocetine was shown to inhibit NF-κB-dependent inflammation via an IKK-dependent but PDE-independent mechanism (Jeon *et al*., 2010). Previously, we showed that nimodipine, a dihydropyridine Ca^{2+} antagonist, selectively inhibits PDE1 in basal- and calmodulin-

Table 1

Cyclic nucleotide phosphodiesterase isozyme families: properties, tissue distribution and reference inhibitors

Family	Specificities	K_m (μM) cAMP/cGMP	Tissue distribution	Inhibitors
PDE1 (3)	Ca2+/calmodulin-stimulated	0.3–124/0.6–6	Heart, brain, lung, smooth muscle	Nimodipine, IC86340, IC224, IC295, dioclein
PDE2 (1)	cGMP-stimulated	15/15	Adrenal gland, heart, lung, liver, platelets, endothelial cells	EHNA, BAY-60–7750, PDP, IC933, oxindole, ND7001
PDE3 (2)	cAMP-selective, cGMP-inhibited	0.2/0.1	Heart, smooth muscle, lung, liver, platelets, adipocytes, immunocytes	Cilostamide, milrinone, siguazodan, cilostazol
PDE4 (4)	cAMP-specific, cGMP-insensitive	2/>300	Brain, Sertolli cells, kidney, liver, heart, smooth muscle, lung, endothelial cells, immunocytes	Rolipram, roflumilast, cilomast, NCS 613
PDE5 (1)	cGMP-specific	150/1	Lung, platelets, smooth muscle, heart, endothelial cells, brain,	Zaprinast, DMPPO, sildenafil tadalafil, vardenafil
PDE6 (3)	cGMP-specific, transducin activated	2000/60	Photoreceptors, pineal gland, lung	Zaprinast, DMPPO, sildenafil, vardenafil
PDE7 (2)	cAMP-specific, high-affinity rolipram-insensitive	0.2/>1000	Skeletal muscle, heart, kidney, brain, pancreas, T lymphocytes	BRL 50481, IC242, ASB16165
PDE8 (2)	cAMP-selective, IBMX insensitive rolipram-insensitive	0.06/NA	Testes, eye, liver, skeletal muscle, heart, kidney, ovary, brain, T lymphocytes, thyroid	PF-04957325
PDE9 (1)	cGMP-specific, IBMX insensitive	NA/0.07–0.17	Kidney, liver, lung, brain	BAY-73–6691, PF-04447943
PDE10 (1)	cGMP-sensitive, cAMP-selective	0.02–1/13	Testes, brain, thyroid	Papaverine, TP-10, MP-10
PDE 11 (1)	cGMP-sensitive, dual specificity	0.5–2/0.3–1	Skeletal muscle, prostate, pituitary gland, liver, heart	None selective

(N) = gene numbers. Adapted from Bender and Beavo (2006), Francis *et al.* (2009), Lugnier (2006). NA, non applicable.

activated states (Lugnier *et al.*, 1984), indicating that this compound is a helpful tool to assess the contribution of PDE1 in tissue or cell homogenates. Indeed, the use of nimodipine in cardiac tissue showed that PDE1 is up-regulated in early cardiac hypertrophy induced by angiotensin II (Mokni *et al.*, 2010). Additionally, this compound, despite its Ca^{2+} antagonist effect, has been used to assess the participation of PDE1 in relaxation of noradrenaline-contracted smooth muscle (Noguera *et al.*, 2001). Use of two potent and specific PDE1 inhibitors, namely IC86340 (IC_{50} from 0.06 to 0.44 μM depending on PDE1 variants; Nagel *et al.*, 2006) and IC295 (Vandeput *et al.*, 2007) synthesized by ICOS Corporation clearly established the contribution of PDE1 in cardiovascular function. Notably, PDE1 inhibition by IC86340 or down-regulation of PDE1A using siRNA allowed demonstrating that PDE1A may regulate specific cGMP pools with elevations of $[Ca^{2+}]i$ during hypertrophic stimulation (Miller *et al.*, 2009). Unfortunately, these compounds are no longer available to the scientific community. We recently showed that dioclein selectively inhibits PDE1 (IC_{50} of 0.62 ± 0.14 μM for basal PDE1 and of 0.55 ± 0.07 μM for activated PDE1) and induces PKG-dependent vasodilatation (Gonçalves *et al.*, 2009), indicating that this compound is helpful for studying basal- and stimulated-PDE1 contributions in cellular signalling.

PDE1 was reported to offer a new target for therapeutic intervention in pulmonary hypertension (PHT). Indeed, a strong up-regulation of PDE1C and PDE1A in pulmonary arterial smooth muscle cells (PASMCs) was noted, respectively, in idiopathic pulmonary arterial hypertension (IPAH) lungs and in lungs from animal models of PHT, and inhibition of PDE1 was reversing structural lung vascular remodelling and right heart hypertrophy in two animal models (Schermuly *et al.*, 2007). The expression of both PDE1A and PDE1C was enhanced in PASMCs from IPAH and secondary pulmonary hypertension (SPH) patients compared with control PASMCs (Murray *et al.*, 2007). PDE1C has been reported to be expressed in proliferating human SMCs but to be absent in quiescent human aorta and to be expressed *in vivo* in human foetal aorta containing proliferating SMCs, making PDE1C a marker of human SMC proliferation *ex vivo* and *in vivo* (Rybalkin *et al.*, 2002). Furthermore, inhibition of PDE1C in SMCs isolated from normal aorta or from lesions of atherosclerosis was resulting in suppression of SMC proliferation, making PDE1C a good target to inhibit proliferating SMCs in lesions of atherosclerosis or restenosis (Rybalkin *et al.*, 2002).

PDE2

PDE2 hydrolyses both cAMP and cGMP. It is encoded by a single gene *PDE2A* (hcl: 11q 13.4) and expressed as PDE2A1, PDE2A2 and PDE2A3. These isozymes differ by their N-terminal residues allowing different subcellular locations: PDE2A1 is cytosolic, whereas PDE2A2 and PDE2A3 are membrane-associated. We showed that PDE2 is associated

with the nuclear envelope (Lugnier *et al*., 1999) and to purified Golgi endosomal fraction, and its phosphorylation by PKC increases its activity (Geoffroy *et al*., 1999). The N-terminal domain of the monomer has two cGMP-binding domains, GAF-A and GAF-B. The GAF-A domain mediates PDE2 dimerization (Martinez *et al*., 2002). Recently, crystal structure studies extended this function to GAF-A and GAF-B domains (Heikaus *et al*., 2009).

The binding of cGMP (1–5 μM) to GAF-B domain allosterically and positively stimulates up to a 30-fold increase in cAMP hydrolysis, inducing Michaelian behaviour with K_m values from 10–30 μM (for review, Martinez, 2006). For this reason, PDE2 was first named cGMP-stimulated PDE (cGS-PDE). This specific regulation gives a major feedback role for PDE2 by restoring the basal cyclic nucleotide level in response to elevated cGMP (Bender and Beavo, 2006), notably following NO, ANP or BNP production. PDE2 is thus able to induce a crosstalk between cAMP and cGMP pathways (Lugnier, 2006).

When activated by cGMP, PDE2 is selectively inhibited by EHNA in comparison with other PDEs (Duncan *et al*., 1982; Podzuweit *et al*., 1995) with an IC_{50} value of 2 μM for activated PDE2 and 38 μM for basal PDE2 (Keravis *et al*., 2006). However, EHNA is also a potent adenosine deaminase inhibitor (K_i = 10 nM; Schaeffer and Schwender, 1974). Bay 60–7550 (Boess *et al*., 2004), IC933 (Snyder *et al*., 2005), PDP (Seybold *et al*., 2005) and oxindole (Chambers *et al*., 2006) are new potent and specific PDE2 inhibitors; however, only Bay 60–7550 is available. Interestingly, it was reported in a cell-based assay that EHNA, Bay 60–7550 and PDP increase cGMP levels only when combined with ANP, indicating that these inhibitors work only on cGMP-stimulated PDE2 (Wunder *et al*., 2009). Recently, our group designed and patented ND7001 as a PDE2 inhibitor that inhibits with the same potency basal and cGMP-stimulated PDE2 (Bourguignon *et al*., 2004). This compound antagonizes the anxiogenic effects of restraint stress on behaviour in mouse, similarly to Bay 60–7550, and in primary cultures of rat cerebral cortical neurons, ND7001 increases cGMP levels similarly to Bay 60–7550 (Masood *et al*., 2009). These data confirm that ND7001 is efficacious both *in vivo* and *in vitro*. Interestingly, in a mouse model of acute lung injury, LPS by intratracheal (i.t.) administration increases lung PDE2A mRNA and protein expression. PDE2A knockdown with an adenovirus administrated i.t. 3 days before LPS decreases PDE2A protein expression and attenuates alveolar inflammation and protein leak (Rentsendorj *et al*., 2011).

For complementary information, an extensive and up-to-date review on PDE2A has recently appeared online (Gesellchen and Zaccolo, 2011).

PDE3

PDE3 hydrolyses both cAMP and cGMP. The V_{max} for cAMP hydrolysis is 10-fold higher than for cGMP hydrolysis and the substrate affinity for cGMP being higher than for cAMP, cGMP behaves as a competitive inhibitor for cAMP hydrolysis and therefore participates in cAMP/cGMP crosstalk (in platelets: Maurice and Haslam, 1990; in vascular smooth muscle: Komas *et al*., 1991; Eckly and Lugnier, 1994). Accordingly, PDE3 was 'cGMP-inhibited PDE' (cGI-PDE; Beavo, 1995). PDE3 is encoded by two genes: *PDE3A* (hcl: 12p12) and *PDE3B* (hcl: 11p15.1). Three variants are expressed for PDE3A (Wechsler *et al*., 2002): PDE3A1 (136 kDa), PDE3A2 (118 kDa) and PDE3A3 (94 kDa), whereas only a single PDE3B1 variant has been identified (Bolger, 2006) even if PDE3B of various sizes have been reported. These variants possess N-terminal hydrophobic membrane association regions (NHRs) and a 44-amino acid insert in the catalytic domain. A PKA phosphorylation site is present on PDE3A1 and PDE3A2, whereas an Akt/protein kinase B (PKB) phosphorylation site is only present on PDE3A1. PDE3A3 lacks PKA and PKB phosphorylation sites (Wechsler *et al*., 2002). PKA phosphorylation activates PDE3A, inducing a negative-feedback regulation in cAMP signalling. PKA phosphorylation of human PDE3B promotes 14-3-3-protein binding and inhibits phosphatase-catalysed inactivation (Palmer *et al*., 2007). PKB-dependent phosphorylation activates PDE3A and PDE3B. PKC phosphorylates and activates PDE3A (Pozuelo Rubio *et al*., 2005). PDE3A1 contains two membrane-associated domains. PDE3A2 contains only one membrane-associated domain, whereas PDE3A3 lacks membrane-associated domains. PDE3 is associated with cardiac sarcoplasmic reticulum (Lugnier *et al*., 1993), cardiac nuclear envelope near nucleopore complexes (Lugnier *et al*., 1999) as well as with liver Golgi endosomal fraction (Geoffroy *et al*., 2001). In primary adipocytes, PDE3B is associated with caveolae (Nilsson *et al*., 2006). PDE3B is present in hepatocyte caveolae and smooth endoplasmic reticulum (Berger *et al*., 2009) and adipocyte caveolae, endoplasmic reticulum and Golgi (Ahmad *et al*., 2009).

PDE3A is mainly present in heart, platelet, vascular smooth muscle and oocytes, whereas PDE3B is mainly associated with adipocytes, hepatocytes and spermatocytes. Reviews have appeared on the implications of PDE3 isoforms in heart failure and hypertension (Movsesian and Smith, 2006) and the role of PDE3B in energy homeostasis (Degerman and Manganiello, 2006). In adipocytes, insulin-mediated phosphorylation/activation of membrane-associated PDE3B leads to a reduction in PKA activity and inhibition of lipolysis. Inhibition of adipocyte PDE3B blocks the antilipolytic action of insulin and reduces insulin-stimulated lipogenesis and glucose uptake (Zmuda-Trzebiatowska *et al*., 2006).

PDE3B has the ability to act as an important scaffold protein with PI3K. A PDE3B-based signalling complex integrates EPAC1 and PI3K signals in human arterial endothelial cells, and this signalosome may be of importance in wound repair and angiogenesis (Wilson *et al*., 2011). Also, a recent study (Perino *et al*., 2011) shows that p110γ-anchored PKA activates PDE3B thus enhancing cAMP degradation and phosphorylating p110γ to inhibit PIP3 production, and this provides a local feedback control of PIP3 and cAMP signalling events. The authors show that pharmacological inhibition of p110γ normalizes β-adrenergic receptor density and improves contractility in failing hearts.

The first potent and selective inhibitor for PDE3 was cilostamide, shown as a platelet anti-aggregant (Hidaka *et al*., 1979). PDE3 was considered as a cardiotonic target. Therefore, medicinal chemistry has been focusing on PDE3 inhibitors, giving rise to amrinone, milrinone and enoximone. Milrinone was the first PDE3 inhibitor given in heart failure. However, due to some mortality in chronic treatment related to tachyarrhythmia and tachycardia, its prescription in

humans is now only for a short period. In these conditions, milrinone does not induce exacerbation of myocardial injury in patients with severe heart failure and low cardiac output but instead is associated with a reduced inflammatory and apoptotic signalling (Lanfear *et al*., 2009). Nevertheless, continuous milrinone therapy use is shown to be safe as bridge to transplant in patients with short waiting time (<100 days) (Assad-Kottner *et al*., 2008). Recently, the PDE3 inhibitor cilostazol was marketed as Pletal® for treatment of intermittent claudication, the most common symptom of peripheral artery disease in which the narrowing and hardening of the arteries that supply legs leads to decreased blood flow. When a patient with peripheral artery disease exercises, the insufficient blood flow can produce muscle ischemia, and walking becomes problematic.

Pharmacological therapy for intermittent claudication in patients with peripheral artery disease is limited. Along with pentoxyfilline, cilostazol is the only medication approved by the Food and Drug Administration (FDA) for intermittent claudication. Cilostazol inhibits platelet aggregation and is a direct arterial vasodilator. Its main effects are dilation of the arteries supplying blood to the legs and decreasing platelet coagulation. Interestingly, cilostazol might increase O_2 delivery by prediabetes erythrocytes whose PDE3B is up-regulated by insulin (Hanson *et al*., 2010). Long-term safety of cilostazol has been questioned because other PDE3 inhibitors, notably milrinone, have been previously associated with excess mortality in patients with heart failure (Movsesian and Kukreja, 2011). However, a recent study has shown that cilostazol demonstrated no increased risk of all-cause mortality (Pande *et al*., 2010).

PDE4

The PDE4 family that selectively hydrolyses cAMP is the most extended family, encoded by four genes, namely *PDE4A* (hcl: 19p13.2), *PDE4B* (hcl: 1p31), *PDE4C* (hcl: 19p13.1) and *PDE4D* (hcl: 5p12) that have different promoters and give rise to a multitude of proteins by alternative splicing. More than 25 human isoforms (from 50 to 125 kDa) are identified (Bolger, 2006). These proteins contain a unique amino acid signature region called upstream conserved regions 1 and 2 (UCR1 and UCR2; Bolger *et al*., 1993). Long PDE4 isozymes exhibit both UCR1 and UCR2, short PDE4 isozymes lack UCR1 and super-short isozymes contain only half of UCR2. UCR1 contains a PKA phosphorylation site that upon phosphorylation attenuates the ability of UCR1 to interact with UCR2 and thereby activates PDE4 activity (Beard *et al*., 2000). The carboxyl-terminal end of the catalytic region contains an ERK phosphorylation site; its phosphorylation induces the activation of PDE4 short forms and the inhibition of PDE4 long forms (Baillie *et al*., 2000). PDE4, like other PDEs, may exist as a dimer. Since the UCR modules mediate dimerization, only long-form PDE4 splice variants containing UCR1 and UCR2 are dimerized (Richter and Conti, 2004). PDE4A1 is a super-short isoform that possesses a specific N-terminal region entirely associated with membranes (Scotland and Houslay, 1995) and formed from two helices separated by a mobile hinge. The helix-2 contains a TAPAS-1 microdomain that allows rapid Ca^{2+}-triggered membrane association with phosphatidic acid (Baillie *et al*., 2002). The helix-1 is important for intracellular targeting of PDE4A1 in living cells, facilitating membrane association, targeting to the *trans*-Golgi stack and conferring Ca^{2+}-stimulated intracellular redistribution in a manner that is dependent on the phospholipase-D-mediated generation of phosphatidic acid (Huston *et al*., 2006). PDE4 variants are specifically localized by A-kinase anchoring protein (AKAPs; Skroblin *et al*., 2010; Welch *et al*., 2010) in subcellular compartments nearby PKA, which regulates functional responses (Dodge *et al*., 2001; for review see Houslay, 2010). We have shown that PDE4 is associated with cardiac sarcoplasmic reticulum (Lugnier *et al*., 1993), and that PDE4B and PDE4D are associated with the nuclear envelope (Lugnier *et al*., 1999). There is a predominant co-localisation of PDE4D3 with centrosome-associated AKAP450 (McCahill *et al*., 2005). Furthermore, PDE4s interact directly with many other intracellular proteins, thereby defining the PDE4 interactome (for a detailed recent review, see Houslay, 2010). PDE4D tethering EPAC1 regulates both the activity and subcellular localization of EPAC1 and controls cAMP-mediated vascular permeability (Rampersad *et al*., 2010). Furthermore, a relationship between PDE4A and PDE4B expression and the regulation of $[Ca^{2+}]i$ was shown in human endothelial cells (Campos-Toimil *et al*., 2008). PDE4s are present in brain, smooth muscle, heart, kidney, endothelial cells and immunocytes.

Rolipram, an antidepressant compound (Schwabe *et al*., 1976), specifically binds with high affinity to membrane brain fractions (Schneider *et al*., 1986). Rolipram was shown to be a potent inhibitor of cAMP hydrolysis in brain homogenates. Rolipram and Ro 20-1724 are highly selective for PDE4 (Lugnier *et al*., 1983, 1986; Komas *et al*., 1989). Accordingly, PDE4 became an archetype for the synthesis of new potent and selective PDE4 inhibitors (Marivet *et al*., 1989). PDE4 subtypes are expressed in a number of cell types that are considered suitable drug targets for the treatment of respiratory diseases such as asthma and COPD (for review, see: Spina, 2008). Suppression of human inflammatory cell function by subtype-selective PDE4 inhibitors correlates with inhibition of PDE4A and PDE4B (Manning *et al*., 1999). Interestingly, PDE4A4 is significantly upregulated in lung macrophages from smokers with COPD when compared with control smokers (Barber *et al*., 2004). Pharmaceutical companies synthesized many PDE4 inhibitors as anti-inflammatory agents for asthma and COPD, since they decreased production of TNF-α and cytokines (for review, Houslay *et al*., 2005; Boswell-Smith *et al*., 2006; Lugnier, 2006; Pagès *et al*., 2009). The PDE4 inhibitor NCS 613, a potent adenine analogue synthesized by our group, inhibits TNFα production in human lymphocytes; interestingly, this compound does not stimulate gastric acid secretion in rats (Boichot *et al*., 2000). Today, roflumilast is the first PDE4 inhibitor on the market (as Daxas®) for COPD treatment (Hatzelmann *et al*., 2010). Recently, a very potent and subtype-specific PDE4D inhibitor was reported (Aspiotis *et al*., 2010) and PDE4D allosteric modulators for enhancing cognition with improved safety have been designed (Burgin *et al*., 2010).

PDE5

PDE5 specifically hydrolyses cGMP and is encoded by one gene *PDE5A* (hcl: 4q27) with three variants being expressed: PDE5A1 (≈100 kDa), PDE5A2 (≈95 kDa) and PDE5A3 (≈95 kDa). Their N-terminal domain contains GAF-A and GAF-B domains. The GAF-A domain is responsible for allos-

teric binding of cGMP that promotes phosphorylation of PDE5 (Turko *et al*., 1998). Such phosphorylation activates catalytic activity and it increases cGMP-binding affinity and converts PDE5 into a stably activated form. The GAF-B domain modulates cGMP binding by GAF-A, thereby contributing to PDE5 dimerization (Francis *et al*., 2006). Despite a similar overall folding of the two GAF domains, only one (GAF-A) binds cGMP. A recent study suggests a complicated and cooperative process of cGMP signalling, which is composed of conformation changes in the GAF-A pocket upon cGMP binding, relay of the cGMP signal from GAF-A to GAF-B, and involvement of N-terminal segment 101–127 in the cGMP signalling (Wang *et al*., 2010). The first three-dimensional structure resolved by electron microscopic analysis of a native PDE revealed a dimeric arrangement for PDE5 having GAF-A, GAF-B and catalytic domains (Kameni-Tcheudji *et al*., 2001). PDE5 is cytosolic in vascular smooth muscle cells (Lugnier *et al*., 1986) and is mainly present in lung, smooth muscles, platelets and corpus cavernosum. Initially, no detectable levels of PDE5 in cardiac ventricles were reported (Komas *et al*., 1989; Wallis *et al*., 1999). PDE5 is associated with Z-bands in cardiac ventricle tissues (Takimoto *et al*., 2005) and with membrane fractions in isolated cardiomyocytes (Keravis *et al*., 2007). PDE5 is localized in endothelial caveolae, where it modulates NOS3 activity (Gebska *et al*., 2011).

Zaprinast (M&B 22948) was the first selective PDE5 inhibitor described (Lugnier *et al*., 1986), and it was used to investigate various functional implications of PDE5. Sildenafil was the first potent and selective PDE5 inhibitor marketed (Viagra™, Pfizer Inc.) for male erectile dysfunction (Francis *et al*., 2008). Thereafter, compounds more potent were developed. Tadalafil (Cialis™, Lilly-ICOS) was synthetized with greater selectivity for PDE5 versus PDE1-4 than sildenafil and with the highest long-lasting effect (>7h) (Daugan *et al*., 2003). Vardenafil (Levitra ™, Bayer-GSK) was synthetized with the lowest IC_{50}. Both sildenafil and vardenafil are also potent PDE6 inhibitor and induce visual perturbation (Cote, 2006). Furthermore, tadalafil although inhibiting PDE11 might not induce cross-reaction in patients (Weeks *et al*., 2005). Pulmonary hypertension (Wilkens *et al*., 2001; Ghofrani *et al*., 2002; Michelakis *et al*., 2002; for review, Croom and Curran, 2008), high altitude mountain sickness (Bates *et al*., 2007), memory dysfunctions (Puzzo *et al*., 2008) and cardiovascular diseases (Kass *et al*., 2007; for reviews, Kumar *et al*., 2009 and Tsai and Kass, 2009) are new therapeutic fields for PDE5 inhibitors. Recently, it has been reported that, human heart PDE5 being present at much lower levels than those seen in animal models, the applicability of PDE5 inhibitors in treating heart failure could be questioned (Movsesian and Kukreja, 2011). However, PDE5 inhibition with sildenafil improves left ventricular diastolic function, cardiac geometry, and clinical status in patients with stable systolic heart failure (Guazzi *et al*., 2011).

Moreover, PDE5 plays a role in cancer. In melanoma cells, oncogenic BRAF induces invasion through down-regulation of PDE5A (Arozarena *et al*., 2011; Houslay, 2011). Nevertheless, PDE5 inhibition is responsible for the breast tumour cell growth inhibitory activity and apoptosis inducing activity of sulindac sulphite and may contribute to the chemopreventive properties of sulindac (Tinsley *et al*., 2009).

PDE6

In contrast to other PDE families, the PDE6 family is restricted to retinal rod and cone cells (Miki *et al*., 1975; Ridge *et al*., 2003) and to pineal gland (Morin *et al*., 2001). PDE6 family, that hydrolyses cGMP, is a key component in the visual transduction cascade and is known as the photoreceptor phosphodiesterase (Cote, 2006). PDE6 is composed of two large catalytic subunits (α and β in rod, 100 and 98 kDa, respectively, and two α′ in cones, 99 kDa) associated with two copies of small inhibitory subunits (γ in rods, 9.5 kDa and γ′ in cones, 8.9 kDa; Cote, 2006). The catalytic subunits are encoded by *PDE6A* for rod α subunit, *PDE6B* for rod β subunit and *PDE6C* for cone α′ subunit. The inhibitory subunits are encoded by *PDE6G* (hcl: 17q25) and *PDE6H* (hcl: 12p13), for γ and γ′ respectively. The *PDE6D* (hcl: 2q35-q36) gene that encodes a δ subunit (17 kDa) co-purified with PDE6 was recently excluded from PDE6 family and revised as a prenyl binding protein (PrBP/δ). Contrary to PDE6, PrBP/δ is present in numerous tissues and may interact with other proteins (Cote, 2006). The structure for the membrane-bound rod PDE6 holoenzyme is αβγ2, whereas it is α′2γ2 for cone PDE6 (Artemyev *et al*., 1996). Each catalytic subunit contains the cGMP-binding GAF-A and GAF-B domains. The GAF-A domain may allow dimerization and binding of the inhibitory subunit γ′. The binding of cGMP is required for high affinity binding of γ subunit. PDE6 activation, mediated by phototransduction, decreases cGMP levels, and it induces the closure of cyclic nucleotide-gated channels (CNGC), thereby converting a light signal into an electrical signal (Wensel, 2008). For now, there is no specific PDE6 inhibitor. However, some PDE5 inhibitors also inhibit PDE6 (for review, Francis *et al*., 2009).

Subtypes of PDE6 have been found outside the eye, especially PDEγ in human embryonic kidney 293 cells (Wan *et al*., 2001) and in mouse lung (Tate *et al*., 2002), which seems to have a critical role in regulating p42/p44 MAPK signalling. PDEγ is also in pulmonary vessels from rats as well as in cultured human pulmonary SMCs, both maintained under chronic hypoxic conditions (Murray *et al*., 2003). Recently, PDE6D and PDE6G/H subtypes have been shown to be present in alveolar epithelial cells and to be altered in lung fibrosis (Nikolova *et al*., 2010). These studies are encouraging to investigate whether some PDE6 subtypes could be therapeutic targets in pulmonary hypertension.

PDE7

The PDE7 family specifically hydrolyses cAMP and is insensitive to rolipram, the specific inhibitor of the PDE4 family, but is sensitive to isobutyl-methylxanthine (IBMX). There is no known regulatory domain on the N-terminal region. This family includes two genes *PDE7A* (hcl: 8q13) and *PDE7B* (hcl: 6q23-q24), with alternative splicing for *PDE7A* (Michaeli, 2006) giving rise to PDE7A1 (57 kDa), PDE7A2 (50 kDa) and PDE7A3 (50 kDa). PDE7A1, expressed in lymphocytes and proinflammatory cells, is a bifunctional protein. In addition to its cAMP hydrolytic activity, its N-terminal domain, by interacting directly with the catalytic subunit of PKA, inhibits kinase activity with high affinity. PDE7A2, present in skeletal and cardiac muscles, has a hydrophobic N-terminus. PDE7A3 lacks a part of the catalytic domain structure and retains the capacity of PDE7A1 to interact and inhibit the catalytic

subunit of PKA. Although no endogenous PDE7B proteins have been yet detected, four alternative splice PDE7B transcripts have been identified: PDE7B1, PDE7B2, PDE7B3 and an additional variant found in GenBank (Michaeli, 2006). Two putative phosphorylation sites for PKA would be on PDE7B1 and PDE7B3. PDE7B1 is the only variant to be activated by D1 agonist through the cAMP/PKA/CREB pathway in striatal neuron, arguing for a role for PDE7B1 in memory function and promoting PDE7B1 as a possible target for Parkinson and Huntington diseases (Sasaki *et al.*, 2004). There are few PDE7 inhibitors. IC242 was the first reported selective PDE7A inhibitor with an IC_{50} value of 0.37 μM (Lee *et al.*, 2002). BRL 50481was discovered as a PDE7 inhibitor (K_i = 180 nM) and has an acceptable *in vitro* selectivity (Smith *et al.*, 2004). The thiadiazoles is a structural class of potent and selective PDE7 inhibitors, synthetized by Pfizer and acting in the nanomolar range (Vergne *et al.*, 2004). Lastly, ASB16165, a novel inhibitor for PDE7A (IC_{50} = 15 nM) was reported to suppress IL-12-induced IFN-γ production by mouse activated T lymphocytes (Kadoshima-Yamaoka *et al.*, 2009).

PDE7 family is a pharmacological target in chronic lymphocytic leukaemia (CLL). Indeed, PDE7A is present in human splenic B lymphocytes from either healthy donors or B-lineage malignancy CLL patients as well as in WSU-CLL, a CLL-derived cell line (Lee *et al.*, 2002). Amongst the different cAMP-PDE isozymes present in WSU-CLL, only PDE7A levels increase after a treatment with methylxanthines; theophylline, IBMX and IC242 inhibit PDE7A activity with respective IC_{50} values of 343.5, 8.6 and 0.84 μM. These data might explain the observation that theophylline, a non-specific methylxanthine PDE inhibitor, induces apoptosis in CLL B-lymphocytes *in vitro* (Mentz *et al.*, 1995). Another study has shown that PDE7B of peripheral blood mononuclear cells (PBMC) was increased at both mRNA (23-fold increase) and protein (10-fold increase) levels in patients with CLL compared with healthy donors and that the PDE7 inhibitors BRL-50481 and IR-202 selectively increased apoptosis in CLL cells compared with normal PBMC or B cells (Zhang *et al.*, 2008).

PDE8

The PDE8 family specifically hydrolyses cAMP with the highest affinity amongst all PDEs and is insensitive to rolipram, IBMX and cGMP (Vasta, 2006). It is encoded by *PDE8A* (hcl: 15q25.3) and *PDE8B* (hcl: 5q13.3). PDE8A mRNA has the highest expression in testis, followed be eye, liver, skeletal muscle, heart, kidney ovary and brain, in decreasing order. The primary structure of PDE8 includes N-terminal REC and PAS domains whose regulatory functions for PDE8 are unknown. Three putative PKA and PKG phosphorylation sites are located between the PAS domain and the catalytic domain. One to five splice variants of PDE8 were cloned from testis and T cells. PDE8A1, the longest (93 kDa) and most frequently expressed variant, contains REC and PAS domains. PDE8A2 lacks the PAS domain, whereas PDE8A3 and the truncated PDE8A4 and PDE8A5 lack both REC and PAS domains (Wang *et al.*, 2001). Five variants of PDE8B have been identified (Gamanuma *et al.*, 2003). PDE8B1 and PDE8B4 contain both REC and PAS domains, whereas PDE8B2 and PDE8B3 have a deletion in PAS domain. RT-PCR studies showed that PDE8B1 is mainly present in thyroid gland, PDE8B1 and PDE8B3 are equally expressed in placenta and PDE8B3 is the most abundant form in brain (Hayashi *et al.*, 2002). Interestingly, PDE8 regulates excitation–contraction coupling in ventricular myocytes, revealing its participation in cardiac function (Patrucco *et al.*, 2010). PF-04957325, the recently produced PDE8 inhibitor (Vang *et al.*, 2010) will be helpful for studying the role of PDE8 in cardiovascular pathologies.

PDE9

The PDE9 family specifically hydrolyses cGMP with the highest affinity amongst all PDE families and is insensitive up to 100 μM to most reference PDE inhibitors, notably to IBMX. However, zaprinast inhibits PDE9 with an IC_{50} value of 35 μM (Fisher *et al.*, 1998). Twenty-one N-terminal mRNA variants have been identified for the *PDE9A* gene (hcl: 21q22.3) (Rentero *et al.*, 2003). There are no regulatory sequences identified on the N-terminal domain of PDE9 (Bender and Beavo, 2006). There is no characterization of PDE9A phosphorylation (Omori and Kotera, 2007). Only PDE9A1 and PDE9A6 (originally named PDE9A5 in the referenced paper) proteins have been expressed and characterized. They are predominant in some immune tissues. PDE9A6 is cytosolic, whereas PDE9A1 is associated to the nucleus (Wang *et al.*, 2003). BAY 73-6691 inhibits PDE9A with a 25-fold greater selectivity compared with all other PDEs (Wunder *et al.*, 2005). The use of BAY 73-6691 in rodents has demonstrated that PDE9 inhibition increases learning and memory (van der Staay *et al.*, 2008). An orally bioavailable PDE9 inhibitor was synthetized for use as a potential hypoglycemic agent (DeNinno *et al.*, 2009). Lastly, PF-04447943, a brain penetrant PDE9A inhibitor that enhances synaptic plasticity and cognitive function in rodents has been published (Hutson *et al.*, 2011).

PDE10

The PDE10 family is a dual-substrate gene family encoded by the unique gene *PDE10A*. Human *PDE10A* maps to chromosome 6q26-27 (Fujishige *et al.*, 1999; 2000). The deduced amino acid sequence contains 779 amino acids (88 kDa), including two GAF domains in the N-terminal region. In contrast to other PDEs, the GAF-A domain apparently binds only cAMP. Due to its kinetic properties for cAMP and cGMP hydrolysis, cGMP hydrolysis by PDE10 is potently inhibited by cAMP, which is notably the opposite compared with the PDE3 family (Bender and Beavo, 2006). The first known potent and specific PDE10A inhibitor is papaverine, with an EC_{50} value of 36 nM (Siuciak *et al.*, 2006). There are 18 splice variants for PDE10A (PDE10A1–PDE10A18; for details, see Strick *et al.*, 2006). PDE10A is expressed mainly in brain, particularly in striatal medium spiny neurons, in pineal gland and, to a lower extent, in testis. TP-10, a new potent and very specific PDE10 inhibitor, synthesized by Pfizer, was under investigation in preclinical study as a new therapeutic approach for the treatment of schizophrenia (Schmidt *et al.*, 2008). Furthermore, papaverine and MP-10 treatments act on the negative symptoms of schizophrenia (Grauer *et al.*, 2009). Recently, it was shown that this PDE10 inhibitor improves striatal and cortical pathology in the R6/2 mouse model of Huntington's disease (Giampà *et al.*, 2010), and that PDE10A

plays a key role in the pathophysiology of Parkinson's disease (Giorgi *et al.*, 2011). A recent study demonstrates for the first time that PDE10A has a central role in progressive pulmonary vascular remodelling and suggests a novel therapeutic approach for the treatment of pulmonary hypertension (Tian *et al.*, 2011).

PDE11

The PDE11 family represents a dual-substrate PDE family having a catalytic site more similar to PDE5 than to PDE10A. PDE11A1 (491 amino acids; predicted molecular mass 56 kDa) was cloned from human skeletal muscle and was shown to contain only one GAF domain. PDE11 is mainly present in prostate and to a lower amount in pituitary gland, liver and heart. Four N-terminal variants are encoded from the *PDE11A* gene mapped on human chromosome 2 (2q31.2): PDE11A1 to PDE11A4. PDE11A2 (65.8 kDa) and PDE11A3 (78 kDa) contain one complete and one incomplete GAF domain in the N-terminal region (Omori and Kotera, 2006). PDE11A4 (100 kDa) is the longest PDE11 protein, including two GAF domains and two phosphorylation sites for PKA and PKG (Yuasa *et al.*, 2000). The role of the GAF domain in PDE11 is still unknown. PDE11A is inhibited by dipyridamole (IC_{50} = 0.9–1.8 μM), zaprinast (IC_{50} = 5–33 μM), IBMX (IC_{50} = 25–81 μM) and is insensitive to EHNA, rolipram and milrinone. There are no specific inhibitors for PDE11A. However, amongst the newly launched PDE5 inhibitors, tadalafil was the most potent (IC_{50} = 37–73 nM), inhibiting both cAMP and cGMP hydrolysis (Saenz de Tejada *et al.*, 2002; for review, see Makhlouf *et al.*, 2006). Although PDE11A is expressed in very restricted brain areas, such as ventral hippocampus, its deletion in a KO mouse model causes psychiatric disease-related phenotypes (Kelly *et al.*, 2010). Carney complex patients possess PDE11A variants with a high frequency, suggesting that PDE11A is a genetic factor for the development of testicular and adrenal tumours (Libé *et al.*, 2011).

The search for specific PDE family inhibitors might take advantage of yeast-based assays, which have been reported to be useful for detecting and characterising inhibitors of either cAMP- or cGMP-metabolizing PDEs (Demirbas *et al.*, 2011).

Intracellular implication of PDEs in regulating signalling cascades

Intracellular cyclic nucleotide targets and PDE regulation

The multiplicity of intracellular cyclic nucleotide targets give rise to an expanding role for PDEs in controlling cellular events. For example, in addition to their very well known stimulating effect on PKA and PKG, binding of cyclic nucleotides to CNGCs results in activation of cation-permeable channels that have key cellular signalling roles (for review, see Kaupp and Seifert, 2002; Matulef and Zagotta, 2003; Biel and Michalakis, 2009). Amongst the direct cAMP targets, EPAC was newly discovered as a Rap1 guanine nucleotide exchange factor that is activated directly by cAMP (de Rooij *et al.*, 1998; Gloerich and Bos, 2010). As described above, cyclic nucleotides regulate PDEs either by their binding to GAF domains or by competitive inhibition. The discovery of

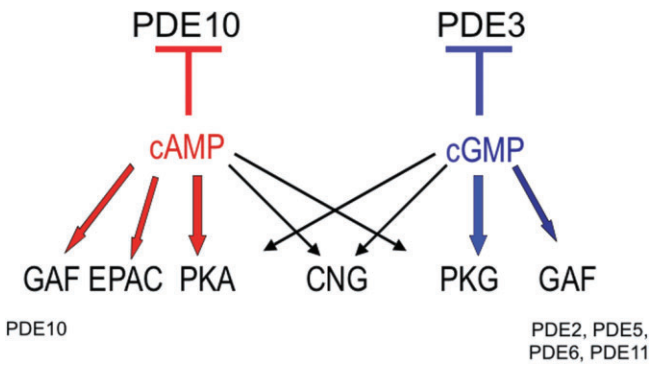

Figure 1
Intracellular targets for cAMP and cGMP.

EPAC enriches the complexity of the network of cyclic nucleotide-mediated pathways in which PDEs play a critical function. EPAC proteins transduce diverse cellular actions of cAMP (for review, see: Borland *et al.*, 2009). Studies of the molecular mechanisms of EPAC-related signalling have demonstrated that these novel cAMP sensors regulate many physiological processes either alone and/or in concert with PKA (Grandoch *et al.*, 2010). Mechanisms for the anti-apoptotic response to cAMP likely involve EPAC. Therapeutic approaches that activate PKA-mediated pro-apoptosis or block EPAC-mediated anti-apoptosis may provide a means to enhance cell killing, such as in certain cancers. In contrast, efforts to block PKA or stimulate EPAC have the potential to be useful in disease settings (such as heart failure) associated with cAMP-promoted apoptosis (Insel *et al.*, 2011).

PKA and PKG that are directly activated, respectively, by cAMP and cGMP control functional cellular responses including intracellular calcium signalling, cell proliferation, inflammation and transcription (for review, see Francis and Corbin, 1999). Since some of these kinases are attached to different cellular ultrastructures via specific A kinase anchoring proteins (AKAPs; Dell'Acqua and Scott, 1997; Moorthy *et al.*, 2011) or G kinase anchoring proteins (GKAPs; Vo *et al.*, 1998; Casteel *et al.*, 2010; Manneville *et al.*, 2010), they participate in intracellular signalling compartmentalization (Wong and Scott, 2004). Moreover, cAMP and cGMP were shown to cross-activate PKG and PKA, respectively (Forte *et al.*, 1992; Eckly-Michel *et al.*, 1997), thereby representing a crossroad in cyclic nucleotide pathways. GAF domains are present in the N-terminal region of PDE2, PDE5, PDE6, PDE10 and PDE11. PDE2, PDE5 and PDE6 contain both GAF-A and GAF-B domains, whereas PDE10 and PDE11 contain one complete GAF domain. The binding of cGMP to PDE2 GAF-A, PDE5 GAF-B and PDE6 GAF-B domains positively regulate cGMP hydrolysis. Furthermore, cAMP hydrolysis by PDE3 is inhibited by cGMP, whereas cGMP hydrolysis by PDE10 is potently inhibited by cAMP. It should be also emphasized that within cells, in the case of other dual-substrate PDEs (PDE1C, PDE2 and PDE11), the hydrolysis of one nucleotide might be competitively inhibited by the other cyclic nucleotide depending on their ratio at the catalytic site (Figure 1).

Beyond cyclic nucleotide targets (PKA, PKG, GAF domains, EPAC, as well competitive substrates), it should be

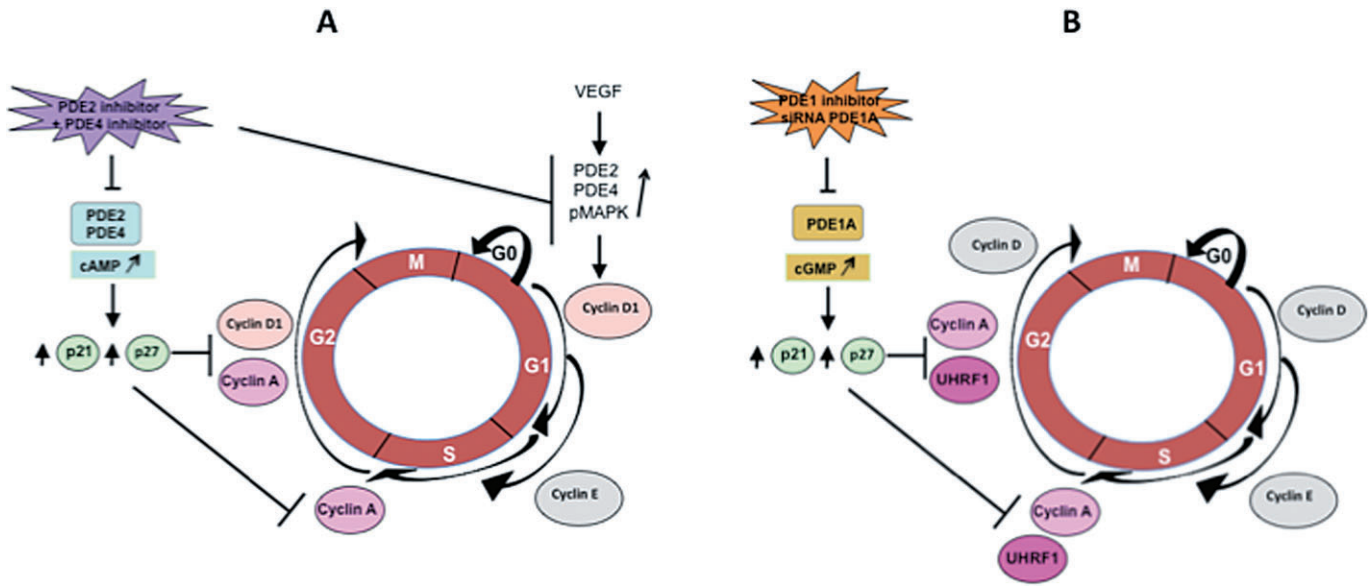

Figure 2

PDE participation in the control of cell cycle. (A) Primary cultured HUVECs. (B) Jurkat cell line.

noticed that CaM, CaM-Kinase, ERK, PKC and phosphatidic acid also participate in short-term regulation of PDEs (Keravis and Lugnier, 2010).

Long-term regulation of PDEs is mediated by PKA-dependent phosphorylation of transcription factors such as CREB and CREM (Lamas and Sassone-Corsi, 1997) and ICER expression (Cho *et al*., 2005). Therefore, these molecular mechanisms will contribute to regulate physiological cellular events as well as dysfunctions associated with various pathologies.

Two examples of PDE regulation in altered signalling

Since the 1980s, the use of various PDE inhibitors in normal and diseased cells and tissues as well as in animal models has allowed to increase and more precisely define the possible implication of specific PDE families in various physiological systems and thereafter in associated pathologies, pointing out their roles in the CNS, in the cardiovascular system and in inflammation. Currently, biochemical and molecular biological tools as well as fluorescent imaging, allow specifying which PDE family and how a variant contribute to the studied dysfunction. In that way, hereafter, we present two examples where we studied the implications of different PDEs in diseases with altered cell cycle: angiogenesis and acute lymphoid leukaemia (ALL).

PDEs in angiogenesis. Angiogenesis, the development of new vessels from pre-existing ones, contributes mainly in tumour growth and metastasis that are dependent on VEGF secretion, which induces endothelial cell proliferation. Endothelial PDEs were first described as PDE2 and PDE4 in pig aortic endothelial cell (Souness *et al*., 1990) and in bovine aortic endothelial cells (Lugnier and Schini, 1990). On one hand, cAMP is anti-proliferative, and on the other hand, VEGF stimulates NOS that increases cGMP levels by stimulating soluble guanylyl cyclase. We wondered whether VEGF could modify PDE activity and expression in angiogenesis. This was investigated on primary HUVECs as a model of *in vitro* angiogenesis and the chicken embryo chorioallantoic membrane (CAM) as an *in vivo* angiogenesis model (Auerbach *et al*., 1974). HUVECs contain PDE2, PDE3, PDE4 and PDE5 families (Favot *et al*., 2003; Netherton and Maurice, 2005). Our results (Favot *et al*., 2003; 2004; Keravis *et al*., 2006) showed for the first time that VEGF specifically up-regulates PDE2 and PDE4 activity and mRNA expression of PDE2A, PDE4A, 4B and 4D without acting on PDE3 and other HUVEC PDEs except for a decrease in PDE5 activity. The treatment of HUVECs with the association of PDE2 and PDE4 inhibitors increases cAMP levels, decreases VEGF-induced HUVEC migration, proliferation and cell cycle progression in the transition phase S-G2/M for the PDE2 inhibitor and G0/G1-S for the PDE4 inhibitor. This is associated with decreases in cyclin A and cyclin D1 expression and with increases in the expression of repressors p21 and p27. The combination of PDE2 and PDE4 inhibitors decreases significantly VEGF-induced phosphorylation of p42/p44 MAPK (Figure 2A). Interestingly, this treatment inhibits *in vivo* angiogenesis, as shown by the dose-dependent reduction of total capillary surface compared with control untreated zones of CAM in the same embryo (Favot *et al*., 2003). PDE2 and PDE4 inhibitors, as well as PDE3 inhibitors, decrease cell migration in other vascular endothelial cells (Netherton and Maurice, 2005). Taken together, our data reveal that targeting specifically altered PDEs in angiogenesis might be an interesting alternative to the currently developed anti-angiogenic therapy with bevacizumab (a humanized monoclonal antibody that inhibits VEGF activity), that has been recently shown to increase mortality in cancer patients (Ranpura *et al*., 2011).

PDEs in ALL. It has been reported that PDE4 inhibitors, which activate cAMP signalling by reducing cAMP catabolism, are known to induce apoptosis in B-lineage CLL cells (Lerner *et al*., 2000), but not in normal human T cells (Meyers *et al*., 2009) and T leukaemic cell lines (Tiwari *et al*., 2005). We recently showed in Jurkat cells (a human T-cell line, model of ALL) that the apoptotic effect of the natural compound thymoquinone is mediated by p73 up-regulation that induces down-regulation of the epigenetic integrator UHRF1 and G1 cell cycle arrest in a dose-dependent manner (Alhosin *et al*., 2010). Since thymoquinone has anti-oxidant and anti-inflammatory properties similarly to PDE4 inhibitors (Houslay *et al*., 2005), since some natural compounds are able to inhibit PDEs and since PDE4 inhibitors decrease cell proliferation in leukaemia (Kim and Lerner, 1998), we wondered whether a specific PDE isoform controlling apoptosis would be responsible for the thymoquinone effects. The determination of the PDE family profile contributing to total cAMP- and cGMP-hydrolysing activities reveals that PDE4 contributes mainly to cAMP hydrolysis, whereas cGMP-hydrolysing activity is mainly due to PDE5, PDE1 contributing only for 1%. Assessed as a PDE inhibitor, thymoquinone only inhibits cAMP hydrolysis by PDE4 with an IC_{50} value of 60 μM, in accordance with its previous described anti-oxidant and anti-inflammatory properties. However, and intriguingly, 24 h thymoquinone treatment dose-dependently decreases cAMP levels but increases cGMP levels, despite that thymoquinone does not act on cGMP hydrolysis by PDE1, PDE2, PDE3 and PDE5. This treatment inhibits Jurkat cell growth in a dose-dependent manner and induces apoptosis. In contrast to PDE3, PDE4 and PDE5 inhibitors, nimodipine, a PDE1 inhibitor, is able, at a concentration selectively active on PDE1, to inhibit the growth and apoptosis of Jurkat cells, similarly to thymoquinone. The effects of nimodipine and thymoquinone induce concomitant decreased expression of PDE1A and UHRF1, whereas p73 expression is upregulated. Knocking down PDE1A by RNA-interference leads to the same results. Conversely, re-expression of PDE1A induces UHRF1 expression and p73 down-regulation. Taken together, these data clearly and newly demonstrate that inhibition of PDE1A expression induces apoptosis of Jurkat cells by up-regulating p73 repressor and down-regulating the expression of the epigenetic integrator UHRF1 (Figure 2B). This also indicates for the first time that a PDE family, such as PDE1A, apparently participates in epigenetic regulation (Abusnina *et al*., 2011).

These two examples of alterations of PDEs participating in cell cycle regulation illustrate one aspect of how PDEs are implicated in cellular functions via critical partners (Figure 2). They point out that, although thymoquinone inhibits only PDE4 activity, its effect in Jurkat cells is not mediated by PDE4 but dependent on PDE1A regulation, illustrating the complexity of cyclic nucleotide pathways. This complexity could be linked to subcellular compartmentalization orchestrated by PDEs, AKAP and protein kinases (Houslay and Adams, 2003), together with subcellular changes in location of PDEs induced by some PDE inhibitors as recently shown for PDE4 inhibitors (Day *et al*., 2011). Furthermore, PDE/protein interactions as largely reviewed for the PDE4 interactome (Houslay, 2010) might well be extended to other 'PDE interactomes', as suggested for PDE1 by our work on PDE1A/UHRF1in Jurkat cells (Abusnina *et al*., 2011), for PDE3B in endothelial cells (Wilson *et al*., 2011). Other ways to disrupt this interactome include the design of specific peptides that would modify critical peptidic interaction during PDE/protein interaction as suggested recently (Keravis and Lugnier, 2010) and the stimulation of changes in microdomain location of a critical PDE variant for a particular cell function.

Perspectives for future therapeutic developments

After numerous disappointments in the development of PDE3 and PDE4 inhibitors due to their adverse effects (mortality and emesis, respectively), the success in PDE5 inhibitor development with Viagra®, associated with increasing knowledge in the PDE field, renews interest in developing selective PDE inhibitors as specific therapeutic agents with few adverse effects.

Since PDEs participate in the interactome that regulates cellular signalling as recently exemplified by PDE3B (Perino *et al*., 2011; Wilson *et al*., 2011), PDEs might be implicated in multiple dysfunctions and pathologies associated with deregulated signals. Recent studies demonstrate the interest of PDE inhibitors to treat and to reverse heart hypertrophy (Takimoto *et al*., 2005), to enhance cognition (Reneerkens *et al*., 2009) and to treat sleep deprivation (Vecsey *et al*., 2009). Concerning alterations of PDE expression, increase in PDE5 expression in human failing left ventricle heart tissue relates to oxidative stress and sildenafil treatment counteracts this alteration (Lu *et al*., 2010). Similarly, expression of PDE5 and PDE9 increases in aging and Alzheimer's disease (Domek-Łopacińska and Strosznajder, 2010). Lastly and interestingly, by using a global PDE8B KO mouse model and a new PDE8-selective inhibitor (PF-04957325), Beavo and colleagues reported that PDE8B controls steroidogenesis in mouse adrenal gland (Tsai *et al*., 2011).

To improve therapeutic index, dual PDE inhibitors inducing synergic potencies with lesser adverse effect have been envisionned. Indeed, dual PDE3/4 inhibitors are now designed as therapeutic agents for chronic obstructive pulmonary disease. By combining both inhibitions, these compounds have additive and synergistic bronchodilatory and anti-inflammatory effects (Banner and Press, 2009). Furthermore, PDE7/PDE4 dual inhibitors would represent a novel class of drugs that could regulate pro-inflammatory and immune T-cell function and be especially useful in treating a wide variety of immune and inflammatory disorders with less undesirable side effects (Castaño *et al*., 2009).

Another way to obtain an efficient therapeutic effect, without inducing adverse effects, may be to specifically target the altered PDE family and in particular the altered variant so that the other PDEs present are not modified. To do so, it is first of all necessary to (i) characterize the various PDE isozymes present in the studied model, (ii) identify the altered PDE isozyme(s) related to the given pathology (therapeutic target) and (iii) validate the therapeutic treatment by the induction of concomitant restoration of the altered PDE(s) and associated cellular function without changing the other non-altered PDEs. This approach might be useful in the case of diseases for which no specific therapy is available. It

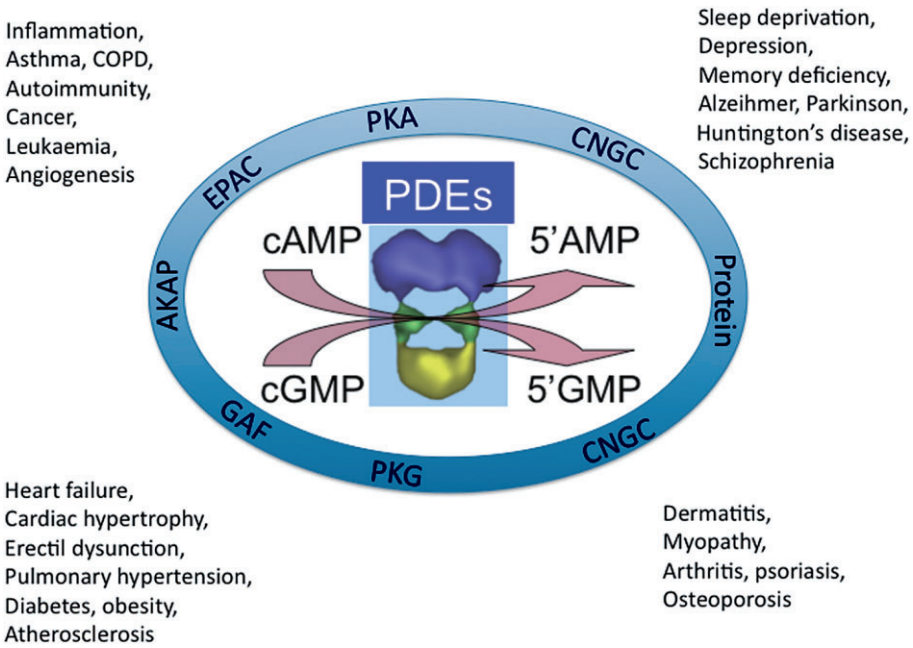

Figure 3

Participation of PDEs in intracellular signalling network: potential therapeutic implications. Local cAMP and cGMP levels controlled by PDEs activate PKA, PKG, CNGC and bind to GAF domains. PDE themselves interact with AKAP and other critical intracellular proteins. These two modes of interaction potentially participate in various diseases.

could also be worth considering in established treatments that induce too many adverse effects.

Throughout this review, many molecular mechanisms interacting with PDE regulation have been discussed (Figure 3), and they may well participate in the normal and pathophysiological regulation of the interactome. Beyond targeting PDEs by the synthesis of potent and very selective PDE family inhibitors, the development of inhibitors of specific PDE variants represents the next focus for therapeutic development.

Furthermore, disrupting agents for PDE/protein interaction might be worth considering for controlling PDE-mediated signalling pathways, as recently demonstrated with the use of a peptide in the hypertrophic response of cardiac myocytes (Sin *et al.*, 2011). Peptides or peptidomimetics acting on critical extra- or intra-PDE-binding sites and able to induce PDE activation could be of interest when the targeted PDE should be upregulated as a therapeutic goal (Keravis and Lugnier, 2010).

In conclusion, considering the participation of multiple PDE variants in the signalling network, their specific and various regulatory mechanisms together with their subcellular compartmentalization and association with diverse critical proteins for cellular function, there is no doubt that PDE super family should become a major target to treat various pathologies.

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Conflict of interest

There is no conflict of interest on behalf both authors.

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