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MINIREVIEW

Cyclic Nucleotide Phosphodiesterase Activity, Expression, and Targeting in Cells of the Cardiovascular System

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ABSTRACT

Cyclic AMP (cAMP) and cGMP regulate a myriad of cellular functions, such as metabolism, contractility, motility, and transcription in virtually all cell types, including those of the cardiovascular system. Considerable effort over the last 20 years has allowed identification of the cellular components involved in the synthesis of cyclic nucleotides, as well as effectors of cyclic nucleotide-mediated signaling. More recently, a central role for cyclic nucleotide phosphodiesterase (PDE) has also been elaborated in many cell types, including those involved in regulating the activities of the cardiovascular system. In this review, we introduce the PDE families whose members are expressed in cells of the cardiovascular system including cardiomyocytes, vascular smooth muscle cells, and vascular endothelial cells. Because cell behavior is a dynamic process influenced by numerous factors, we will attempt to emphasize how changes in the activity, expression, and targeting of PDE influence cyclic nucleotide-mediated regulation of the behavior of these cells.

The cyclic nucleotides cAMP and cGMP regulate a myriad of cellular functions, including metabolism, contractility, motility, and transcription in virtually all cell types, including those of the cardiovascular system (Antoni, 2000; Klein, 2002). Although early work identified cAMP and cGMP as second messengers and led to the discovery of the proteins involved in coordinating the synthesis, degradation, and cellular actions of cyclic nucleotides, early models describing how these systems allowed cyclic nucleotide-mediated regulation of multiple cellular functions underestimated the levels of flexibility and specialization involved. In this context, recent work has identified large numbers of receptor (Marchese et al., 1999; Lucas et al., 2000), adenylyl cyclase (Hanoune and Defer, 2001), guanylyl cyclase (Garbers, 1999; Lucas et al., 2000), heterotrimeric G-protein (Marchese et al., 1999), and cyclic nucleotide phosphodiesterase (PDE) (Beavo and Reifsnyder, 1990; Beavo, 1995; Conti et al., 1995; Manganiello and Degerman, 1999; Conti, 2000; Conti and Jin,

2000; Houslay and Kolch, 2000; Soderling and Beavo, 2000; Francis et al., 2001; Houslay and Adams, 2003) protein families. Although many of the cellular effects of cAMP and cGMP are coordinated through their activation of cyclic nucleotide-dependent protein kinases (Lincoln et al., 1995), several other effectors are now known. Thus, cyclic nucleotide-gated ion channels (Yau, 1994), cAMP-activated guanine nucleotide exchange factors (de Rooij et al., 1998; Kawasaki et al., 1998), and cyclic nucleotide PDEs have each been shown to transduce cyclic nucleotide-encoded information (Beavo and Reifsnyder, 1990; Beavo, 1995; Conti et al., 1995; Manganiello and Degerman, 1999; Conti, 2000; Conti and Jin, 2000; Houslay and Kolch, 2000; Soderling and Beavo, 2000; Francis et al., 2001; Houslay and Adams, 2003). More recently, an appreciation of the impact of regulated anchoring/targeting of cyclic nucleotide-regulated proteins to discrete subcellular domains (Rubin, 1994; Vo et al., 1998; Michel and Scott, 2002), has also challenged several earlier assumptions. In this review, we introduce the PDE families whose members are expressed in cardiomyocytes, the terminally differentiated cells that allow the continuous rhythmic

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ABBREVIATIONS: PDE, phosphodiesterase; VSMC, vascular smooth muscle cells; VEC, vascular endothelial cells; AC, adneylyl cyclase; GAF, cGMP phosphodiesterase, adenylyl cyclase, Fh1A; PKA, protein kinase A; PKB, protein kinase B; UCR, upstream conserved region; AKAP, A-kinase–anchoring protein; mAKAP, muscle A-kinase–anchoring protein; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; ED, erectile dysfunction; PKG, protein kinase G; Ro 20-1724, 4-[(3-butoxy-4-methoxyphenyl)-methyl]-2-imidazolidinone.

contractility of the heart, as well as cell types that regulate blood vessel function, namely vascular smooth muscle cells (VSMC) and vascular endothelial cells (VEC). Although cardiac fibroblasts constitute about 20% of ventricular mass, and blood vessel adventitial layer-derived fibroblasts may participate in blood vessel functions, PDE expression in these vascular cells will not be discussed in this review. Because cell behavior is a dynamic process influenced by numerous factors, we will attempt to emphasize how changes in the activity, expression, and targeting of PDE influence cyclic nucleotide-mediated regulation of the behavior of these cells. Although our focus is on cells of the cardiovascular system, we contend that the basic concepts are probably more broadly relevant.

Cyclic Nucleotide PDEs: General Considerations

Despite early difficulties in resolving and characterizing a heterogeneous pool of cyclic nucleotide hydrolyzing activities, a great deal is now known about the composition of the PDE superfamily. At present, mammalian PDE enzymes are subdivided into 11 distinct enzyme families based on primary amino acid sequence, overall domain structure, and catalytic and regulatory considerations. Each of the PDE families contains multiple genes that, through the use of alternate promoter initiation site usage and/or alternate splicing of mRNA, yield more than 50 distinct isoenzyme variants (Table 1) (Beavo and Reifsnyder, 1990; Beavo, 1995; Conti et al., 1995; Manganiello and Degerman, 1999; Conti, 2000; Conti and Jin, 2000; Houslay and Kolch, 2000; Soderling and Beavo, 2000; Francis et al., 2001; Houslay and Adams, 2003). Although sequence identity within the catalytic domain is relatively high between families (20–45%), amino- and carboxyl-terminal sequences are considerably dissimilar and encode those sequences allowing family-selective regulatory characteristics (Beavo and Reifsnyder, 1990; Beavo, 1995; Conti et al., 1995; Manganiello and Degerman, 1999; Conti, 2000; Soderling and Beavo, 2000; Conti and Jin, 2000; Francis et al., 2001; Houslay and Adams, 2003; Houslay and Kolch, 2000). A nomenclature system allows identification of the many PDEs. For example, HSPDE4D3 identifies a human (HS, *Homo sapiens*) enzyme of the PDE4 family that is encoded by the D gene of this family. Because many PDE genes generate several distinct variants, a final number, in this case 3, identifies the specific splice variant.

PDE Activity, Expression, and Targeting in Cells of the Cardiovascular System

Cardiomyocytes, which account for approximately 60% of the cells of the heart, are terminally differentiated cells that allow the continuous rhythmic contractility of the heart (Nadal-Ginard et al., 2003). VSMC and VEC constitute the bulk of the cells of blood vessels and cooperate to regulate blood vessel structure and function (Thyberg, 1998; Regan et al., 2000). Although they are markedly different cell types, several convergent signaling pathways regulate the physiological actions of cardiomyocytes, VSMC, and VEC and the capacity of each to function "normally" in situations of cardiovascular stress. Thus, although cardiomyocytes do not normally undergo significant phenotypic modulation in the adult, these cells, when under conditions of prolonged cardiovascular stress, such as hypertension or after a myocardial infarct, can undergo a process of hypertrophic growth in which their contractile function becomes deregulated (Thyberg, 1998; Regan et al., 2000). Similarly, when stressed, VSMC and VEC undergo morphologic growth-related alterations that reduce their ability to respond to normal variations in cardiovascular demand. Thus, VSMC and VEC of healthy undamaged arteries function primarily to regulate blood vessel lumen diameter by coordinating contractile and

TABLE 1
Properties of cyclic nucleotide PDE families

PDE Family	Cyclic Nucleotide Substrates	Inhibitors (K_i)	No. of Genes	Regulation
PDE1: Ca^{2+}/CAM stimulated PDE	cAMP and cGMP	Vinpocetine (14 μM), W-7 (300 μM)	3	(+) Ca^{2+}/CAM (−) PKA, CamKII
PDE2: cGMP-stimulated PDE	cAMP and cGMP	EHNA (1 μM)	1	(+) cGMP, PKC (+/−) N-terminal targeting domain
PDE3: cGMP-inhibited PDE	cAMP > cGMP	Cilostamide (20 nM), milrinone (150 nM), zardaverine (IC_{50} 0.5–2 μM)	2	(+) PKA, PKB (−) cGMP (+/−) N-terminal targeting domain
PDE4: High affinity, Rolipram-sensitive cAMP-specific PDE	cAMP	Rolipram (1 μM), Ro 20-1724 (5 μM), piclamilast (1 nM), zardaverine (IC_{50} 0.8–4 μM)	4	(+) PKA, ERK, Phosphatidic Acid (−) ERK, Caspases (+/−) N-terminal targeting domains
PDE5: cGMP-specific PDE	cGMP	Zaprinast (130 nM), sildenafil (10 nM), vardenafil (1 nM), tadalafil (10 nM)	1	(+) cGMP, PKG, PKA (−) Caspases
PDE6: Photoreceptor cGMP-specific PDE	cGMP	Zaprinast (400 nM), dipyridamole (125 nM), sildenafil (50 nM)	3	(+) Trasnsducin (−) cGMP, gamma and delta subunits
PDE7: High-affinity, Rolipram-insensitive cAMP-specific PDE	cAMP	IBMX (4 μM), dipyridamole (600 nM)	2	(+/−) PKA
PDE8: High-affinity and IBMX-insensitive cAMP-specific PDE	cAMP	Dipyridamole (9 μM)	2	PAS domain
PDE9: High-affinity cGMP-specific PDE	cGMP	Zaprinast (35 μM)	1	
PDE10: cAMP-Inhibited cGMP PDE	cAMP < cGMP	Dipyridamole (1 μM),	1	(−) cAMP
PDE11: Dual specificity cGMP-binding PDE	cAMP and cGMP	Zaprinast (12 μM), dipyridamole (0.4 μM), tadalafil (60 μM)	1	

W7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; IBMX, 3-isobutyl-1-methylxanthine; Ca^{2+}/CAM, calcium/calmodulin.

relaxant cues. These cells are said to display a "contractile/quiescent" phenotype (Thyberg, 1998; Regan et al., 2000). In contrast, during development, or in response to cardiovascular stresses in the adult, VSMC and VEC undergo a process of phenotypic modulation that results in the development of cells with migratory and proliferative phenotypes. Such "synthetic/activated" VSMC and VEC are no longer contractile and become sensitive to the growth-promoting and chemoattractant influences of plasma- and cell-derived factors (e.g., platelet-derived growth factor) (Thyberg, 1998; Regan et al., 2000). VSMC and VEC isolated from blood vessels and propagated in tissue culture share many of the attributes of synthetic/activated cells (Rose et al., 1997; Liu and Maurice, 1998; Rybalkin and Bornfeldt, 1999). Left untreated, these alterations in cardiomyocytes, VSMC, and VEC growth responses contribute to the establishment of severe cardiovascular complications, such as heart failure, a progressive syndrome leading to heart damage and inadequate blood supply to distal tissues, or severe vasculopathies, such as atherosclerosis or angioplasty-induced restenotic injury. Because cAMP and cGMP influence nearly all facets of the biology of cardiomyocytes (strength and frequency of excitation-contraction coupling), VSMCs (relaxation-contraction coupling, proliferation, migration, and cellular metabolism), and VECs (proliferation, migration, cellular metabolism, and permeability) (Pang, 1992; Rybalkin and Bornfeldt, 1999; Movsesian, 2000), it could be anticipated that changes in the activity, expression, or subcellular targeting of PDE in these cells might significantly alter their behavior and overall cardiovascular function and health.

Activity, expression and targeting of PDE have been investigated in cardiomyocytes, VSMC, and VEC isolated from several species, including human, rat, mouse, bovine, pig, and dog. In studies of cardiac tissue, or isolated cardiomyocytes, multiple variants of several PDE families have been identified (Pang, 1992; Movsesian, 2000). Although studies with isolated cardiac tissues were consistent with the expression of enzymes from the PDE1, PDE2, PDE3, PDE4, and PDE5 families, data with isolated cardiomyocytes were not always consistent with PDE1 or PDE5 expression in these cells, in all species, and may reflect the contribution of cardiac fibroblasts or endothelial cells (Pang, 1992; Movsesian, 2000). In an earlier review, Polson and Strada (1996) concluded that variants from the PDE1, PDE3, PDE4, and PDE5 families of enzymes were expressed in contractile/quiescent and synthetic/activated VSMC of numerous blood vessels from several species, including human, bovine, pig, rat, dog, guinea pig, and rabbit. Interestingly, in their review, Polson and Strada (1996) reported that levels of PDE activity of some families were significantly different between contractile/quiescent and synthetic/activated VSMC. In this context, recent studies of this phenomenon have concluded that these differences relate, at least in part, to authentic differences in the expression profile of PDE genes in the two distinct VSMC phenotypes (Rybalkin et al., 1997; Rybalkin and Bornfeldt, 1999; Dunkerley et al., 2002). Although many fewer studies have assessed PDE activities and expression in VEC (and in most instances only cultured synthetic/activated VEC were studied), the available literature identifies possible roles for PDE1, PDE2, PDE3, PDE4, and PDE5 family variants in these cells (Ashikaga et al., 1997; Zhao et al., 1997; Keravis et al., 2000; Thompson et al., 2002). Given the central role of

VEC in processes such as blood vessel contractility, coagulation, inflammation, angiogenesis, and vascular remodelling, the therapeutic value of targeting VEC PDE will probably spur further analysis of PDE in these important cells.

PDE1

General Characteristics. PDE1 family variants are activated upon Ca^{2+}/calmodulin binding and as such are known as Ca^{2+}/calmodulin-activated PDE (Zhao et al., 1997; Kakkar et al., 1999). Three *PDE1* genes (*PDE1A*, *PDE1B*, and *PDE1C*) encode PDE1 variants exhibiting different cyclic nucleotide hydrolysis kinetics and Ca^{2+}/calmodulin selectivity (Table 1). Although PDE1A and PDE1B enzymes selectively hydrolyze cGMP, PDE1C variants hydrolyze both cAMP and cGMP with high affinity (Zhao et al., 1997). *PDE1A* and *PDE1B* genes each encode two splice variants (PDE1A1, PDE1A2 and PDE1B1, PDE1B2, respectively), whereas five *PDE1C* gene products have been described (PDE1C1–5). Levels of PDE1 activity and protein in cells are regulated transcriptionally and post-translationally; cAMP- and Ca^{2+}-dependent signaling both contribute. Roles for PDE1C have been demonstrated in human VSMC proliferation (Rybalkin et al., 1997), olfactory fatigue (Jaworsky et al., 1995), and insulin-secretion (Han et al., 1999). In addition, increased PDE1A expression was reported to contribute to the development of nitroglycerin tolerance, a multifactorial phenomenon leading to reduced efficacy of this important antianginal drug (Kim et al., 2001). Calmodulin antagonists inhibit PDE1 activity, although their lack of PDE selectivity has limited their utility. Similarly, although direct catalytic site inhibitors such as vinpocetine and 8-methoxy-1-methyl-3-isobutylxanthine inhibit PDE1 activity, these drugs have limited inter-PDE family selectivity and, within the PDE1 family of enzymes, are less potent inhibitors of PDE1C enzymes (Zhao et al., 1997; Kakkar et al., 1999).

Cardiomyocyte-PDE1. Two *PDE1* gene products (PDE1A and PDE1C) are expressed in cardiac tissues from several species (Rybalkin et al., 1997; Zhao et al., 1997; Kakkar et al., 1999), although studies with isolated cardiomyocytes and cardiac fibroblasts are consistent with a predominant expression of these enzymes in a nonmyocyte fraction, rather than cardiomyocytes (Bode et al., 1991). Obviously, a thorough characterization of PDE1 expression in cardiomyocytes from several species using sensitive methods, perhaps reverse transcription-polymerase chain reaction, may be required to decisively address this issue. Because Ca^{2+} has been shown to regulate cardiomyocyte adenylyl cyclases (AC5/6) (Hanoune and Defer, 2001) and nitric-oxide synthase activities in cardiomyocytes, PDE1 expression in these cells might identify a role for Ca^{2+} in a dynamic regulatory system for cAMP and cGMP levels during cardiomyocyte contractions (Fig. 1a). Although the data available concerning PDE1 expression in cardiomyocytes isolated from most species, including human, are currently incomplete and sufficiently potent and selective PDE1 inhibitors are not available, this hypothesis must at present remain untested. No changes in PDE1 expression have been reported to accompany the cardiovascular stresses that predispose to heart disease; however, a role for increased PDE1C expression in the cardio-protective effect of the stable prostacyclin derivative, 7-oxo-prostacyclin, indicates that PDE1C variants may be

involved in tissue responses to cardiovascular stresses (Kostic et al., 1997).

Vascular Smooth Muscle Cells-PDE1. Several reports have confirmed PDE1 expression in contractile/quiescent and synthetic/activated VSMC from several blood vessels, harvested from several species. The most comprehensive study to date identified PDE1A1 and PDE1B2 variants in human, rat, bovine, and monkey aortic contractile/quiescent VSMC, albeit in species-selective proportions (Rybalkin et al., 1997).

The absence of PDE1C expression in all contractile/quiescent VSMC studied so far indicates that Ca^{2+}-stimulated hydrolysis is unlikely to regulate cAMP levels in this VSMC phenotype. However, the expression of PDE1A and PDE1B variants in contractile/quiescent VSMC, in combination with the expression of Ca^{2+}-activated nitric-oxide synthase in these cells, may define a Ca^{2+}-dependent system allowing coordinated control of cGMP levels (Fig. 1a). Although the absence of selective PDE1 inhibitors has significantly hampered ef-
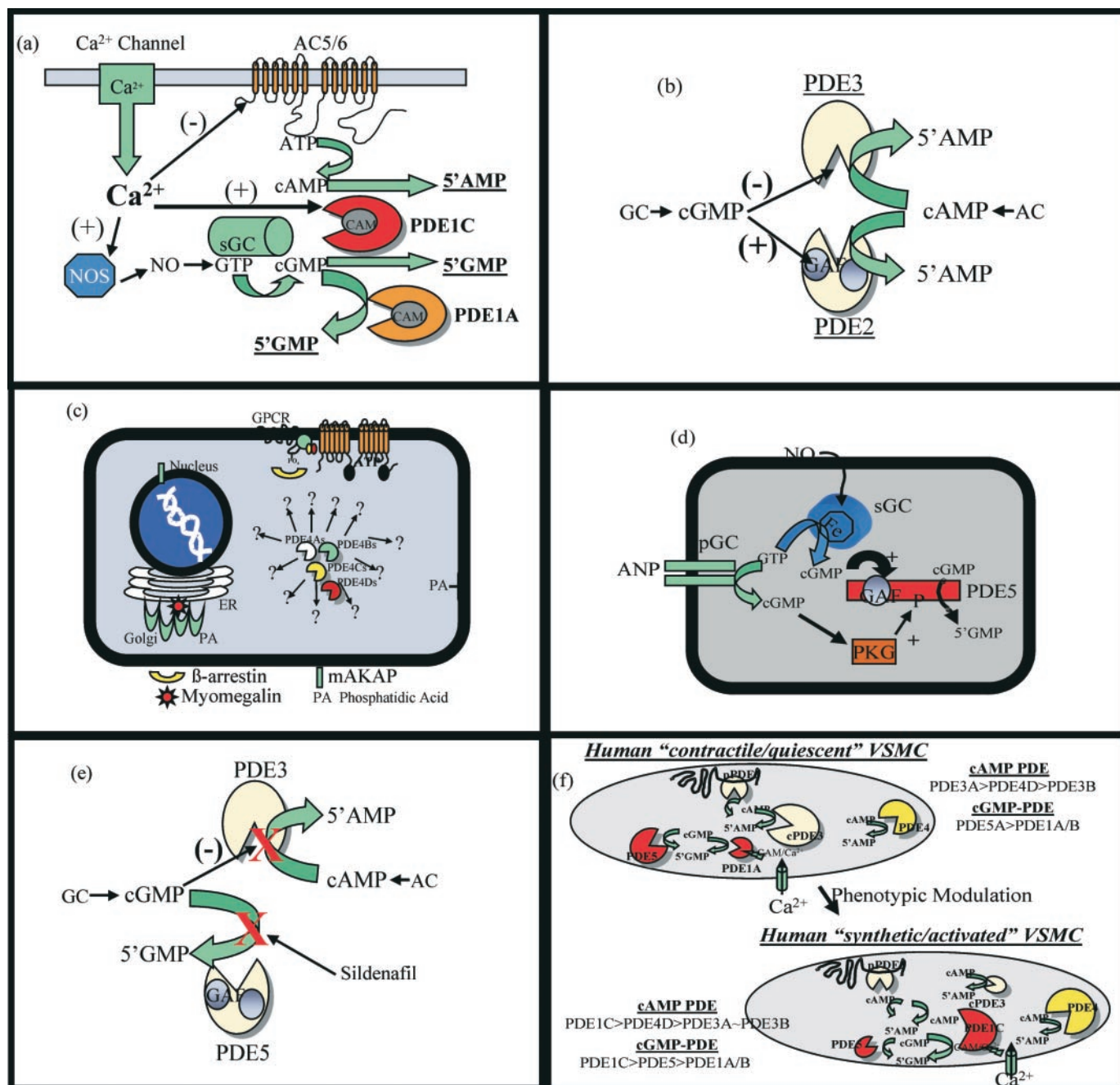
Fig. 1. a, model depicting potential role of Ca^{2+}-mediated regulation of cardiomyocyte cAMP and cGMP levels. b, scheme of the potential interplay between cGMP-mediated activation of PDE2 and inhibition of PDE3 in cells expressing both PDE family variants (cardiomyocytes, VEC). c, PDE4 variants interact with several proteins, including AKAPs, myomegalin, β-arrestins, and PA-rich membrane structures in cells. Question marks indicate that relative proportions of *PDE4* gene products in each "pool" and the mechanisms involved in targeting are poorly defined. d, PDE5 is activated by cGMP binding to regulatory GAF domains as well as by PKG-dependent phosphorylation. e, model of sildenafil-mediated indirect inhibition of PDE3 in cells. Although there is evidence for this mechanism in VSMC, no evidence supports a role for this mechanism in human cardiomyocytes. f, scheme of changes in PDE variants expressed in contractile/quiescent and synthetic/activated VSMC as related to the process of phenotypic modulation.

forts to assess the relative role of PDE1 activity in cells of the cardiovascular system, a report indicating that increased expression of PDE1A1 in rat aorta may contribute to the development of nitroglycerin tolerance underscores the importance of this enzyme in contractile/quiescent VSMC, at least in this situation (Kim et al., 2001).

Although several synthetic/activated VSMC, including those derived from human, rat, and monkey, also expressed one or both PDE1A and PDE1B variants, a marked species-dependent difference in expression of PDE1C variants was reported in these cells (Rybalkin et al., 1997; Palmer and Maurice, 2000). Thus, whereas PDE1C expression was markedly induced in VSMC cultured from several human arteries, no such induction was observed when rat, monkey, or bovine VSMC were similarly cultured (Rose et al., 1997; Liu and Maurice, 1998; Palmer and Maurice, 2000). Based on these findings, some have proposed that PDE1C induction in cultured synthetic/activated human VSMC may represent a useful marker of the phenotypic switch of these cells and that PDE1C induction may be required for proliferation of synthetic/activated VSMC (Rybalkin et al., 2002). Should a similar induction in PDE1C expression accompany the cardiovascular stress-mediated VSMC phenotypic switch in vivo in humans, selective approaches aimed at inhibiting PDE1C activity, or expression, might represent a *new* therapeutic target. Indeed, these drugs could be of use in continuing attempts to reduce VSMC proliferation in conditions such as atherosclerosis or restenosis after angioplasty. Because VSMC harvested from older more stable vascular lesions have been shown to re-establish a contractile/quiescent phenotype, a test of this concept will require the development of experimental systems in which synthetic/activated human VSMC may be harvested early during lesion formation, as is routinely done in experiments with experimental animals.

Vascular Endothelial Cells-PDE1. PDE1 activity is present in lysates of bovine and human aortic VEC. Reports indicating that VEC PDE1 enzymes catalyze the hydrolysis of both cAMP and cGMP may suggest that PDE1C is expressed in some VEC (Ashikaga et al., 1997; Keravis et al., 2000; Thompson et al., 2002). However, the PDE1 variants expressed in VEC have not yet been identified. Although PDE1 activity was reportedly lower in VEC cultured for greater numbers of passages may suggest that expression of these enzymes is regulated by changes in VEC phenotype, because PDE1 expression in VEC in blood vessels, in situ, has not been investigated, further studies will be required for the testing of this hypothesis.

Potential Physiologic and Therapeutic Implications of Differing PDE1 Activity, Expression and Targeting in Cells of the Cardiovascular System. The potential expression of PDE1C in cardiomyocytes coupled with the documented absence of this variant in contractile/quiescent VSMC may represent a potential mechanism to selectively regulate cyclic nucleotide signaling in cardiomyocytes. In addition, although PDE1C inhibitors might prove effective when used alone, their use in combination with activators of adenylyl or guanylyl cyclase variants expressed selectively in cardiovascular cell types might be expected to allow more significant cardiomyocytes-selective effects. Although PDE1C inhibitors might lack the greater range of target cells afforded to PDE3 or PDE4 inhibitors (see below), the elevation of both cAMP and cGMP that could be obtained with

PDE1C inhibitors might allow combined effects on heart rate and force of contraction and synergistic functional effects. As outlined in Fig. 1a, effects of Ca^{2+} on nitric-oxide synthase and AC5/6 variants may also influence the results of such approaches; the influence of Ca^{2+} is probably cell type-dependent.

PDE2

General Characteristics. A single *PDE2* gene encodes three PDE2 splice variants (Yang et al., 1994; Rosman et al., 1997) (Table 1). PDE2 enzymes hydrolyze either cAMP or cGMP; hydrolysis is stimulated by cGMP binding to amino terminal allosteric regulatory sites known as GAF domains (Martinez et al., 2002). A role for PDE2 in regulating cGMP-mediated effects in blood platelets (Dickinson et al., 1997), cardiomyocyte and VEC (Mery et al., 1995), and adrenal granulosa cells (Juilfs et al., 1997) have each been reported. Indeed, natriuretic peptides or organic nitrates/nitric oxide donors elevate cellular cGMP and activate PDE2 in some of these cells (Mery et al., 1995; Dickinson et al., 1997). Although few selective PDE2 inhibitors are available, cGMP-mediated activation of PDE2 is inhibited by *erythro*-9-(2-hydroxyl-3-nonyl)adenine, a potent inhibitor of adenosine deaminase.

Cardiomyocytes-PDE2. One *PDE2A* variant, PDE2A2, is expressed in cardiac tissues and in isolated cardiomyocytes of several species, including rat and human (Sadhu et al., 1999). Although direct pharmacological inhibition of PDE2 activity with *erythro*-9-(2-hydroxyl-3-nonyl)adenine increases L-type Ca^{2+} currents in cardiomyocytes, and contracts these cells, the overall magnitude of this effect is species-specific and probably influenced by several factors, including basal levels of adenylyl and guanylyl cyclases as well as the level of PDE3 activity in these cells (Vandecasteele et al., 2001). In addition to the positive inotropy resultant from pharmacological inhibition of PDE2, considerable evidence implicates cGMP-mediated activation of this enzyme, in conjunction with PDE3, in the regulation of cardiomyocyte Ca^{2+} currents and contractility (Fig. 1b).

Vascular Smooth Muscle Cells-PDE2. Although low levels of PDE2 activity have been reported in some endothelium-denuded vascular preparations and an *erythro*-9-(2-hydroxyl-3-nonyl)adenine -inhibited PDE activity was involved in hypoxic pulmonary vasoconstriction in rat lung (Haynes et al., 1996), a role for PDE2 family enzymes in the regulation of VSMC function has not generally been reported and may be suggestive of species-specific differences akin to that described for PDE1 isoform expression. In one study in which PDE2 antisera were used to study PDE2 expression in blood vessels in situ, PDE2 was expressed in some VEC, but no PDE2-selective staining was reported in VSMC (Sadhu et al., 1999).

Vascular Endothelial Cells-PDE2. Expression of PDE2 in VEC contained within sections of human cardiac and renal tissues, as well as in several human, bovine, and porcine synthetic/activated cultured arterial VEC, is consistent with a role for this enzyme in VEC (Sadhu et al., 1999). However, marked differences in the abundance of PDE2 expressed in VEC in situ and in culture have been reported (Sadhu et al., 1999). Indeed, in the most definitive study of VEC PDE2 expression published so far, capillary and venous VEC in human tissue sections were shown to express significant

PDE2, whereas arterial VEC in these sections were uniformly negative for PDE2 expression (Sadhu et al., 1999). It is not yet clear whether these differences are methodological and related to lower levels of PDE2 expression in arterial VEC in situ or related to phenotypic differences between VEC in large and small blood vessels in situ. Perhaps speaking to a potential role for phenotypic modulation of VEC in directing PDE2 expression, PDE2 levels have been reported to decrease in bovine aortic VEC with prolonged subculture. Similarly, PDE2 activity was different in bovine aortic VEC with "cobblestone" or "spindle" phenotypes (Ashikaga et al., 1997; Keravis et al., 2000; Thompson et al., 2002). Clearly, this issue will require further study.

PDE3

General Characteristics. The PDE3 family is composed of two genes, *PDE3A* and *PDE3B* (Table 1). *PDE3A* mRNA is enriched in blood vessels, heart, megakaryocytes, and oocytes (Reinhardt et al., 1995). In contrast, *PDE3B* mRNA is highest in adipocytes, hepatocytes, brain, renal collecting duct epithelium, and developing spermatocytes (Reinhardt et al., 1995). Although some tissues express both PDE3A and PDE3B, in cells expressing both these PDE3 gene products, levels of PDE3A are usually dominant (Liu and Maurice, 1998; Manganiello and Degerman, 1999). Alternate startcodon usages in *PDE3A* give rise to three different PDE3A isoforms (PDE3A1–3) (Movsesian, 2002; Wechsler et al., 2002), whereas *PDE3B* encodes a single enzyme (Movsesian, 2002; Wechsler et al., 2002). Both full-length PDE3A and PDE3B enzymes encode C-terminal catalytic domains and two N-terminal hydrophobic membrane association regions, named NHR1 and NHR2. Catalytic domains of PDE3A and PDE3B each contain 44 amino acid inserts that are unique to this PDE family and contribute to both catalytic activity and inhibitor selectivity of these enzymes (Manganiello and Degerman, 1999). For PDE3A, different variants are predicted to contain one, or both, N-terminal hydrophobic regions. Thus, whereas PDE3A1 is predicted to express both NHR1 and NHR2, PDE3A2 is predicted to yield two variants, one expressing NHR2 and the other not. PDE3A3 is predicted to encode neither NHR1 nor NHR2 but to be derived from a third transcription initiation site (Movsesian, 2002; Wechsler et al., 2002). Consistent with these predictions, heterologous expression of PDE3A1 yields an entirely particulate protein, whereas PDE3A2 is both particulate and soluble. Consistent with its structure, expression of PDE3A3 yields an exclusively cytosolic enzyme (Wechsler et al., 2002). PDE3A and PDE3B are activated by PKA or PKB phosphorylation and consensus sites of phosphorylation for each kinase are located between NHR1 and NHR2 in both PDE3A and PDE3B (Manganiello and Degerman, 1999). In addition to being subject to phosphorylation-induced activation, PDE3 variants are also directly inhibited by cGMP-mediated competition for cAMP binding to the active site. For this reason, PDE3 was also referred to as cGMP-inhibited cAMP PDE in earlier literature. This effect of cGMP can represent a significant mode of regulation of PDE3A in cells expressing this enzyme (Manganiello and Degerman, 1999). For example, incubation of PDE3A-expressing cells with guanylyl cyclase-activating organic nitrates, nitric oxide donors, or natriuretic peptides, results in a significant increase in *both* cGMP and cAMP and a synergistic increase in cAMP when these agents are combined with activators of adenylyl cyclases (Maurice and Haslam, 1990a,b; Maurice et al., 1991). Because PDE3B is only ~10% as sensitive to cGMP inhibition, incubation of adipocytes, a cell type expressing only PDE3B, with nitrate donors, does not increase adipocyte cAMP (Manganiello and Degerman, 1999). In addition to these short-term regulatory effects, levels of PDE3 expression are increased after incubation of cells with cAMP-elevating agents, although the magnitude of this effect is PDE3 variant- and cell type-specific (Manganiello and Degerman, 1999; Shakur et al., 2001). Historically, PDE3 enzymes have garnered the most sustained interest as therapeutic targets in the CV system (Beavo and Reifsnyder, 1990; Manganiello and Degerman, 1999; Shakur et al., 2001), and a very large number of PDE3-family selective inhibitors exist, including milrinone, amrinone, cilostamide, and cilostazol (Table 1). To date, no PDE3-selective inhibitors differentiate between *PDE3A*- and *PDE3B*-derived enzymes.

Cardiomyocytes-PDE3. PDE3 activity is abundant in cardiac tissues and in cardiomyocytes from several species, including human, rat, guinea pig, and pig (Movsesian, 2002; Wechsler et al., 2002). Consistent with this, three distinct anti-PDE3A-immunoreactive proteins are expressed in human, canine, rabbit, and guinea pig cardiac tissues and in human cardiomyocytes (Movsesian, 2002; Wechsler et al., 2002). These proteins include an exclusively particulate protein (PDE3A-136 kDa) and two others that are both particulate and cytosolic (PDE3A-118 kDa and PDE3A-94 kDA). Expression studies are consistent; PDE3A1 mRNA yields the PDE3A-136-kDa variant, whereas PDE3A2 mRNA is thought to yield both PDE3A-118-kDa and -94-kDa proteins. Because of their transcription initiation sites, PDE3A-118-kDa and -94-kDA enzymes are predicted to not encode PKB phosphorylation sites and, as such, to not be subject to PKB-mediated regulation (Wechsler et al., 2002). This supposition has not yet been tested formally in cells expressing these enzymes.

Direct pharmacological inhibition of PDE3 activity increases L-type Ca^{2+} currents in cardiomyocytes isolated from human, rat, and frog hearts, an effect that contributes to the positive inotropic effects of these inhibitors (Verde et al., 1999; Vandecasteele et al., 2001). In addition, considerable evidence implicates this enzyme, in concert with PDE2, in mediating the effects of cGMP on cardiomyocyte Ca^{2+} currents and contractions (Fig. 1b). Thus, whereas cGMP-mediated inhibition of PDE3 allows nitrate donors or atrial natriuretic peptides to increase cardiomyocyte cAMP levels and Ca^{2+} currents, cGMP-mediated activation of PDE2 acts to limit the cGMP-mediated effect at PDE3 (Haynes et al., 1996; Vandecasteele et al., 2001) (Fig. 1b). Species differences in the dominance of PDE2 or PDE3 in regulating cGMP-mediated effects on cardiomyocyte Ca^{2+} currents and contractility have been reported (Haynes et al., 1996; Vandecasteele et al., 2001). It is likely that these differences are dependent on several factors, including basal and stimulated levels of guanylyl cyclase and adenylyl cyclase activities, as well as the abundance and intracellular compartmentation of PDE2, PDE3, and PKA in these cells. Because of their positive inotropic effects, PDE3 inhibitors were tested for their potential value in treating heart failure (Packer et al., 1991; Movsesian, 2000). Although the short-term hemodynamic benefits of this class of drugs were impressive, their effects

were lost at later times and prolonged use was positively correlated with increased treatment-associated mortality (Packer et al., 1991; Movsesian, 2000). Although the molecular basis for this temporally biphasic effect of PDE3 inhibitors has not yet been resolved, results with β-adrenoceptor agonists such as dobutamine strongly implicate a role for cAMP. Recognizing that cAMP signaling is spatially restricted in most cells, including cardiomyocytes, some have proposed that selectively increasing certain "pools" of cardiomyocyte cAMP, rather than total cellular cAMP, might be more effective (Movsesian, 2002). This concept is consistent with the differential effects of agents acting through activation of adenylyl cyclase. Thus, although β-adrenergic agonists, such as isoproterenol, increase cardiomyocyte membrane-associated cAMP and PKA activity and have positive inotropic effects, other activators of cardiomyocyte adenylyl cyclase activity, such as prostaglandin E_1, fail to increase membrane-associated cAMP levels and have only weak inotropic effects (Buxton and Brunton, 1985; Movsesian, 2000). Although data indicating that PDE3A1 and PDE3A2 are expressed in distinct subcellular domains in human cardiomyocytes may imply that selective cAMP pools could be regulated by directly targeting one of these enzymes (Wechsler et al., 2002), no pharmacologic inhibitors have thus far been generated that distinguish among the many different PDE3 variants. As such, a formal test of this idea must necessarily await the development of more selective tools.

Mechanisms involved in regulating PDE3A-136 kDa, PDE3A-118 kDa, and PDE3B membrane association have not been identified. Although it is clear that NHR1 allows stable membrane association of proteins encoding this domain, the role for NHR2 is less certain (Manganiello and Degerman, 1999; Shakur et al., 2000). Clearly, further work directed at assessing the potential for NHR2-dependent protein-protein interactions in regulating PDE3A2 membrane association is warranted.

Vascular Smooth Muscle Cells-PDE3. Several PDE3 variants are expressed in human, pig, and rat contractile/quiescent and synthetic/activated VSMC, and evidence is consistent with expression of both *PDE3A* and *PDE3B* gene products in both VSMC phenotypes (Polson and Strada, 1996; Rose et al., 1997; Liu and Maurice, 1998; Manganiello and Degerman, 1999; Palmer and Maurice, 2000; Choi et al., 2001; Dunkerley et al., 2002). Thus, a ~118-kDa PDE3A variant, probably PDE3A2, is detected in cytosolic (human, rat) and particulate (human) fractions of contractile/quiescent and "activated/synthetic" aortic VSMC, and a ~135-kDa PDE3B variant is present in particulate fractions of both these VSMC phenotypic variants in both rat and human (Liu and Maurice, 1998; Palmer and Maurice, 2000). Although prolonged nitrate administration to rats in vivo was associated with increased aortic contractile/quiescent VSMC PDE1A1 expression (Kim et al., 2001), prolonged treatments with cAMP-elevating agents in vivo increased both PDE3A2 and PDE3B in rat aortic and femoral artery contractile/quiescent VSMC through a mechanism involving de novo mRNA and protein synthesis (Tilley and Maurice, 2002). In contrast to these in vivo effects, prolonged cAMP elevation increased PDE3B in cultured synthetic/activated rat and human aortic VSMC but had no effect on PDE3A (Liu and Maurice, 1998; Palmer and Maurice, 2000). The molecular basis and potential significance for the differential induction of *PDE3B* and

PDE3A by cAMP-elevating agents in contractile/quiescent and synthetic/activated VSMC in vitro are not yet clear but again might form the basis for selected regulation of these two PDE3 populations.

In addition to their positive inotropic effects in heart, PDE3 inhibitors also relax isolated arterial and venous tissues, dilate blood vessels in vivo, inhibit VSMC proliferation in vitro, and limit accumulation of neointimal VSMC in arteries after in vivo vascular damage (Schoeffter et al., 1987; Lindgren et al., 1989; Maurice et al., 1991; Souness et al., 1992; Pan et al., 1994; Palmer et al., 1998; Manganiello and Degerman, 1999; Inoue et al., 2000; Netherton et al., 2002). Although it is likely that VSMC PDE3A and PDE3B variants differentially control some of these effects, the lack of pharmacological agents capable of differentially inhibiting PDE3A and PDE3B has hindered an assessment of the potential of this phenomenon. Of potential note, we recently identified a hypermigratory phenotype in synthetic/activated VSMC isolated from a leptin receptor-deficient model of type II diabetes that results, at least in part, from increased PDE3B activity in these cells (Netherton et al., 2002). In this context, leptin-mediated, leptin receptor-dependent activation of PDE3B in several cells has been reported (Zhao et al., 1998, 2002), although these data do not clarify why the absence of leptin receptors in VSMC in our model would associate with increased PDE3B.

In addition to the above effects attributed to direct pharmacological inhibition of PDE3, earlier work identified an important role for PDE3 in coordinating and amplifying the vasodilatory effects of cAMP- and cGMP-elevating agents (Maurice and Haslam, 1990b; Maurice et al., 1991; Jang et al., 1993; Eckly and Lugnier, 1994). Thus, cGMP inhibition of cAMP hydrolysis by PDE3 allowed guanylyl cyclase-dependent vasodilators, such as NO-releasing drugs or ANP, to increase levels of contractile/quiescent VSMC cAMP and to synergize with VSMC adenylyl cyclase activating agents, such as prostaglandin I_2 or isoproterenol. Although this mechanism is identical to that discussed previously for cardiomyocytes, the absence of PDE2 in VSMC predicts that cGMP-mediated inhibition of PDE3 should dominate in these cells and that the biphasic effect attributed to cGMP-mediated PDE2 activation would not be seen in VSMC (Fig. 1b). Several reports demonstrating that cGMP can regulate VSMC functions through inhibition of PDE3 have been published (Maurice and Haslam, 1990b; Maurice et al., 1991; Jang et al., 1993; Eckly and Lugnier, 1994; Osinski and Schror, 2000; Osinski et al., 2001). For example, we reported previously that nitrovasodilators, such as nitroprusside or 3-morpholinylsydnoneimine, potentiated the relaxant effects of isoproterenol in rat aorta and that this effect occurred through a cGMP-mediated inhibition of PDE3 in rat aortic VSMC (Maurice and Haslam, 1990b). A similar effect is suggested to allow inhibitors of PDE5 to synergize with activators of adenylyl and inhibit proliferation of synthetic/activated VSMC (Osinski and Schror, 2000; Osinski et al., 2001).

Although VSMC expressing either contractile/quiescent or synthetic/activated phenotypes express similar levels of PDE3B, we reported previously that expression of PDE3A in contractile/quiescent and synthetic/activated VSMC was markedly different (Liu and Maurice, 1998; Palmer and Maurice, 2000; Dunkerley et al., 2002). Thus, although PDE3 activity represented $\geq$60% of cAMP PDE activity in rat or

human aortic contractile/quiescent VSMC, PDE3 activity and PDE3A expression were markedly reduced in synthetic/activated VSMC of both species (Liu and Maurice, 1998; Dunkerley et al., 2002). Attesting to the potential pathophysiological relevance of this observation, a similar reduction in PDE3 activity and PDE3A expression was observed in neointimal synthetic/activated VSMC when this tissue was harvested from damaged rat aorta (Dunkerley et al., 2002). Because PDE3 inhibitors, such as cilostamide and cilostazol, inhibit proliferation of synthetic/activated VSMC and markedly reduce the accumulation of synthetic/activated VSMC in the intimal layer of damaged blood vessels, it may be that these agents are acting via inhibition of VSMC PDE3B in these cells or perhaps are able to inhibit indirectly the proliferation and migration of synthetic/activated VSMC through reductions in aggregation of blood platelets, a cell type that expresses PDE3A and whose participation in vascular lesion-mediated events is reduced by PDE3 inhibitors (Maurice and Haslam, 1990a,b; Maurice et al., 1991; Jang et al., 1993; Eckly and Lugnier, 1994). Further experiments to delineate the role of platelet PDE3A inhibition in the in vivo effects of PDE3 inhibitors in this context would address this issue.

Vascular Endothelial Cells-PDE3. Although PDE3 inhibitors reduce VEC proliferation and expression of adhesion molecules on VEC, little is known concerning the PDE3 variants expressed in these cells (Lugnier and Schini, 1990; Suttorp et al., 1993; Blease et al., 1998). Indeed, although PDE3 activity was reportedly altered during culture of bovine aortic VEC, and PDE3 activity in bovine aortic VEC with "cobblestone" and "spindly" phenotypes were reported to differ (Keravis et al., 2000), the PDE3 variants involved in these situations are not known.

PDE4

General Characteristics. Four *PDE4* genes (*PDE4A—D*; Table 1) yield a large number of distinct PDE4 variants. These enzymes, which result from the use of alternate promoters and extensive splicing of PDE4 mRNAs, are stratified into long or short forms (Conti et al., 2003; Houslay and Adams, 2003). Whereas long PDE4 variants contain two amino-terminal conserved regions called upstream conserved regions 1 (UCR1) and UCR2 in addition to splice-specific amino termini, short PDE4 isoforms lack a complete UCR1 domain and can have a truncated UCR2 domain. PDE4 variants are expressed in almost all cell types, except blood platelets. Selected PDE4 variants localize to subcellular domains through interaction with various scaffolding/anchoring structures, including 1) proline-rich sequence-binding (SH3) proteins, 2) A-kinase–anchoring proteins (AKAPs), 3) receptor for activated c kinase (RACK1), 4) β-arrestins 1 and 2, or 5) phosphatidic acid-rich regions of cellular membranes. These interactions are thought to play a dominant role in the regulation of cAMP signaling by these enzymes (for review, see Conti et al., 2003; Houslay and Adams, 2003) (Fig. 1c). PKA-dependent phosphorylation selectively activates many long PDE4 isoforms (Houslay and Adams, 2003; Conti et al., 2003), whereas long and short forms of PDE4 are differentially regulated by ERK-mediated phosphorylation of a conserved catalytic domain serine residue (Conti et al., 2003; Houslay and Adams, 2003). Indeed, although ERK-mediated phosphorylation can inhibit long PDE4 variants, phosphory-

lation of short PDE4 variants results in a slight activation of these enzymes. Interestingly, because ERK-mediated phosphorylation of PDE4 long forms inhibits the hydrolysis of cAMP by these enzymes, under some conditions, the inhibitory effect can be readily overturned by cAMP-mediated activation of PKA and its phosphorylation of PDE4 (Baillie et al., 2001). The ERK-mediated phosphorylation of PDE4, and the overall impact of this event on cellular PDE4 activity, will probably be defined by the nature of the PDE4 involved, as well as the subcellular domain in which the PDE4 variant is expressed. The dynamic regulation of PDE4D3 and PDE4D5 variants through these mechanisms is important in cardiomyocytes and VSMC (Liu and Maurice, 1999a; Liu et al., 2000; Baillie et al., 2001). Myriad hormones, drugs, and cytokines alter levels of PDE4 expression in several cell types, although the *PDE4* genes altered are highly cell-type specific (Houslay et al., 1998). Several PDE4-selective inhibitors have been developed for use in the treatment of immune and inflammatory conditions. For example, cilomilast (Ariflo), a new oral PDE4 inhibitor, is in the final stages of development for use in the treatment of chronic obstructive pulmonary disease and asthma (Giembycz, 2001), an indication consistent with the altered airway responsiveness associated with the *PDE4D* gene null genotype in mice (Hansen et al., 2000).

Cardiomyocytes-PDE4. One *PDE4A*, three *PDE4B* (PDE4B1, PDE4B2, and PDE4B3), and three *PDE4D* (PDE4D1, PDE4D2 and PDE4D3) variants are expressed in rat and human cardiac tissues (Kostic et al., 1997; Baillie et al., 2001; Houslay and Adams, 2003). Although selective pharmacological PDE4 inhibition increased cardiomyocyte cAMP, slightly increased cardiomyocyte Ca^{2+} currents, and promoted cardiac contractility in certain species (Mery et al., 1995; Verde et al., 1999; Abi-Gerges et al., 2000; Vandecasteele et al., 2001), virtually nothing is known about the contribution of individual PDE4 variants to these effects. Notwithstanding that the roles of PDE4 enzymes in controlling cardiomyocyte functions are not clearly defined, regulated targeting of one PDE4 variant, PDE4D3, has been studied in cardiomyocytes. Indeed, cardiomyocyte PDE4D3 has been shown to associate with proteins involved in anchoring components of cAMP signaling in cells. Thus, PDE4D3 association with mAKAP, a striated muscle-specific AKAP scaffold to nuclear membranes, promoted more efficient control of PKA-mediated phosphorylation of several proteins, including PDE4D3 itself, in cardiomyocytes and in the L6 cell line (Dodge et al., 2001) (Fig. 1c). Because hypertrophic stimuli increase cardiomyocyte mAKAP expression, a role for increased efficiency in PDE4D3 regulation after redistribution of this enzyme to mAKAP under similar conditions in vivo has been proposed but has not yet been tested formally. Indeed, further studies should clarify whether the PDE4D3 interaction with mAKAP is specific to that PDE4 variant and whether other cardiomyocyte PDE4 variants associate with the large number of distinct AKAP expressed in cardiac tissue. It is worth considering, however, that genetic evidence (*PDE4D*- and *PDE4B*-null mice) to date does not reveal a significant role for PDE4 activity in regulated cardiac function per se. Several PDE4 variants were recently shown to associate with β-arrestins 1 and 2. In these studies, β-arrestin protein binding to phosphorylated G protein-coupled receptors was shown to allow recruitment of PDE4 vari-

ants to the membrane and the participation of these PDEs in desensitization of cAMP-signaling (Perry et al., 2002; Houslay and Adams, 2003) (Fig. 1c). Because these studies showed that both short and long variants of PDE4A, PDE4B, and PDE4D could interact with β-arrestins 1 and 2 and that each β-arrestin is known to interact with multiple G protein-coupled receptors, the contribution of this mechanism to regulated cAMP- and non–cAMP-dependent cellular signaling will require further study. Very recently, a role for the interaction between PDE4D3 and PDE4D5 and β-arrestin in mediating the relative coupling of β_2-adrenergic receptors with G_s or G_i in cardiomyocytes was reported (Baillie et al., 2003). Clearly, these findings imply that PDE4 targeting plays a central role in G-protein coupling beyond its role in general cAMP catabolism.

Vascular Smooth Muscle Cells-PDE4. Two *PDE4D* gene-derived variants, PDE4D3 and PDE4D5, are expressed in human and rat aortic, mesenteric, and femoral contractile/quiescent and synthetic/activated VSMC (Liu and Maurice, 1999a; Liu et al., 2000). Whereas PDE4D3 in human or rat aortic VSMC was predominately cytosolic, PDE4D5 was almost exclusively particulate in these cells. Whether any of the PDE4 targeting systems described above are involved in selectively localizing PDE4 variants in VSMC remains unknown. However, given that PDE4D3 and PDE4D5 are located in different subcellular fractions in VSMC and that increases in PDE4 activity and expression are also important in the heterologous desensitization to cAMP signaling in these cells (Rose et al., 1997), it may be reasonably assumed that they will be important in VSMC as well.

In experiments in which levels of PDE4 activity between contractile/quiescent and synthetic/activated VSMC were compared, marked differences were noted. Thus, although PDE4 activity represented ~35% of cAMP PDE activity in contractile/quiescent rat aortic VSMC, ~75% of cAMP PDE activity was attributable to PDE4 in synthetic/activated rat aortic VSMC (Dunkerley et al., 2002; Rybalkin et al., 2002). The increased percentage of PDE4 activity in synthetic/activated VSMC was attributed solely to a phenotype-dependent reduction in PDE3A expression in synthetic/activated VSMC. Similar phenotype-based reductions in PDE3A expression in human aortic VSMC also increased the fraction of PDE4 activity in these cells; however, because of the large induction of PDE1C expression in synthetic/activated human VSMC, this effect was more modest in human VSMC (Palmer and Maurice, 2000). Incubation of rat or human VSMC with cAMP-elevating agents caused time- and concentration-dependent increases in PDE4 activity and expression (Liu and Maurice, 1999b; Liu et al., 2000; Palmer and Maurice, 2000; Tilley and Maurice, 2002). However, as with PDE3, significant differences were observed between response of contractile/quiescent and synthetic/activated VSMC to such treatments (Liu and Maurice, 1999b; Liu et al., 2000; Tilley and Maurice, 2002). During short-term treatments (2–30 min) of contractile/quiescent rat aortic or femoral artery VSMC with cAMP-elevating agents, PKA-dependent phosphorylation of PDE4D3 and of PDE4D5 activated these enzymes. Longer treatment periods (1–4 h) of these cells with cAMP-elevating agents increased expression of PDE4D3 but did not cause induction of short PDE4D variants (PDE4D1, PDE4D2) (Tilley and Maurice, 2002). Although similar treatments of synthetic/activated VSMC also caused the acute PKA-medi-

ated, phosphorylation-dependent activation of PDE4D3 and PDE4D5 activity in these cells, longer-term treatments of synthetic/activated VSMC resulted in the marked induction of PDE4D1 and PDE4D2 with no change in PDE4D3 or PDE4D5 levels (Liu and Maurice, 1999a; Liu et al., 2000). The molecular basis of this difference in response to prolonged increases in cAMP in contractile/quiescent and synthetic/activated VSMC is not known but presumably involves differential access of the intronic promoter controlling PDE4D short-form expression in these cells.

In contrast to the effects of PDE3 inhibitors on VSMC contractions and proliferation, PDE4 inhibitors are generally poor vasodilators of contractile/quiescent VMSC in vitro and do not inhibit VSMC proliferation (reviewed in Polson and Strada, 1996). However, when PDE4-selective inhibitors are used with activators of adenylyl cyclase, with PDE3 inhibitors, or in experiments in which the vessels maintain an intact endothelial lining, PDE4 inhibitors are efficient vasorelaxants of contractile/quiescent VSMC and inhibit synthetic/activated VSMC proliferation (Maurice et al., 1991; Jang et al., 1993; Eckly and Lugnier, 1994; Polson and Strada, 1996). These characteristics of PDE4 inhibitors are consistent with the concepts that 1) endothelial NO regulates PDE3 activity in contractile/quiescent VSMC presumably through a NO-cGMP-inhibitory dependent mechanism, and 2) PDE3 and PDE4 regulate distinct, although overlapping, cAMP pools in VSMC. Similar findings concerning the roles of PDE3 and PDE4 activities in mesangial cells may imply that this represents a more general phenomenon (Chini et al., 1997).

In addition to cAMP-elevating agents, those that activate mitogen-activated protein kinase signaling, more specifically the protein kinase C-MEK-ERK signaling cascade, also regulate PDE4D activity and expression in VSMC, although perhaps in a cell type-specific fashion (Liu and Maurice, 1999a; Liu et al., 2000; Baillie et al., 2001). Although phorbol ester- or angiotensin II-treatment of synthetic/activated rat aortic VSMC resulted in an ERK-dependent activation of the particulate PDE4D3 fraction in these cells, this treatment did not alter the phosphorylation level or activity of the cytosolic fraction of PDE4D3 in these cells. Although similar studies in synthetic/activated human aortic VSMC demonstrated that an ERK-mediated feedback system allowing activation of PKA accounted for a PMA-mediated activation of this PDE4D5 in these cells (Baillie et al., 2001), the role of this system on the effects in rat aortic VSMC remains to be assessed (Liu and Maurice, 1999a; Liu et al., 2000). Although the ERK-mediated, PKA-dependent, activation of PDE4D5 in synthetic/activated human VSMC did not alter the anchoring characteristics of this enzyme, simultaneous incubation of synthetic/activated rat aortic VSMC with ERK-activating and cAMP-elevating agents resulted in the translocation of particulate PDE4D3 to the cytosol in these cells. In addition to these complex effects on the activation state of PDE4D in VSMC, ERK-activating agents also regulate *PDE4D* expression in synthetic/activated rat VSMC. Thus, agents capable of activating the protein kinase C-MEK-ERK-signaling axis blunted cAMP-dependent increases in PDE4D1 and PDE4D2 in these cells (Liu et al., 2000). Although the molecular basis of this effect was related to destabilization of *PDE4D* mRNA and required induction of protein synthesis, the protein(s) involved have not yet been identified. To date, the role of this

post-translational regulatory mechanism of *PDE4D* expression has not been investigated in human VSMC.

Vascular Endothelial Cells-PDE4. As with PDE3 inhibition, PDE4 inhibitors reduce VEC proliferation and the expression of adhesion molecules in these cells (Lugnier and Schini, 1990; Suttorp et al., 1993; Blease et al., 1998). In addition, PDE4 inhibitory drugs reduced VEC permeability, as determined by in vitro assays (Lugnier and Schini, 1990; Suttorp et al., 1993; Blease et al., 1998) or in vivo using the chorioallantoic membrane of fertilized avian eggs (Defouw and Defouw, 2001). The PDE4 variants expressed in VEC, and their relative role(s), remain unknown.

Potential Physiologic and Therapeutic Implications of Alterations in PDE3 and PDE4 Activity, Expression, and Targeting in Cells of the Cardiovascular System. Although both PDE3 and PDE4 inhibitors have demonstrable pharmacological effects in each of the cardiovascular cell types discussed in this review, and PDE3 inhibitors have been used successfully in several cardiovascular conditions, no PDE4-selective agent has yet been tested for cardiovascular indications. In addition to their potential effects when used alone, the synergistic effects of simultaneous inhibition of PDE4 and PDE3 activities using selective agents discussed in the preceding sections may hint at an untapped potential for dual PDE3/4-inhibiton in certain situations. Ideally, under this paradigm, toxicities attributed to PDE3 inhibition might be lessened by combining lower doses of PDE3 inhibitors with PDE4 inhibitors or by the use of single molecules processing dual PDE3/PDE4 inhibitory activity, such as the inhibitor zardaverine (Table 1).

PDE5

General Characteristics. Specific cGMP hydrolysis in many cells is carried out by variants of the PDE5 family, and, like PDE2, PDE5 have GAF domains (McAllister-Lucas et al., 1995). Although cGMP binding has been reported not to directly activate PDE5 but rather to facilitate activation by PKG- or PKA-mediated phosphorylation, very recent data indicate that cGMP binding, without PKG-mediated phosphorylation of this enzyme, may also activate this enzyme (Rybalkin et al., 2003) (Fig. 1d). The PDE5 family consists of a single *PDE5* gene that can encode three distinct proteins (PDE5A1–3) (McAllister-Lucas et al., 1995; Loughney et al., 1998). Recent evidence suggests that an inhibitory subunit, typically associated with PDE6 isoforms, may also regulate PDE5 activity, perhaps through proteolysis by caspases or simply through inhibition of the activation of PDE5 enzymes (Francis et al., 2001). PDE5 is expressed in several tissues, including brain, lung, platelets, vascular and visceral smooth muscle, and kidney. Very little information is available concerning the differential targeting of PDE5 variants in cells or the potential consequences of these events (McAllister-Lucas et al., 1995; Loughney et al., 1998; Wallis et al., 1999; Francis et al., 2001; Giordano et al., 2001; Senzaki et al., 2001; Rybalkin et al., 2003). Adopting the paradigm elaborated above for PDE4D variants, one might predict that PDE5 could be found in association with proteins involved in coordinating PKG-targeting, such as the IP$_3$ receptor I, IRAG, cGMP kinase Iβ complex (Ammendola et al., 2001). Therapeutic strategies have investigated the possibility of inhibiting PDE5 in vascular, thrombotic, or pulmonary disorders (Francis et al., 2001; Corbin and Francis, 2002). In particu-

lar, the success of sildenafil (Viagra) (Francis et al., 2001; Corbin and Francis, 2002), a selective PDE5 inhibitor, in the treatment of male erectile dysfunction (ED) has validated these efforts and further increased the interest in this approach. Indeed, several other PDE5-selective inhibitors should soon become available for several indications.

Cardiomyocytes-PDE5. Although a *PDE5A* variant, PDE5A1, was detected in human, rat, and dog cardiac tissues, and the presence of an abundant anti-PDE5A immunoreactive protein has been reported in experiments with isolated canine cardiomyocytes, convincing evidence of PDE5 expression in human cardiomyocytes is presently lacking (McAllister-Lucas et al., 1995; Loughney et al., 1998; Wallis et al., 1999; Giordano et al., 2001; Senzaki et al., 2001; Rybalkin et al., 2003). What is certain, however, is that *if* PDE5 is expressed in human cardiomyocytes, the impact of its inhibition by selective PDE5 inhibitors such as sildenafil, vardenafil (Levitra), or tadalafil (Cialis) on cardiac function is probably modest (Arruda-Olson et al., 2002). Indeed, an extensive literature dealing with the issue of cardiac effects of these potent and selective PDE5 inhibitors has consistently reported few, if any, direct effects of these agents on indices of cardiac function (Arruda-Olson et al., 2002). However, given the potential ramifications of PDE5 expression in human cardiomyocytes, (Fig. 1e), it is likely that this issue will receive further consideration.

Vascular Smooth Muscle Cells-PDE5. Two *PDE5* variants, PDE5A1 and PDE5A2, are expressed in rat, bovine, and human contractile/quiescent VSMC (Rybalkin et al., 1997, 2002; Murray et al., 2002). Until recently, the absence of highly potent and sufficiently selective inhibitors had made an analysis of PDE5 in these cells difficult. However, because of the recent introduction of the above-listed PDE5 inhibitors, the impact of PDE5 inhibition on blood vessel function has been revisited. In this context, sildenafil and the other selective PDE5 inhibitors potently relax several arterial contractile/quiescent VSMC, in addition to the smooth muscle of the corpus cavernosum (Corbin and Francis, 2002). In addition to ED, PDE5 has been recognized to be a valid therapeutic target for use in the treatment of pulmonary hypertension, a disorder with limited treatment options and a poor outcome (Michelakis et al., 2002). Because the NO-cGMP signaling-axis mediates normal pulmonary vascular tone and pulmonary hypertension was shown to associate with reduced vascular reactivity to NO-dependent vasodilators, PDE5 inhibitors were predicted to be effective (Michelakis et al., 2002; Murray et al., 2002). In this context, several case reports and investigational studies have shown that dipyridamole or zaprinast, two PDE5 inhibitors with limited selectivity, and sildenafil selectively dilated the pulmonary vasculature in experiments with both humans and rats. Indeed, in a small number of clinical trials, sildenafil augmented pulmonary vasodilator effects of inhaled NO, prevented rebound pulmonary hypertension after cessation of NO inhalation, attenuated hypoxia-induced pulmonary hypertension, and selectively decreased pulmonary versus systemic vascular resistance (reviewed in Galie et al., 2002). At a molecular level, these effects of PDE5 inhibition are consistent with increased PDE5 activity during hypoxia in several animal models of pulmonary hypertension and may imply that the therapeutic value of PDE5 inhibitors in this condition is related to an underlying role for increased PDE5 expression

in pulmonary hypertension (Murray et al., 2002). This contrasts with ED, in which reduced NO-mediated guanylyl cyclase activation is usually thought of as the dominant effect.

Although PDE5 in contractile/quiescent and synthetic/activated VSMC of rat and murine aorta are activated by PKG-mediated phosphorylation, recent reports indicate that activation of this enzyme may occur upon cGMP binding, without the need for PKG-mediated phosphorylation (Rybalkin et al., 2003). In addition to these short-term effects, our unpublished data indicate that in rat vena cava VSMC, PDE5 is increased in animals treated for prolonged periods with NO-releasing agents, whereas the level of this enzyme in aortic VSMC was unaltered by this treatment paradigm (H. A. Dunkerley, D. H. Maurice, and B. Bennett, unpublished data). How these data relate to the development of tolerance to nitroglycerin, and previous reports of elevated levels of PDE1A1 in aortae of tolerant animals, will require further analysis. Relatively little is known about PDE5 activity and expression in synthetic/activated VSMC. Thus, although PDE5A1 and PDE5A2 are each expressed in synthetic/activated VSMC of human and rat and these enzymes are activated by PKG-mediated phosphorylation (Rybalkin et al., 1997, 2002, 2003), little is known of their influence on synthetic/activated VSMC functions. In one report, selective inhibition of PDE5 activity in cultured synthetic/activated bovine coronary artery VSMC with sildenafil significantly increased cGMP and cAMP, activated PKA, and inhibited proliferation and migration of these cells (Osinski and Schror, 2000; Osinski et al., 2001). Consistent with an important role for a mechanism involving cGMP-mediated inhibition of PDE3 in this effect, direct activation with cGMP analogs that did not inhibit PDE3 were without effect. Whether PDE5 inhibitors affect VSMC function independent of the indirect effect on PDE3 will require further study.

Vascular Endothelial Cells-PDE5. As described in the previous sections, very little is known concerning PDE5 activity and expression in VEC. Given the importance of NO-mediated regulation of blood vessel function, it is clear that this area requires more study.

Potential Physiologic and Therapeutic Implications of Alterations in PDE2, PDE3, and PDE5 Activity, Expression and Targeting in Cells of the Cardiovascular System. The different combinations of PDE2, PDE3 and PDE5 expression in cardiomyocytes versus VSMC may allow for selective effects in these cells. Thus, PDE5 inhibitors may integrate cGMP signals selectively in VSMC, although they may not influence cGMP signaling in cardiomyocytes. Indeed, there is little evidence to suggest that PDE5 inhibitors significantly alter indexes of cardiac function, although PDE5 inhibitor-mediated effects in VSMC attributed both to PKG activation and PDE3 inhibition have been reported. As outlined in the PDE5 section of this review, this characteristic of PDE5 inhibition represents a significant opportunity in situations in which cGMP catabolism is limiting, such as ED and perhaps pulmonary hypertension. In addition, as described above, binding of cGMP to PDE2 and PDE5 activates these enzymes, whereas cGMP-mediated competitive inhibition of cAMP binding to PDE3 results in a cGMP-mediated inhibition of this enzyme. As such, PDE inhibition in cells expressing different combinations of these enzymes will probably be complex and depend on several factors, including levels of adenylyl cyclase and guanylyl cyclase activities. In human platelets and cardiomyocytes, cell types expressing both PDE2 and PDE3 (see *PDE2* and *PDE3*, above), potent stimulation of guanylyl cyclase could blunt effects of PDE3 inhibitors in these cells (e.g., Dickinson et al., 1997). Several interesting, as-yet untested hypotheses flow from these ideas. First, because NO-mediated increases in cardiomyocyte cGMP would be expected to activate PDE2 in these cells, cGMP-mediated activation of PDE2 may allow pharmacological PDE3 inhibitors to selectively increase cAMP in cardiomyocytes present in regions of the myocardium in which NO or natriuretic peptide-mediated activation of guanylyl cyclase is low (i.e., endothelial cell damaged). Similarly, because of NO-dependent, cGMP-mediated inhibition of PDE3 in VSMC, PDE3 inhibitors may act selectively on VSMC in regions in which endothelial NO release is reduced, or inhibited, but may be less effective in regions of the vasculature in which endothelial layer-mediated release of NO is normal. Similarly, for the same reason, PDE5 inhibition-mediated increases in cGMP may selectively alter VSMC PDE3-mediated cAMP hydrolysis in regions of the vasculature in which NO release is normal or elevated but not where it is low. Atherosclerosis and other conditions that affect VEC release of prostacyclin and NO may alter cardiovascular cell reactivity to PDE inhibitors because of these regioselective events. Indeed, because hormone-mediated release of prostacyclin and NO is often coupled (de Nucci et al., 1988), PDE3 may operate as an integrator of these short-lived substances in a physiological setting.

PDE7–11

As a result of bioinformatics-based genomic screening, several novel *PDE* gene family variants (PDE 7–11) have been identified (reviewed in Soderling and Beavo, 2000) (Table 1). Incomplete information regarding the tissue distribution of these novel PDEs, and, more importantly, a lack of selective inhibitors, has severely limited efforts aimed at assessing their involvement in the cardiovascular system. Although it may be that these novel PDE variants have as-yet undiscovered physiological significance in cardiac and vascular functions, a formal test of this possibility must await the development of selective reagents.

Potential Physiologic and Therapeutic Implications of Differential PDE Expression in Contractile/Quiescent and Synthetic/Activated VSMC and of Selected PDE Compartmentation. The marked changes in PDE1C (human) and PDE3A (rat and human) expression that accompany the phenotypic conversion of VSMC from contractile/quiescent to synthetic/activated phenotypes may be predicted to significantly impact cyclic nucleotide fluxes in these cells as well as the ability of PDE family-selective agents to alter cellular functions (Fig. 1f). Indeed, based on these considerations, PDE1C-selective inhibitors should represent a potentially important class of molecules to reduce proliferation and perhaps migration of the synthetic/activated VSMC phenotype. Similarly, the increased role of PDE4 in synthetic/activated VSMC may identify this class of agents as useful drugs to stimulate cAMP-mediated effects in synthetic/activated VSMC compared with contractile/quiescent cells. Although PDE1C-selective agents are not currently available, clinical trials currently underway to test the effectiveness of PDE4-selective agents in asthma and chronic obstructive

pulmonary disease may allow the impact of PDE4 inhibition on VSMC function to be assessed, if only indirectly.

As described, prolonged increases in cAMP or cGMP often cause a compensatory increase in the activity, or expression, of PDE. This effect may limit the benefits of PDE inhibition to cells less capable of reacting to the continued presence of cAMP- or cGMP-elevating agents and, in the context of atherosclerosis or angioplasty-mediated vascular restenosis, may be of therapeutic interest. Thus, as discussed above, prolonged treatment with cAMP-elevating agents induced a modest increase in PDE4D3 levels in contractile/quiescent and a robust increase in PDE4D1 and PDE4D2 expression in synthetic/activated VSMC. Should similar differences occur in vivo, PDE4 inhibitors may have reduced effectiveness when used on synthetic/activated VSMC. Similarly, the reduced level of PDE3A in synthetic/activated VSMC, coupled with the selective induction of PDE3B expression, may hint at the need to target PDE3B in VSMC rather than both PDE3s. Because growth factor signaling stimulates both PDE3 and PDE4 activities, it is also likely that the agents responsible for proliferation and migration of VSMC in these vasculopathies will also play an important role in the success of strategies involving selective PDE inhibition. At present, the available data do not allow a similar description of phenotype-based changes in cardiomyocytes and VEC to be undertaken.

The veritable explosion in technologies permitting more detailed analysis of intermolecular interactions, in both in vitro and in vivo settings, has allowed for further analysis of the importance of compartmentation of PDEs in their precise regulation of cellular functions. As outlined above, distinctive compartmentation of PDEs is a strategy commonly employed by cells to allow selective cyclic nucleotide-mediated signaling. Membrane/organelle, cytoskeletal, or soluble distributions of PDE are probably all significant in the regulation of global cyclic nucleotide levels while also providing sufficient flexibility for tighter dynamic control of specific cellular functions. As discussed previously, significant information is available concerning the targeting of PDE4 family variants to structures via interactions with AKAPs, β-arrestin proteins, and/or phosphatidic acid-rich membrane structures. Indeed, recent work has pointed to a role for PDE4D3 interactions with AKAP in the development of cardiac hypertrophy and of the interaction of either PDE4D3 or PDE4D5 with β-arrestin proteins in β_2-adrenergic receptor coupling in cardiomyocytes. Although recent studies in our laboratory have confirmed that PDE4D3 also interacts with β-arrestin proteins in VSMC, they are not consistent with a role for PDE4D3 interactions with AKAPs in these cells (D. Raymond and D. H. Maurice, unpublished observations). Although these findings are consistent with the possibility that altering PDE4D3-AKAP interactions may allow cardiomyocyte-specific effects, further work is required to validate this hypothesis. Elucidation of the molecular basis for particulate PDE4D3 targeting in VSMC, in the absence of significant PDE4D3-AKAP interactions in these cells, will also require further study but may represent an avenue for cell-specific targeting. Although PDE3 are dominant enzymes in cardiovascular cells, significantly less is known concerning the mechanisms controlling targeting of these enzymes in cells. Indeed, although PDE3B can be shown to interact with either the insulin receptor, or IRS-1, by immunoprecipitation

in certain adipocyte cell lines, the role of these interactions in regulating the function of this enzyme in cells of the cardiovascular system is unclear. Similarly, PDE3B was shown to interact with 14-3-3β, potentially through interaction of this scaffolding protein with PKA and PKB phosphorylation motifs in this protein. Again, however, the functional consequences of this interaction remain unclear. Recently, we reported an example of the potential impact of altered PDE3B-dependent protein-protein interactions on the activity of this enzyme. Indeed, in this recent work, we demonstrated that a hyperproliferative and motile phenotype observed in VSMC isolated from a leptin receptor-deficient rodent model of diabetes was attributable to increased PDE3B specific activity and reversed with PDE3 inhibitors. Recent evidence that PDE3 variants and receptors for leptin interact may eventually allow the mechanism responsible for this phenomenon to be clarified (H. S. Elbatarny and D. H. Maurice, unpublished observation).

Conclusion

Cells of the cardiovascular system express numerous variants of several PDE families. These different PDE variants are recruited to allow specific control of a myriad of cAMP- and cGMP-dependent events and to coordinate cyclic nucleotide and non-cyclic nucleotide-dependent signaling. It is our contention that through a more complete understanding of the varied mechanisms through which these cells regulate the activity, expression, and subcellular targeting of these enzymes, more specific pharmaceutical targeting of these enzymes will be possible, and far greater promise for the control of cardiovascular indications will be possible. Although the preceding discussion of the differential expression and regulation of PDE family variants in cells of the cardiovascular system does not radically alter the established rationale for the use of PDE3 and PDE5 inhibitors in the treatment of cardiovascular conditions such as heart failure, post-angioplasty restenotic injury, diabetes-associated vascular disease, or pulmonary hypertension, it does unmask the potential of targeting other PDE family variants in some of these conditions. In addition, this analysis highlights the complexity of the system controlling cyclic nucleotide hydrolysis in cells and identifies points of intersection between the actions of individual PDE family enzymes that might be amenable to therapeutic intervention.

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