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CYCLIC NUCLEOTIDE PHOSPHODIESTERASE-MEDIATED INTEGRATION OF cGMP AND cAMP SIGNALING IN CELLS OF THE CARDIOVASCULAR SYSTEM

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1. ABSTRACT

Numerous pharmacological and physiological agents acting *via* either cAMP- or cGMP-mediated impact the activities of cells of the cardiovascular system. While most define cAMP and cGMP signaling systems as separate and independent, recent advances in our understanding of cyclic nucleotide signaling, and more specifically, of the roles which cyclic nucleotide phosphodiesterases (PDEs) play in these events, have altered this view. In this short chapter, I will review the data identifying expression of several PDEs in cells of the cardiovascular system. In addition, I will review the data that identify PDEs as enzymes capable of allowing integration between cAMP and cGMP signaling in cells, and propose that cAMP and cGMP signaling systems can represent parallel and interdependent signaling systems. Moreover, I will propose that cGMP-mediated effects on the activities of variants of the Phosphodiesterase 2 (PDE2), PDE3 and PDE5 families may act to coordinate linkage between cAMP and cGMP signaling in these cells.

2. INTRODUCTION

2.1. Cyclic Nucleotide-Mediated Actions In Cells Of The Cardiovascular System

The cyclic nucleotides, cyclic AMP (cAMP) and cGMP, each regulate a myriad of events in virtually all cells, including those of the cardiovascular system. Indeed, the ability of i) vascular smooth muscle cells (VSMC) and vascular endothelial cells (VEC) to co-ordinately regulate blood pressure, of ii) cardiomyocytes to regulate the rhythmic pumping of the heart and of iii) blood platelets to limit injury-associated blood volume loss, are each independently and powerfully regulated by numerous natural agents (hormones) and pharmaceutical agents (drugs) that act by altering levels of either cAMP or cGMP (1-10). While cAMP- or cGMP-regulated events are usually ascribed as having arisen from events mediated by parallel and independent systems, it is my goal here to develop the idea that in certain situations cAMP and cGMP signaling systems regulate cell functions by operating as highly integrated systems in cells. More specifically, I will present evidence that cGMP, by acting through certain Phosphodiesterase 2 (PDE2), PDE3 and PDE5 family variants, plays a critical role in coordinating an integration between cAMP and cGMP signaling in cells.

3. CYCLIC NUCLEOTIDE PHOSPHODIESTERASES (PDES)

3.1. General Considerations

During the last two decades our knowledge concerning the composition of the PDE super-family of enzymes, as well as the mechanisms by which these various PDEs allow selective regulation of cyclic nucleotide-mediated cellular events, has increased markedly (reviewed in 10-16). Briefly, mammalian PDEs are subdivided into 11 distinct families based on primary amino acid sequence, overall domain structure as well as catalytic and regulatory characteristics. Most PDE families contain multiple homologous *PDE* genes. Although sequence identity within the PDE catalytic domain is relatively high between families (20–45%), amino- and carboxyl-terminal

Table 1. Numbers of PDE-family Variants Expressed in Human Cardiovascular Tissues

PDE Family	VSMCs	VECs	Cardiomyocytes	Platelets
PDE1	3	0	0	0
PDE2	0	1	1	1
PDE3	3	2	3	1
PDE4	4	3	7	0
PDE5	2	1	0	1

sequences are considerably dissimilar and encode sequences allowing family-selective regulatory characteristics as well as selected subcellular targeting (10-16). Individual *PDE* genes can yield numerous variants by making use of alternate promoter initiation sites and/or alternate splicing. Individual PDE variants can have unique catalytic and/or regulatory characteristics and can be differentially localized to selected subcellular compartments (10-16). In this context, studies of the impact of selected PDE subcellular targeting on cyclic nucleotide regulated cell functions are consistent with the hypothesis that this phenomenon represents a fundamental component for proper integration of cyclic nucleotide fluxes in cells (16). A recently established nomenclature system allows identification of the many PDEs. For example, HSPDE3A2 identifies a human (HS, *Homo sapiens*) enzyme of the PDE3 family encoded by the A gene of this family. Since several PDE genes can generate distinct variants, a final number, in this case 2, identifies the specific variant.

3.2. PDEs In Cells Of The Cardiovascular System

As is generally the case for most, if not all cells, human VSMCs, VECs, cardiomyocytes and blood platelets each express enzymes from several individual PDE families (Table 1). In addition, in some cases, these cells can in fact express several distinct variants of each PDE enzymes (Table). Briefly, variants of the PDE1, PDE3, PDE4 and PDE5 families are expressed in human arterial and venous VSMCs (reviewed in 10-11). While fewer studied have directly addressed the issue of PDE expression in human VECs, recent studies from some laboratories (17), including ours (11,18), identify expression of variants of PDE1, PDE2, PDE3, PDE4 and PDE5 families in these cells. For their parts, cardiomyocytes have been shown to express PDE2, PDE3 and PDE4 family variants while blood platelets express PDE2, PDE3 and PDE5 family enzymes (10-12). Following a short description of the main characteristics of the PDE2, PDE3 and PDE5 family enzymes expressed in cardiovascular cells I will review the established functional consequences which arise from differential PDE expression in cells of the cardiovascular system. Subsequently I will propose less formally tested consequences of these expression patterns. For a more detailed description of PDE expression in cells of the cardiovascular system the reader is referred to more comprehensive recent review (11).

3.2.1. Phosphodiesterase 2 (PDE2)

Enzymes of the PDE2 family were initially differentiated from other PDEs by virtue of the fact that addition of cGMP to these enzymes was shown to stimulate their catalysis of either cGMP itself, or cAMP (19, 20). In fact, these kinetic characteristics led to the initial characterization of PDE2 enzymes as cGMP-stimulated cyclic nucleotide PDEs. Since cGMP is the preferred stimulating cyclic nucleotide and, at physiological concentrations, cAMP the preferred substrate, it is generally accepted that these enzymes most likely function as cGMP-stimulated cAMP PDEs in most cells (11).

To date the PDE2 family has been shown to contain a single gene (*PDE2A*) that can encode three distinct mRNA transcripts, namely PDE2A1, PDE2A2 and PDE2A3 (20). Since the mRNA encoding these three PDE2A variants yield enzymes with distinct amino-termini and generate either soluble (PDE2A1), or particulate (PDE2A2 and PDE2A3) enzymes, the distinct amino termini likely provide the required information for selective targeting of these proteins in cells. It has been suggested that targeted expression of PDE2 variants in cells allows these enzymes to regulate selected sub-cellular "pools" of cAMP. Indeed, recent data has shown that PDE2 variants are differentially localized in cells and that this may impact their ability to regulate cellular functions (17-21). In addition to expressing amino-termial sequences responsible for subcellular targeting, each PDE2 monomer encodes two GAF domains, GAFa and GAFb. GAF domains are structural motifs named because of their presence in cGMP-regulated PDE, adenylyl cyclases, and the *E. coli* protein Fh1A (22-23). While it is clear that GAFb in PDE2 binds cGMP, and that this interaction likely coordinates the cGMP-mediated stimulation of catalytic activity in these enzymes, the involvement of GAFa in PDE2 basal and stimulated activity is unclear (23). Interestingly, cGMP-stimulated hydrolysis of itself, or of cAMP, provides a potential point of convergence for cGMP and cAMP signaling in cells, an effect that in principle could allow cGMP-elevating agents to perhaps oppose cAMP-mediated processes in cells in which significant PDE2 is expressed. In this context, while PDE2 is not expressed in VSMCs, significant PDE2 expression has been reported in VECs, platelets and cardiomyocytes, as well as in brain, olfactory neurons, liver and granulosa cells of the adrenal gland (reviewed in 11). The impact of PDE2 expression on cGMP and cAMP signaling in VECs, cardiomyocytes and platelets is further discussed below.

3.2.2. Phosphodiesterase 3 (PDE3)

In marked contrast to the impact of cGMP on PDE2 hydrolysis of cyclic nucleotides, addition of cGMP to purified PDE3s inhibits the hydrolysis of cAMP by these enzymes (9-11, 24). This competitive inhibition of cAMP hydrolysis by PDE3s is related to the fact that PDE3s have virtually identical K_m's for both cAMP and cGMP (~0.1 μM) but hydrolyze the latter at a rate approximately 10-fold slower than the former. This characteristic led to these

enzymes initially being referred to as cGMP-inhibited cAMP PDEs (24). Two genes, named *PDE3A* and *PDE3B*, encode several distinct PDE3 variants (9,24). The *PDE3A* gene gives rise to three PDE3A isoforms, PDE3A1, PDE3A2 and PDE3A3, whereas the *PDE3B* gene encodes a single protein product, namely PDE3B (11). Both PDE3A1 and PDE3B encode 135 kDa enzymes that incorporate all of the predicted coding regions within the full-length mRNA transcripts of these genes (9,11). Experimental evidence is consistent with the idea that both PDE3A1 and PDE3B are each entirely particulate (9,11), although the structures with which these enzymes interact within these particulate domains have not yet been fully defined. PDE3A2 results from the translation of an mRNA truncated within a region encoding a cluster of putative transmembrane-spanning helices and encodes a 118 kDa protein and is expressed in both soluble and particulate fractions of cells. The shortest PDE3A is the 80 kDa PDE3A3 enzyme and this variant is entirely cytosolic when expressed. Differential subcellular targeting of distinct PDE3s may significantly impact their actions in cells and some evidence of this has been recently reported (9, 11).

PDE3A mRNA is highly enriched in vascular smooth muscle, heart, megakaryocytes, and oocytes whereas PDE3B mRNA is highest in adipocytes, hepatocytes, brain, renal collecting duct epithelium, and developing spermatocytes (24). Notwithstanding these mRNA expression profiles, analysis of PDE3A and PDE3B protein expression is suggestive of significant overlap in the expression of these enzymes in cells. Thus, while rat and human VSMC, bovine aortic endothelial cells (bAEC) and certain hepatoma cell lines express both PDE3A and PDE3B proteins, platelets and cardiomyocytes express only PDE3A (11). PDE3B is a prominent PDE3 variant in lymphocytes, macrophages, and pancreatic β-cells (reviewed in 11).

Since PDE3s are activated following PKA-mediated phosphorylation, these enzymes are actively involved in desensitizing cells to continued cAMP-mediated signaling (24). Indeed, all three PDE3As and the PDE3B are phosphorylated by PKA and activated following addition of activators of adenylyl cyclases including prostanoids or catecholamines in several cell types, including those of the cardiovascular system (reviewed in 11). PDE3s are also subject to regulation by PKB/Akt following insulin or IGF-1 treatment of certain cells (reviewed in 11, 24), although some evidence suggests that this effect would be limited to PDE3A1 and PDE3B (9).

3.2.3. Phosphodieserase 5 (PDE5)

Specific hydrolysis of cGMP in many cells is carried out by variants of the PDE5 family of enzymes. Analogously with PDE2 family enzymes, PDE5 variants also encode two GAF domains and are activated subsequent to binding of cGMP to one of these domains (22, 23, 26). In contrast to PDE2, however, where cGMP binding to GAFb correlated with increased hydrolytic activity, PDE5 hydrolysis is activated when cGMP binds to the GAFa domain of this enzyme and not GAFb (25, 26). In addition to the activation caused by cGMP binding to GAFa, PDE5s can also be activated secondary to PKG-mediated phosphorylation (25). In addition to these short-term effects, our unpublished data indicate that expression of venous VSMC PDE5A1 and PDE5A2 is elevated in animals treated for prolonged periods with NO-releasing agents (H. A. Dunkerley, B. Bennett, D. H. Maurice, unpublished data). The high degree of cGMP selectivity of PDE5 enzymes, coupled with their cGMP-dependent activation, is why these enzymes were initially described as cGMP-binding cGMP PDEs (15). The PDE5 family consists of a single gene, namely *PDE5A* that encodes three distinct PDE5 variants, PDE5A1, PDE5A2 and PDE5A3 (15, 26). Interestingly, recent evidence suggests that an inhibitory subunit, typically associated with retinal PDEs of the PDE6 family, may also regulate PDE5 activity, perhaps through proteolysis by caspases or simply through inhibition (27-28). PDE5 is expressed in several tissues, including brain, lung, kidney, VSMC, VECs and blood platelets (reviewed in 11). While the expression of three distinct variants might suggest that PDE5s could be differentially targeted to selected structures in cells, very little information is available concerning the subcellular targeting of PDE5 variants in cells, or the consequences of such an event. Adopting the paradigm elaborated for PDE4D variants (16), one might predict that PDE5 could be found in association with proteins involved in coordinating PKG-targeting.

Therapeutic strategies based on inhibition of PDE5 in vascular, thrombotic, or pulmonary disorders have been successful (10, 11). In particular, the success of the selective PDE5 inhibitor sildenafil (Viagra™) in the treatment of erectile dysfunction (ED) has validated these efforts and further increased the interest in this approach. Indeed, several other PDE5-selective inhibitors are now available for treatment of ED. 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 (reviewed in 11). 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 (Viagra™), vardenafil (Levitra™), or tadalafil (Cialis™) on cardiac function is likely modest. 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 (reviewed in 11). However, given the potential ramifications of PDE5 expression in human cardiomyocytes, it is likely that this issue will receive further consideration. While two PDE5A variants, PDE5A1 and PDE5A2, are each expressed in rat, bovine, and human VSMCs, VECs only express PDE5A1. Consistent with their potent and selective inhibition of PDE5, sildenafil and the other selective PDE5 inhibitors relax several blood vessel VSMC, in addition to the smooth muscle of the corpus cavernosum, and are potent inhibitors of platelet aggregation, especially in the presence of nitric oxide releasing drugs, or endothelium-derived NO (EDRF)

(29). 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 very poor outcome (30-31). 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 31). 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 (32). This contrasts with ED, in which reduced NO-mediated guanylyl cyclase activation is usually thought the dominant basis of pathology.

4.0 ESTABLISHED AND POTENTIAL PHYSIOLOGICAL, PATHOLOGICAL AND THERAPEUTIC EFFECTS ASSOCIATED WITH DIFFERENTIAL cGMP EFFECTS ON PDE2, PDE3 AND PDE5 ACTIVITIES IN CELLS OF THE CARDIOVASCULAR SYSTEM

4.1. General Considerations

As described above, and shown in the Table, human VSMCs, VECs, cardiomyocytes and blood platelets differentially express enzymes of the PDE2, PDE3 and PDE5 families of enzymes and, in some instances, in fact express several distinct variants of these PDE families of enzymes. Since the activities of each of these enzymes are impacted by intracellular cGMP, I propose that differences in their expression in cells of the cardiovascular system could allow for selective regulation of cellular functions by cGMP and data consistent with this proposal have been reported (reviewed in 10). In this context, in the following subsections I will describe these data and discuss how they support the idea that cGMP and cAMP signaling systems are often highly integrated and that cGMP-mediated effects at PDE2, PDE3 or PDE5 is an important factor in this integration. Moreover, I will discuss how integration of cGMP and cAMP signaling can markedly alter cellular responses to drugs and how these concepts may be therapeutically important.

4.2. *VSMC*

As discussed previously herein and in more detail in (10, 11), cAMP hydrolysis in VSMCs is catalyzed by cGMP-inhibited PDE3 and the cAMP-specific, cGMP-insensitive, PDE4 enzymes and also by PDE1C in actively proliferating human VSMCs (33). A substantial literature confirms that direct pharmacological inhibition of VSMC PDE3 activity with drugs such as milrinone, cilostamide or cilostazol can directly influence VSMC functions such as relaxation-contraction coupling or proliferation and confirms that these agents further potentiate actions of activators of adenylyl cyclases in these cells (reviewed in 11). Contemporaneous with these reports, evidence was also presented that inhibition of VSMC PDE3 activity by endogenous cGMP might account for some of the effects of activators of VSMC guanylyl cyclases such as sodium nitroprusside (SNP), and atrial natriuretic peptide (ANP) and that this effect could begin to explain synergistic effects of activators of guanylyl cyclases and those that activated adenylyl cyclases in VSMCs (34, 35). Thus, evidence consistent with *in situ* cGMP-mediated inhibition of cAMP hydrolysis by PDE3 was reported to allow guanylyl cyclase-dependent vasodilators, such as SNP or ANP to significantly increase VSMC cAMP, in addition to cGMP, and to synergistically elevate cAMP when combined with adenylyl cyclase-activating vasodilators such as PGI2, adenosine or isoproterenol (34-38). For example, in the late 1980's, Dr. Richard Haslam of McMaster University and I first reported that nitrovasodilators such as sodium nitroprusside (SNP), or the active metabolite of molsidomine, SIN-1, markedly potentiated the relaxant effects of isoproterenol in rat aorta (34, 35). Measurement of cAMP and cGMP in these studies identified a significant increase in **BOTH** cGMP and cAMP following incubation of VSMC with the NO-donors in the absence of adenylyl cyclase activating stimuli and a supra-additive increase in cAMP when adenylyl cyclase was activated with isoproterenol (34, 35). Consistent with our earlier work, numerous subsequent reports have since shown that cGMP-mediated inhibition of VSMC PDE3 occurs upon addition of activators of either VSMC soluble or particulate guanylyl cyclases (36-38). Interestingly, this mechanism was recently shown to allow integration of cGMP and cAMP signaling when cGMP levels were elevated subsequent to PDE5 inhibition (36). Thus, while the PDE5 inhibitor sildenafil was shown to inhibit VSMC mitogenesis and to synergize with NO-donors in this respect, the effect was shown to be independent of PKG, and rather, related to cGMP-mediated inhibition of PDE3 and a cAMP-dependent activation of PKA (36). The absolute PDE3-dependence of this effect was validated when attempts to inhibit mitogenesis by direct activation of PKG were shown to be ineffective (36).

The preceding discussion focused exclusively on effects associated with cGMP-mediated inhibition of PDE3-catalyzed cAMP hydrolysis in VSMCs. The relationship between these effects, and those attributable to direct cGMP-mediated activation of either PKG, or PKA, will likely be controlled by several factors. Thus, since inhibition of PDE3 by cGMP occurs at lower concentrations of cGMP than are required for full activation of PKG by this cyclic nucleotide, the magnitude of the increase in cGMP may be one factor (39). Also, since PDE3 variants can be differentially targeted in cells, including VSMCs, local dynamics of cGMP, may also be a factor worthy of consideration (10). In this context, it is perhaps relevant that VSMCs express both cytosolic and particulate guanylyl cyclases. Clearly, intact cell based cGMP measurements, such as those made possible by the development of recent fluorescent indicators, will likely be required to fully address these issues (40).

In addition to those established effects attributable to cGMP-mediated inhibition of VSMC PDE3 which I described above, other "less fully tested" effects might be anticipated to arise from this effects in VSMCs. Briefly, a basal level of VSMC PDE3 inhibition, as might be predicted to arise from the constitutive release of NO from VECs, could in principle be involved in coordinating the effects of the short-lived VEC-derived vasoactive agents, PGI_2 and NO (42). Thus, VEC-derived NO, by activating VSMC guanylyl cyclase, could maintain VSMC cGMP levels within the concentration range required for efficient inhibition of PDE3 and, as such, provide a basal level of "physiological" PDE3 inhibition. This suggestion should be readily testable using eNOS null mice or pharmacological eNOS selective inhibition. By extension, if constitutively released VEC NO regulated VSMC PDE3, local VSMC tone may be higher at sites of vascular damage and, in turn, more sensitive to PDE3 inhibition. Again, these ideas should be readily testable using available reagents. In terms of pathological and therapeutic implications of cGMP-mediated inhibition of PDE3 in VSMCs, it may be relevant that we recently showed that PDE3A levels were markedly reduced in VSMC activated following vascular damage (42). As described in that earlier report, reduced PDE3A in these cells will likely reduce the impact of PDE3 inhibitors following vascular damage (42).

4.3. VECs

As described above, much of our data, and that published by others, identify expression of PDE2, PDE3, PDE4 and PDE5 in VECs and cGMP would be predicted to influence cAMP hydrolysis by both PDE2 and PDE3 enzymes in these cells (17-19). In this context both cAMP and cGMP have been shown to dynamically regulate several important events in VECs, and some studies with selective PDE2 or PDE3 have shown that these enzymes can regulate cAMP pools that mediate these events (19-21). For instance, treatment of VECs with pharmacological PDE2 or PDE3 inhibitors reduce VEC proliferation, migration and permeability (17, 18). Most importantly within the context of this chapter, the relative importance of cGMP-mediated effects through PKG, PDE2 or PDE3 activities has not been directly assessed in VECs. However, since levels of PDE2 are higher in VECs isolated from smaller blood vessels, one might predict that cGMP effects via PDE2 and PDE3 may be different in selected vascular structures.

4.4. Cardiomyocytes

While direct pharmacological inhibition of PDE2 activity increases L-type Ca^{2+} currents in cardiomyocytes, and contracts these cells, the overall magnitude of this effect is influenced by several factors including basal levels of adenylyl and guanylyl cyclases (43-44). In addition to the positive inotropic effect caused by direct pharmacological inhibition of PDE2, considerable evidence implicates direct cGMP activation of PDE2 in the control of cardiomyocyte Ca^{2+} currents and contractions. Indeed, cGMP can inhibit cAMP-induced contractions in cardiomyocytes by activating PDE2 (43-44). Similarly, 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 (10, 43-44) and considerable evidence implicates a cGMP-mediated inhibition of cAMP hydrolysis by PDE3 as contributing to the pro-contractile effects of cGMP-elevating agents in these cells (43-44). Expression of PDE2 and PDE3 in these cells results in complex cGMP effects on cell function. Thus, while cGMP-mediated inhibition of PDE3 allows cGMP-elevating agents, such as nitrate donors, or the atrial natriuretic peptide, to increase cardiomyocyte cAMP levels, and Ca^{2+} currents, cGMP-mediated activation of PDE2 stimulates PDE2-mediated cAMP hydrolysis and limits the cGMP-mediated effect mediated by PDE3 (43, 44). Species differences in the dominance of PDE2, or PDE3, in regulating cGMP-mediated effects on cardiomyocyte Ca^{2+} currents, and contractility, have been reported and will likely profoundly alter the relative effect of cGMP in cardiomyocytes. Selective targeting of PDEs in cardiomyocytes has been described and, using fluorescence-based assays of cellular cAMP changes, these enzymes have been shown to regulate selective pools of cyclic nucleotides (45). The overall impact of targeting will likely be influenced by the extent to which cyclic nucleotides are increased with cAMP or cGMP elevating agents.

4.5. Blood platelets

Coordinated cGMP-mediated regulation of cAMP hydrolysis by PDE2 and PDE3 also plays an important role allowing cGMP-elevating agents to affect platelet functions. Indeed, we reported previously that low concentrations of NO-donors such as SNP, or SIN-1, significantly increased cGMP AND cAMP in rabbit platelets and showed that the increase in cAMP resulted from cGMP-dependent inhibition of PDE3 in these cells (46). Attesting to mechanism, NO donor-dependent increases in platelet cAMP were abolished by addition of haemoglobin to binds NO and potentiated by addition of PDE5 inhibitors. In these earlier experiments we showed that simultaneous addition of guanylyl cyclase activators and adenylyl cyclase activators resulted in a synergistic increase in cAMP and supra-additive inhibition of activation (46). While this situation is reminiscent of that described above for VSMC, responses in human platelets were more complex than those in rabbit platelets. Thus, since human platelets express both PDE2 and PDE3, but rabbit platelets only express PDE3, cGMP-elevating agents affected human platelet functions in a manner more similar to that described above in cardiomyocytes (47, 48). Indeed, while low concentrations of cGMP-elevating agents potentiated the effects of adenylyl cyclase activators, at higher concentrations, cGMP increases antagonized the effects of adenylyl cyclases by activating platelet PDE2 (47, 48).

One potentially important implication of the cGMP-mediated regulation of both PDE2 and PDE3 in human blood platelets may relate to the anti-thrombotic effects of NO and PGI_2 released by VECs. Indeed, any alteration in the release of these agents would be predicted to alter platelet reactivity, especially locally. Since damage

to VECs can reduce their release of NO and PGI_2 and inflammatory mediators can increase the release of these substances from VECs, one could predict that local platelet reactivity would be differentially influenced by these events. Indeed, this mechanism may be involved in mediating the increased platelet reactivity that associates with disease-associated endothelial dysfunction and the reduced local platelet reactivity that associates with infections (49).

5. SUMMARY AND CONCLUSIONS

PDE2 and PDE3 enzymes each hydrolyse cAMP and are both sensitive to the intracellular concentration of cGMP. As such, cells exposed to cGMP-elevating agents that also express either PDE2, or PDE3, or both of these PDE, will be expected to regulate their cAMP levels in a dynamic fashion dependent on the relative amounts of PDE2 or PDE3 that they express, as well as the magnitude of the increase in cGMP. These effects should be incorporated into the analysis of effects of cGMP-elevating agents in these cells.

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