

Invited Review**Phosphodiesterases in the Vascular System**Takayuki MATSUMOTO¹, Tsuneo KOBAYASHI¹ and Katsuo KAMATA¹¹*Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan***Abstract**

Cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) are second messengers involved in the intracellular signal transduction of a variety of extracellular stimuli in several tissues. In the vascular system, these nucleotides play important roles in the regulation of vascular tone and in the maintenance of the mature contractile phenotype in smooth muscle cells. Given that cyclic nucleotide signaling regulates a wide variety of cellular functions, it is not surprising that cyclic nucleotide phosphodiesterases (PDEs). In particular, the accumulating data showing that there are a large number of different PDE isozymes have triggered an equally large increase in interest about these enzymes. At least 11 different gene families of PDEs are currently known to exist in mammalian tissues. Most families contain several distinct genes, and many of these genes are expressed in different tissues as functionally unique alternative splice variants. This article reviews many of the important aspects about the structure, cellular localization, and regulation of each family of PDEs. Particular emphasis is placed on new information obtained in the last few years about vascular disease. The development of novel methods to deliver more potent and selective PDE inhibitors to individual cell types and subcellular locations will lead to new therapeutic uses for this class of drugs in diseases of the vascular system.

Key words: phosphodiesterases, cyclic AMP, vascular tissue

Abbreviations

ACh, acetylcholine; CaM, calmodulin; cAMP, cyclic adenosine 3',5'-monophosphate; cGMP, cyclic guanosine 3',5'-monophosphate; DEAE, diethylaminoethyl; EDHF, endothelium-derived hyperpolarizing factor; FMD, flow-mediated dilation; GAF, cGMP-regulated cyclic nucleotide PDEs, certain adenyl cyclases, and the bacterial transcription factor Φ h1A; GTP, guanine triphosphate; IBMX, 3-isobutyl-1-methylxanthine; NF- κ B, nuclear factor-kappaB; NO, nitric oxide; ODQ, 1H-(1,2,4)oxadiazolo[4,3-a]quinoxalin-1-one; PAS, PER-ARNT-SIM; PDE, phosphodiesterase; PGI₂, prostacyclin; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; SAH, subarachnoid hemorrhage; SMCs, smooth muscle cells; SNP, sodium nitroprusside; STZ, streptozotocin.

Introduction

The second messengers, cyclic nucleotides (cAMP, cGMP), play pivotal regulatory roles in a wide variety of signal transduction pathways and in various tissues (Beavo, 1995). For example, they mediate processes such as vision, olfaction, platelet aggregation, aldosterone synthesis, insulin secretion, T cell activation, and smooth muscle relaxation. In particular, in the vascular system they play important roles in the regulation of vascular tone and in the maintenance of the mature contractile phenotype in smooth muscle cells. The intracellular levels of cAMP and cGMP are tightly controlled both by their rate of synthesis (by adenylyl and guanylyl cyclases, respectively) in response to extracellular signals, and by their rate of hydrolysis [by cyclic nucleotide phosphodiesterases (PDEs)]. PDEs form a superfamily of enzymes that catalyze the hydrolysis of 3',5'-cyclic nucleotides to the corresponding nucleotide 5'-monophosphates (which do not activate cyclic nucleotides' effector proteins).

To date — on the basis of their substrate specificities, kinetics, allosteric regulators, inhibitor sensitivities, and amino acid sequences — at least 11 distinct PDE families have been identified, in total containing more than 50 different PDE enzyme variants, each encoded by several genes (Beavo, 1995; Soderling and Beavo, 2000). Furthermore, each family, and even members within a family, exhibits distinct tissue, cell, and subcellular expression patterns. They are hence likely to participate in discrete signal transduction pathways and thus in discrete physiological and pathophysiological processes e.g., penile erection, asthma, pulmonary hypertension, atherosclerosis, heart failure, and diabetes. Consequently, PDEs are of both fundamental and pharmacological interest.

Here, we review recent advances relating to the structure, distribution, and regulation of PDEs and their role in vascular cell signaling, and we shall conclude by highlighting possible applications of the pharmacology of PDEs to the treatment of vascular disorders.

Classification of PDEs

PDE1 family

The PDEs in the PDE1 gene family are dependent on calcium-calmodulin (CaM) for activity, and were previously termed CaM-PDEs. Three different PDE1 gene products have been cloned: PDE1A, PDE1B, and PDE1C. The first two, PDE1A and PDE1B, hydrolyze cGMP more efficiently than cAMP, whereas PDE1C hydrolyses cAMP and cGMP with equal efficiency (Beavo, 1995). PDE1A and PDE1B have been extensively characterized (Bentley *et al.*, 1992). Two splice variants of the PDE1A gene, PDE1A1 and PDE1A2, have been isolated from bovine heart and brain, respectively (Sonnenburg *et al.*, 1993, 1995). PDE1A1 and PDE1A2 encode the bovine heart 59-kDa and bovine brain 61-kDa CaM-PDE isozymes, respectively, and they differ only in their N-termini (Novack *et al.*, 1991). For PDE1B1, which encodes the bovine brain 63-kDa CaM-PDE isozyme, only one mRNA product has been isolated so far (Bentley *et al.*, 1992; Repaske *et al.*, 1992). Five PDE1C splice variants, PDE1C1 to 5, have been identified in human and mouse tissues (Loughney *et al.*, 1996; Yan *et al.*, 1996). In the vascular system, PDE1 activity has been demonstrated by DEAE ion exchange chromatography in soluble extracts from

a variety of vascular smooth muscle sources, including bovine (Lugnier *et al.*, 1986; Ivorra *et al.*, 1992; Ahn *et al.*, 1992), canine (Pagani *et al.*, 1992; Silver *et al.*, 1988), guinea pig (Silver *et al.*, 1988), human (Lugnier *et al.*, 1986; Hidaka and Endo, 1984), porcine (Saeki and Saito, 1993), rabbit (Hagiwara *et al.*, 1984; Ahn *et al.*, 1989), and rat (Lugnier *et al.*, 1986; Souness *et al.*, 1989) aortas, as well as bovine (Weishaar *et al.*, 1986) and pig (Keravis *et al.*, 1980; Wells *et al.*, 1975) coronary arteries, rat mesenteric (Komas *et al.*, 1991) arteries, and human saphenous vein (Pagani *et al.*, 1992).

The “classical” PDE1A isozymes (59- and 61-kDa CaM-PDEs) have been reported to have values for the Michaelis constant (K_m) for cAMP within the range 34–40 μM , and K_m values for cGMP within the range 2–3 μM (Wang *et al.*, 1990). The maximum velocity (V_{max}) ratio for cAMP/cGMP is ~ 2 for PDE1A isozymes. The PDE1B isozymes (63-kDa CaM-PDEs) have reported K_m values for cAMP and cGMP of ~ 11 and 1 μM , respectively, but a V_{max} ratio of only 0.3. In general, a wide range of kinetic constants for CaM-PDE activity has been reported (Wang *et al.*, 1990).

The PDE1 gene products are called Ca^{2+} /CaM-dependent PDEs because they require both Ca^{2+} and CaM for activity. Of interest is the fact that the affinity for Ca^{2+} /CaM is different among the different PDE proteins. The PDE1A gene encodes two splicing variants, PDE1A1 and PDE1A2. CaM is 10 times more potent in activating A1 than A2, indicating that splicing is a means of regulating the sensitivity to Ca^{2+} and CaM (Sonnenburg *et al.*, 1995). Additional data comparing isoenzymes from brain, heart, and lung have shown differences in the affinity of PDE1B and PDE1C for CaM (Yan *et al.*, 1996). CaM binding is also regulated by PDE1 phosphorylation. At least three of the CaM-dependent PDEs are regulated *in vitro* by phosphorylation/dephosphorylation. Both the 59-kDa heart isozyme and the 61-kDa brain isozyme (PDE1A1 and PDE1A2) are phosphorylated by cAMP-dependent protein kinase (PKA) (Hashimoto *et al.*, 1989; Sharma and Wang, 1985). The 63-kDa isozyme (PDE1B) is phosphorylated by CaM kinase II (Hashimoto *et al.*, 1989). This phosphorylation is accompanied by a decrease in the isozyme affinity towards CaM- Ca^{2+} , and can be reversed by a CaM-dependent phosphatase (Sharma and Wang, 1985; Hashimoto *et al.*, 1989).

PDE2 family

To date, only one gene has been identified for the PDE2 family; however, at least two different 5'-splice variants are known (Yang *et al.*, 1994). Both cAMP and cGMP are hydrolyzed by PDE2, and in fact the V_{max} values for both are very similar (Martins *et al.*, 1982). The two substrates show positively cooperative kinetic effects, with a contribution of the two having Hill coefficients of 1.9 and 1.3 for cAMP and cGMP, respectively. PDE2 has not been detected in most of the studies on vascular smooth muscle isozymes, although a very low PDE2 activity has been reported in soluble extracts of porcine aorta (Saeki and Saito, 1993).

PDE3 family

PDE3s, purified to apparent homogeneity from a variety of tissues, can be distinguished from other PDEs by their high affinities for both cAMP and cGMP, with K_m values within the range 0.1–0.8 μM , and a V_{max} for cAMP 4–10 times higher than that for cGMP (Beavo, 1995).

So far, two different gene products have been identified as being part of the PDE3 family (Meacci *et al.*, 1992; Taira *et al.*, 1993; Degerman *et al.*, 1997). The first, PDE3A, has been identified in smooth muscle, platelets, and cardiac tissues. The second, PDE3B, is most abundant in adipocytes and liver. Both forms are found in smaller amounts in other tissues. PDE3s are activated when phosphorylated either by cAMP-dependent protein kinase (PKA) or by phosphatidyl inositol-3-phosphate-dependent protein kinase (Manganiello and Degerman, 1999). In addition, cAMP-elevating agents increase PDE3 activities and levels in several cell types (Degerman *et al.*, 1997), including cultured rat and human aortic vascular smooth muscle cells (Rose *et al.*, 1997; Liu and Maurice, 1998; Palmer and Maurice, 2000). PDE3 isoforms are found in a variety of intracellular locations, being predominantly membrane-associated in adipocytes (Degerman *et al.*, 1997), cytosolic in platelets (Degerman *et al.*, 1994), and cytosolic as well as associated with the sarcoplasmic reticulum in the myocardium (Kauffman *et al.*, 1986; Muller *et al.*, 1992; Smith *et al.*, 1993). Specific PDE3 inhibitors promote smooth muscle relaxation, stimulate myocardial contractility, and inhibit platelet aggregation, suggesting involvements of PDE3 in the regulation of these physiological (Degerman *et al.*, 1996) and pathophysiological processes (see below).

PDE4 family

cAMP-specific PDE (PDE4) activity that is insensitive to cGMP and/or that is inhibited by rolipram and Ro-20-1724 has been demonstrated in a number of vascular smooth muscle tissues. To date, more than 16 different PDE4, cAMP-specific isoforms have been identified (Houslay *et al.*, 1998; Conti *et al.*, 2003). Four separate genes (A, B, C, and D) encode these various isoforms, with additional multiplicity due to alternative mRNA splicing and the use of different promoters. Perhaps because of the weak vasorelaxation caused by PDE4 inhibitors (Polson and Strada, 1996), the expressions of PDE4s in blood vessels have not been studied extensively. In the vascular system, PDE4 activity has been found in bovine (Ivorra *et al.*, 1992; Prigent *et al.*, 1988), porcine (Saeki and Saito, 1993), and rat (Komas *et al.*, 1991) aortas and in rat mesenteric (Komas *et al.*, 1991) and human pulmonary (Rabe *et al.*, 1994) arteries. It was recently reported that two PDE4D “long forms” (PDE4D3, PDE4D5) are expressed in rat and human vascular smooth muscle cells (Liu and Maurice, 1999; Liu *et al.*, 2000). In cultured rat and human aortic vascular smooth muscle cells, incubation with cAMP-elevating agents induces expressions of two PDE4D “short forms”: PDE4D1 and PDE4D2 (Liu *et al.*, 2000). In addition to its effect on PDE4D3 activity (Liu and Maurice, 1999; MacKenzie *et al.*, 2000; Baillie *et al.*, 2001), the mitogen-activated protein kinase cascade also regulates *PDE4D* expression, inhibiting cAMP-induced increases in the PDE4D “short forms” through a mechanism involving mRNA destabilization (Liu *et al.*, 2000). Selective and regulated targeting of PDE4s also regulates the impact of these enzymes on cell function (Beard *et al.*, 1999; Liu and Maurice, 1999; McPhee *et al.*, 1999; Yarwood *et al.*, 1999; Grange *et al.*, 2000; Conti *et al.*, 2003). PDE3 and PDE4 activities are elevated after incubation with cAMP-elevating agents, and this increase attenuates the responses to further stimulation with cAMP-elevating agents both *in vitro* (Rose *et al.*, 1997; Liu and Maurice, 1999; Liu *et al.*, 2000; Palmer and Maurice, 2000) and *in vivo* (Tilley and Maurice, 2002). These findings need to be discussed in terms of the notion that agents aimed at specific

PDE3 or PDE4 variants may allow greater control of the cAMP-mediated regulation of those vascular smooth muscle cells behaviors that are phenotype-dependent.

Recently, it has been reported that mice deficient in PDE4D exhibit delayed growth as well as reduced viability and female fertility. The decrease in fertility of the null female is caused both by impaired ovulation and by diminished sensitivity of the granulosa cells to gonadotropins. Thus, a critical and indispensable role of PDE4 and its regulation in cell homeostasis has been demonstrated (Jin *et al.*, 1999).

PDE5 family

A cGMP-binding, cGMP-specific PDE (PDE5) is abundant in lung, platelets, and vascular smooth muscle (Lincoln *et al.*, 1976; Francis *et al.*, 1980; Hamet and Coquil, 1978; Coquil, 1983; Francis, 1985; Hamet and Tremblay, 1988). PDE5 is highly specific for cGMP, both in its catalytic site, and in the two cGMP-binding allosteric sites located within the amino-terminal half of the protein (Thomas *et al.*, 1992; McAllister-Lucas *et al.*, 1995). In smooth muscle, nitric oxide (NO) and natriuretic peptides regulate vascular tone by inducing relaxation through stimulation of cGMP synthesis (Sausbier *et al.*, 2000). Degradation of cGMP is controlled by PDEs, and PDE5 is the most highly expressed cGMP-hydrolyzing PDE in these cells. The physiological importance of PDE5 in the regulation of smooth muscle tone has been demonstrated most clearly by the clinical use of its specific inhibitor, sildenafil (Viagra®), in the treatment of erectile dysfunction (Ballard *et al.*, 1998). Recently, the development of other drugs targeting PDE5 in vascular smooth muscle has also been reported. For example, both tadalafil (Cialis™) and vardenafil (Levitra™) can specifically inhibit PDE5 activity in the nanomolar concentration range, but they differ somewhat in their inhibitory profiles towards PDEs from other families (Saenz de Tejada *et al.*, 2001; Eardley and Cartledge, 2002). PDE5 is a multi-domain protein that appears to be regulated intricately by phosphorylation as well as by the binding of cGMP to allosteric cGMP-binding sites at the N-terminus of the protein (Turko *et al.*, 1998; Rybalkin *et al.*, 2003). On the basis of sequence homology in the full-length PDE5 protein, it appears that there are two cGMP-binding domains arranged in tandem that (a) follow an N-terminal domain harboring a site for phosphorylation, and (b) precede the C-terminal catalytic domain of the enzyme (McAllister-Lucas *et al.*, 1993). PDE5 is a dimer, and regions mediating the dimerization have been suggested to be present in the allosteric cGMP-binding domain (Martinez *et al.*, 2002; Thomas *et al.*, 1990; Muradov *et al.*, 2003). The cGMP-binding sites of PDE5 have been found to be necessary for the enhancement of Ser92 (bovine PDE5) phosphorylation by PKA or PKG *in vitro* (Thomas *et al.*, 1990). As a result of PDE5 phosphorylation, the K_d for cGMP binding shifted from 0.13 to 0.03 μM (Corbin *et al.*, 2000). Recently, it was suggested that PDE5, partially purified from rat cerebellum, was allosterically activated by cGMP when a fluorescent analog of cGMP was employed as a substrate (Okada and Asakawa, 2002).

PDE6 family

Photoreceptor cGMP phosphodiesterases (PDE6 family) function as effector proteins in vertebrate visual transduction, which is mediated by the rhodopsin-coupled G protein,

transducin (Chabre and Deterre, 1989; Yarfitz and Hurley, 1994; Beavo, 1995). Retinal rod PDE6 is composed of two catalytic PDE6 $\alpha\beta$ subunits, each tightly associated with the smaller inhibitory γ subunit (P γ) (Baehr *et al.*, 1979; Hurley and Stryer, 1982; Deterre *et al.*, 1988). Cone PDE6 consists of two identical PDE α' subunits complexed with two copies of the cone-specific P γ subunit (Gillespie and Beavo, 1988; Li *et al.*, 1990; Hamilton and Hurley, 1990). The catalytic subunits of rod and cone PDE, as well as the respective P γ subunits, share a high degree of homology (Hamilton and Hurley, 1990; Lipkin *et al.*, 1990). The key role of P γ is to inhibit cGMP hydrolysis by the catalytic subunits in the dark. Upon light stimulation of photoreceptors, PDE6 is activated by GTP-bound transducin- α , which displaces P γ from the enzyme catalytic core. PDE5 and PDE6 share a common domain organization, *i.e.*, two noncatalytic cGMP-binding sites are located N-terminally to the conserved PDE catalytic domain (McAllister-Lucas *et al.*, 1993). Furthermore, PDE5 and PDE6 display a high homology (45–48% identity) between catalytic domains, a strong substrate preference for cGMP, and similar patterns of inhibition by competitive inhibitors such as zaprinast, dipyrindamole, and sildenafil (McAllister-Lucas *et al.*, 1993; Gillespie and Beavo, 1989; Turko *et al.*, 1999; Ballard *et al.*, 1998).

PDE7 family

PDE7 was first isolated at the gene level in 1993 from a human glioblastoma cDNA library, and expressed in a cAMP-deficient strain of the yeast *Saccharomyces cerevisiae* (Michaeli *et al.*, 1993). PDE7A encodes a cAMP-specific PDE that is insensitive both to cGMP and to inhibitors of PDE3 and PDE4, and it has an amino acid sequence distinct from those of the other cAMP PDEs (Michaeli *et al.*, 1993). In humans (Gardner *et al.*, 2000; Hetman *et al.*, 2000; Sasaki *et al.*, 2000) and mice (Gardner *et al.*, 2000; Hetman *et al.*, 2000), two genes (PDE7A and PDE7B) have been identified that encode PDE7. With respect to PDE7A, three isoenzymes (PDE7A1, PDE7A2, and PDE7A3) can theoretically be derived from the same gene by alternative mRNA splicing. PDE7A2 is generated from a 5'-splice variant and, therefore, differs from PDE7A1 in its N-terminal domain (Bloom and Beavo, 1996; Han *et al.*, 1997). In mice and humans, PDE7A2 mRNA is expressed abundantly in skeletal muscle, heart, and kidney, whereas the testis, lung, and immune system (thymus, spleen, lymph node, blood leukocytes) are rich sources of HSPDE7A1, where HS refers to *Homo sapiens* (Bloom and Beavo, 1996; Han *et al.*, 1997; Wang *et al.*, 2000). In the vascular system, the mRNAs for PDE7A1 and also PDE7A2 and also PDE7A1 protein, have been found to be expressed in vascular smooth muscle cells obtained from lung-derived pulmonary artery (Smith *et al.*, 2003), and the mRNAs for PDE7A1 and PDE7A2 are expressed in vascular endothelial cells (Miro *et al.*, 2000).

PDE8 family

PDE8A expression is highest in testis, followed by eye, liver, kidney, skeletal muscle, embryo, ovary, and brain in mice (Soderling *et al.*, 1998). In humans, it has a similar tissue distribution (Fisher *et al.*, 1998a). PDE8A is specific for the hydrolysis of cAMP, with a low K_m of approximately 70 nM (Soderling *et al.*, 1998; Fisher *et al.*, 1998a). PDE8 was the first example (PDE9 is now the second) of a PDE that is not inhibited effectively by IBMX, a non-selective PDE inhibitor. Thus, it should be emphasized that the lack of an effect of IBMX may

not necessarily be a useful indicator that PDEs do not regulate a particular physiological function (Soderling and Beavo, 2000). On the basis of sequence homology with a domain found in proteins from bacteria to eukariots, a PAS (Period, Arnt, Sim) domain has been identified in PDE8 (Soderling *et al.*, 1998). This domain functions as a signal detector, and is usually associated with a heme or a chromophore cofactor (Zhulin *et al.*, 1997). Although the function of the PAS domain in PDE8 is not known, it may be important for protein-protein interaction or for sensing the concentration of a small ligand (Soderling and Beavo, 2000), suggesting a mode of regulation novel among PDEs. To date, it is not known whether PDE8 expression and activities are present within the vascular system.

PDE9 family

PDE9 is specific for the high-affinity hydrolysis of cGMP, with a K_m of 70 nM (Fisher *et al.*, 1998b; Soderling *et al.*, 1998). Like PDE8, PDE9 is not effectively inhibited by IBMX. PDE9 is expressed in smooth muscle in the small intestine, as well as in kidney, liver, lung, brain, testis, skeletal muscle, heart, thymus, and spleen (Fisher *et al.*, 1998b; Soderling *et al.*, 1998; Soderling and Beavo, 2000). PDE9A mRNA is widely distributed throughout the brain, with a varying regional expression, and its expression pattern closely resembles that of soluble guanylyl cyclase in the rat brain, suggesting a possible functional association or coupling of these two enzymes in the regulation of cGMP levels (Andreeva *et al.*, 2001). To date, four 5' alternative splice variants have been identified for PDE9; however, the functional implications of these variants remain as yet currently unknown (Guipponi *et al.*, 1998).

PDE10 family

The PDE10 family was originally isolated from both human (Fujishige *et al.*, 1999a; Loughney *et al.*, 1999) and mouse (Soderling *et al.*, 1999). These studies demonstrated that PDE10 can hydrolyze both cAMP and cGMP, but may function as a cAMP-inhibited cGMP PDE. PDE10A contains two GAF (cGMP binding and stimulated phosphodiesterases, *Anabaena* adenylate cyclases, and *Escherichia coli* FlhA) domains in the N-terminal (Aravind and Ponting, 1997) and a catalytic domain in the C-terminal portions of the molecule (Fujishige *et al.*, 1999a; Soderling *et al.*, 1999). The amino-acid sequence of the catalytic domain bears a closer similarity (40–47% identical) to those of human PDE2A (Rosman *et al.*, 1997), PDE5A (Yanaka *et al.*, 1998), PDE6A (Pittler *et al.*, 1990), PDE6B (Collins *et al.*, 1992), PDE6C (Feshchenko *et al.*, 1996), and PDE11A (Yuasa *et al.*, 2000) than to those of other PDEs. These PDEs contain two GAF domains and show rather low sequence similarity (19–32% identities within catalytic domains) to those of other PDE families lacking these domains. Thus, PDE2, PDE5, PDE6s, PDE10A, and PDE11A constitute a group of PDEs containing GAF domains. PDE10A transcripts are particularly abundant in human putamen, caudate nucleus, and testis. In situ hybridization analysis has demonstrated expressions of PDE10A transcripts in striatal neurons in the rat brain (Fugishike *et al.*, 1999a). The presence of PDE10A activities as high-affinity cAMP PDE and cAMP-inhibited cGMP PDE in extracts of rat striatum and testis indicates that PDE10A functions to control cyclic nucleotide levels, suggesting some physiological roles in these tissues.

PDE11 family

At the moment, the final PDE family is represented by PDE11A, which catalyzes the hydrolysis of both cAMP and cGMP (Fawcett *et al.*, 2000; Yuasa *et al.*, 2000). It has unique splice variants (Yuasa *et al.*, 2000), and a unique structural feature of PDE11A is the presence of multiple forms of GAF (see above). The amino-acid sequence of the PDE11A catalytic domain situated in the C-terminal moiety bears a closer resemblance (42–51% identical) to those of human GAF-PDEs than to those of other PDEs lacking the GAF domain (27–35% identities). PDE11A4 contains two complete GAF domains, whereas PDE11A3 has one complete and one incomplete GAF domain (Yuasa *et al.*, 2000). On the other hand, PDE11A1 has an incomplete GAF domain that lacks the N-terminal part of the GAF consensus sequence (Fawcett *et al.*, 2000). With regard to tissue-specific expression, PDE11A3 transcripts are specifically expressed in testis, whereas PDE11A4 transcripts are particularly abundant in prostate, suggesting some distinct physiological roles of PDE11A via cyclic nucleotide metabolism in these tissues (Yuasa *et al.*, 2000). To date, it is not known whether PDE11A expression and activities are present within the vascular system.

Phosphodiesterases in Vascular Disease

Atherosclerosis

A normal artery consists of quiescent arterial smooth muscle cells (SMCs) covered by a monolayer of endothelial cells lining the interior of the blood vessel. If the artery is injured by an excess amount of atherogenic lipid, by oxidative stress, diabetes (see below), smoking, viruses, or by mechanical means, the SMCs respond by proliferating and forming a neointimal lesion (Ross, 1999). Atherosclerotic lesions occur in the context of endothelial cell dysfunction and involve activation, migration, and proliferation of SMCs. Therefore, considerable effort has been devoted to the identification of factors that regulate SMC proliferation. Endothelial-derived relaxing factors, such as NO or prostacyclin (PGI₂), relax blood vessels and inhibit the proliferation and migration of SMCs by increasing the synthesis of the cyclic nucleotides cAMP or cGMP. In fact, cAMP and cGMP inhibit the proliferation of arterial SMCs (Koyama *et al.*, 2001), and elevation of cyclic nucleotides reduces neointimal formation after angioplasty in animal models. Oral administration for 3–21 days of milrinone (0.3–3.0 mg/kg), a bipyridine derivative that specifically inhibits PDE3, suppressed intimal thickening by up to 56% in a dose- and time-dependent manner in a mouse model of photochemically-induced vascular injury (Kondo *et al.*, 1999). In this model, oral administration of milrinone decreased the number of activated SMC and consequently suppressed intimal thickening by preventing SMC proliferation within the media. PDE1C is expressed in proliferating human SMCs, but is absent from the quiescent human aorta. Inhibition of PDE1C in SMCs isolated from normal aorta or from atherosclerotic lesions, using antisense oligonucleotides or a PDE1 inhibitor, results in suppression of SMC proliferation. Because PDE1C is absent from quiescent SMCs, PDE1C inhibitors may target proliferating SMCs in atherosclerotic lesions or during restenosis (Rybalkin *et al.*, 2002).

Diabetes

Atherosclerosis and other cardiovascular diseases are much more prevalent in diabetics than in the human population at large, and they represent a significant cause of morbidity and early mortality in diabetes (Stern, 1995; Taegtmeier, 1996; Sowers, 1997). It has been reported that alterations in PDEs occur in diabetes-associated cardiovascular disease (Nagaoka *et al.*, 1998; Netherton *et al.*, 2002). For example, Nagaoka *et al.* (1998) reported an increased PDE3 activity in the aorta of atherosclerosis-prone insulin-resistant *cp/cp* rats that correlated positively with increase in the amount of PDE3A mRNA. Netherton *et al.* (2002) reported that in this same animal model, arterial SMCs from homozygous obese (*cp/cp*) rats, but not from age-matched healthy (+/+ or +/*cp*, collectively termed +/?) littermates, display an “activated” phenotype both *in vitro* and *in vivo*, and have an elevated level of PDE activity. Thus, these data are consistent with an increased role for PDE3 in regulating cAMP-dependent signaling in *cp/cp* SMCs, and they identify PDE3 as having a cellular activity potentially responsible for the phenotype of *cp/cp* SMCs.

We recently reported that the impaired EDHF-type relaxation in the mesenteric artery that is seen in STZ (streptozotocin)-induced diabetic rats might be attributable to a reduced action of cAMP, in turn resulting from increased PDE3 activity (Matsumoto *et al.*, 2003). We believe that our findings should stimulate further interest in PDE3 as a potential therapeutic target in the continuing efforts to reduce diabetes-associated vascular disease. In clinical studies, flow-mediated dilation (FMD), induced by occlusion of the brachial artery, is an index of NO-dependent endothelial function, and this is impaired in patients with type 2 diabetes. Desouza *et al.* (2002) assessed the acute and prolonged effects of a low dose of sildenafil (25 mg), an inhibitor of PDE5, on FMD in patients with type 2 diabetes. One hour after oral administration of sildenafil 25 mg, FMD had increased the brachial artery diameter significantly, whereas it did not change with placebo. After treatment with sildenafil 25 mg daily for 2 weeks, and testing 24 hours after the last dose, the mean FMD was found to be 14%. In contrast, the mean FMD with placebo was 9%. These results suggest that acute or prolonged sildenafil treatment has a favorable effect on brachial artery FMD that persists for at least 24 hours after the last dose (Desouza *et al.*, 2002). Further investigation is needed to determine whether this prolonged effect has clinical implications in patients with type 2 diabetes.

Pulmonary hypertension

The pulmonary vascular bed is a low-pressure system with a resistance approximately one-tenth that of the systemic circulation. In the normal lung, pulmonary vascular tone is regulated by a balance between the effects of vasodilators/anti-proliferative agents (such as isoprenaline and PGI₂) and vasoconstrictors/co-mitogens (such as serotonin and endothelin-1). Acute hypoxia causes pulmonary arterial vasoconstriction and increased pulmonary arterial pressure. Chronic hypoxia induces sustained increases in both pulmonary arterial pressure and pulmonary vascular SMC proliferation, and the chronically hypoxic rat is widely used as a model for the study of chronic hypoxia-induced pulmonary hypertension (Jeffery and Wanstall, 2001; Strange *et al.*, 2002). There are several reports suggesting that PDEs play important roles in the development and maintenance of pulmonary hypertension. PDEs activity is increased in

pulmonary arteries from rats with chronic hypoxia-induced pulmonary hypertension (MacLean *et al.*, 1997), and this is correlated with decrease in the intracellular cAMP and cGMP levels (MacLean *et al.*, 1996). In chronic hypoxic-treated pulmonary hypertension-model rats, PDE3A/B gene transcript levels have been found to be increased in the main, first, intrapulmonary and resistance pulmonary arteries. PDE5A2 mRNA transcript and protein levels of in the main and first branch pulmonary arteries were also found to be increased by chronic hypoxia, with no effect on PDE5A1/A2 in the intra-pulmonary and resistance vessels. In the same model, it has been suggested that PDE5 expression might be regulated by the NF- κ B signaling pathway (Murray *et al.*, 2002). A recent study showed that inhibition of PDE3 and PDE4 activities can significantly improve pulmonary hypertension, and that PDE3 mRNA expression was significantly increased in pulmonary artery rings obtained from Sprague-Dawley rats suffering from hypoxia-induced pulmonary hypertension, while PDE4B mRNA expression tended to be reduced, although not significantly (Wagner *et al.*, 1997). Chronic PDE5 inhibition has been shown to elevate pulmonary cGMP levels and abrogate hypoxia-induced pulmonary hypertension and vascular remodeling in animal models, and to reduce pulmonary artery pressure in primary pulmonary hypertension (Hanasato *et al.*, 1999; Wilkens *et al.*, 2001; Zhao *et al.*, 2001; Michelakis *et al.*, 2002).

Inhaled vasodilators have been shown to achieve selective pulmonary vasorelaxation and supraselective vasodilation in well-ventilated (*i.e.*, inhaled vasodilator-accessible) regions within the lung in both experimental and clinical studies. Inhalation of gaseous NO (Troncy *et al.*, 1998; Papazian *et al.*, 1999) and nebulization of PGI₂ (Walrath *et al.*, 1993; Olschewski *et al.*, 1996; Walrath *et al.*, 1996; Zwissler *et al.*, 1996) have been noted both to improve ventilation-perfusion matching and to lower pulmonary arterial pressure in patients suffering from acute respiratory distress syndrome or chronic pulmonary hypertension. The prostanoid PGI₂ doses, however, possess a very short biological half-life (2–3 min) at a physiological pH, and after inhalation of aerosolized PGI₂ the pulmonary vasodilator effect is lost within <30 min both under experimental conditions and when tested in patients (Olschewski *et al.*, 1996; Schermuly *et al.*, 1999). In intact rabbits with acute pulmonary hypertension (Schermuly *et al.*, 1999), subthreshold intravenous doses of nonselective PDE3, PDE4, and PDE5 inhibitors have been noted to augment and prolong the pulmonary vasodilator response to inhaled PGI₂ while limiting the hypotensive effect in the pulmonary circulation. Recently, a prior administration of subthreshold doses of the clinically approved PDE inhibitors theophylline, dipyridamole, and pentoxifylline via the intravascular or inhalational route, which did not itself influence pulmonary hemodynamics, caused more than a doubling of the immediate drop in pulmonary arterial pressure that occurred in response to PGI₂, and also a marked prolongation of the post-PGI₂ vasorelaxation to >60 min (all the PDE inhibitors being effective when given route) (Schermuly *et al.*, 2001). Thus, coaerosolization of PGI₂ and PDE inhibitors should be considered as a postulated therapeutic strategy against pulmonary hypertension.

Subarachnoid hemorrhage

It is well established that endothelium-dependent, NO-induced cerebral vasodilator responses are impaired in a variety of animal models of subarachnoid hemorrhage (SAH)

(Kanamaru *et al.*, 1989; Kim *et al.*, 1989; Edwards *et al.*, 1992; Sobey *et al.*, 1996), and also in patients with SAH (Hatake *et al.*, 1992; Onoue *et al.*, 1995). This may be partly due to reduced NO release resulting from damaged to endothelial cells (Smith *et al.*, 1985; Clower *et al.*, 1994), but importantly it seems that other mechanisms may also contribute. For example, numerous studies have reported that the cerebral vasodilator responses to NO-donor drugs are also impaired after SAH (Kim *et al.*, 1989; Onoue *et al.*, 1995; Zuccarello *et al.*, 1996; Yamamoto *et al.*, 1997), suggesting that the responsiveness of cerebral vascular smooth muscle to NO is altered. Moreover, the vasodilator responses to ACh, sodium nitroprusside (SNP), and low concentrations of zaprinast, an inhibitor of PDE5, are all impaired in SAH rats (Sobey and Quan, 1999). In contrast, the vasodilator responses to adenosine and 8Br-cGMP were similar between control and SAH rats, and the vasoconstrictor response to ODQ, an inhibitor of soluble guanylate cyclase, were unaffected by SAH. In the presence of zaprinast, the responses to ACh and SNP were similar between control and SAH rats. In a canine model of SAH, PDE5 activity was increased to above control levels within the basilar artery seven days after SAH, and the PDE5 expression was most prominent in SMCs following SAH (Inoha *et al.*, 2002). Thus, an increased rate of cGMP hydrolysis by PDE5 may be a major factor contributing to the impairment of NO-mediated cerebral vasodilation after SAH. On the other hand, in a canine model of acute cerebral vasospasm, BRL61063, rolipram, and denbufylline, a selective inhibitor of PDE4, reversed the basilar artery spasm produced by autologous blood without altering mean arterial blood pressure. In contrast, prolonged treatment with BRL61063 failed to alter the development of basilar spasm in two canine hemorrhage models of chronic cerebral vasospasm. Denbufylline-induced relaxation *in vitro* was also significantly impaired in basilar arteries obtained from a model of chronic vasospasm. In conclusion, PDE4 appears to be the predominant isozyme regulating vascular tone via cAMP hydrolysis in cerebral vessels (Willette *et al.*, 1997). Further investigation is needed to determine whether inhibition of PDEs activity might be a useful approach in patients with SAH.

In conclusion, we believe that manipulation of the activities of PDEs within vascular system may have considerable therapeutic potential. Once the full repertoire of the PDEs expressed within the vascular system has been established, it should not be long before new generations of selective PDE inhibitors are available to manipulate vascular cell responses.

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