

PERSPECTIVE

Cyclic Nucleotide Phosphodiesterase 5 and Sildenafil: Promises Realized

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PDEs belong to a large superfamily [11 different gene families (PDEs 1–11)] of structurally related, functionally distinct, and highly regulated enzymes (Francis et al., 2001). Most PDE families comprise more than one gene (~20 PDE genes), which generate multiple protein products (>50 PDE proteins) via alternative mRNA splicing or use of different promoters/transcription initiation sites. Most cells contain representatives of multiple PDE gene families, but in different amounts, proportions, and subcellular locations. PDE5 isoforms, for example, are relatively abundant in vascular smooth muscle, including the pulmonary vasculature and corpus cavernosum of the penis, where they apparently regulate hydrolysis of cGMP pools that modulate vasodilation (Corbin and Francis, 2002; Corbin et al., 2002).

This *Perspective* will focus on the confluence of our understanding of the biological and pharmacological roles of NO, cGMP, one specific PDE, PDE5, and PDE5 inhibitors in regulation of smooth muscle relaxation, penile erection, and treatment of erectile dysfunction, as well as on important contributions of Jackie Corbin, Sharron Francis, and others in elucidating the complex biology of PDE5 and thus facilitating the bench-to-bedside transition of PDE5 inhibitors (Francis et al., 2001; Corbin and Francis, 2002; Corbin et al., 2002). The emergence of the PDE5 inhibitor, sildenafil (Viagra), as effective therapy for erectile dysfunction finally realizes the promise and potential of specific PDEs to serve as important therapeutic targets and of “family-specific” PDE inhibitors to function as safe and efficacious drugs.

Mammalian PDEs exhibit a common structural organization, with a conserved catalytic domain (~250–300 amino acids) in the C-terminal portion of the molecules and divergent regulatory domains and modules in N-terminal portions. The catalytic core, more highly conserved among members of each gene family than among different gene families, contains a signature motif, common to all PDEs, and consensus metal-binding domains (Francis et al., 2001). In addition

to common structural elements, the catalytic core contains family-specific sequences, responsible for differences in substrate affinities, catalytic activities, and sensitivities to specific inhibitors. Some PDE families are relatively specific for cAMP (PDEs 4, 7, 8) or for cGMP (PDEs 5,6,9); others hydrolyze both (PDEs 1, 2, 3, 10, 11) (Francis et al., 2001). Although methylxanthines inhibit almost all PDEs, selective inhibitors that are relatively specific for individual families are available (i.e., for PDEs 1–6). Sildenafil, vardenafil, and tadalafil are potent, selective PDE5 inhibitors (Francis et al., 2001; Corbin and Francis, 2002; Corbin et al., 2002).

N-terminal portions of PDE molecules are highly divergent, containing structural determinants that allow different PDEs to respond selectively to specific regulatory signals (Francis et al., 2001). These regulatory regions and modules include, for example, sites and domains that are subject to different types of covalent modification (e.g., phosphorylation) or that interact with allosteric ligands, protein partners, or molecular scaffolds and thereby regulate catalytic activity, protein/protein interactions, and/or subcellular compartmentation. Five PDE families (PDEs 2, 5, 6, 10, 11) contain homologous so-called GAF domains. In three families (PDEs 2, 5, and 6) classified as cGMP-binding PDEs, GAF domains bind cGMP with high affinity but without identical functional consequences (Francis et al., 2001).

Signaling pathways, in general, include mechanisms for negative feedback control. From this perspective, PDEs are critical homeostatic regulators of intracellular cyclic nucleotide concentrations. For example, acute increases in cAMP activate cAMP-dependent protein kinase, phosphorylation/activation of PDE3 and PDE4, and enhanced destruction of cAMP. Long-term elevation of cAMP also provides negative feedback, by increasing transcription of PDE3 and PDE4 genes, indirectly resulting in increased enzymatic activities (Francis et al., 2001).

With respect to PDE5, Corbin and Francis, their cowork-

ers, and others (Francis et al., 2001; Mullerhausen et al., 2001; Okada and Asakawa, 2001; Rybalkin et al., 2002, 2003; Corbin et al., 2003) have reported that binding of cGMP to PDE5 GAF domains induces conformational changes that increase affinity of the catalytic site for cGMP; binding of cGMP to the catalytic site, in turn, increases cGMP binding to GAF domains. cGMP binding to GAF domains also facilitates phosphorylation by cGMP-dependent protein kinase of a specific serine near the N terminus of PDE5, which in turn induces binding of cGMP to GAF domains and allosteric activation of PDE5 (Francis et al., 2001; Mullerhausen et al., 2001; Okada and Asakawa, 2001; Rybalkin et al., 2002, 2003). Thus, elevation of intracellular cGMP provides negative feedback control and enhances its own destruction via direct, cGMP-induced allosteric activation of PDE5 as well as indirect activation caused by phosphorylation of PDE5 by cGMP-dependent protein kinase (Francis et al., 2001; Mullerhausen et al., 2001; Okada and Asakawa, 2001; Rybalkin et al., 2002, 2003). Corbin, Francis, and coworkers have also examined interactions between the catalytic and GAF domains in PDE5 using [³H]sildenafil, which selectively interacts with the cGMP-binding site in the catalytic domain (Francis et al., 2001; Corbin and Francis, 2002; Corbin et al., 2002, 2003). Inhibition of PDE5 by sildenafil can increase cGMP that binds to PDE5 GAF domains; this in turn increases binding affinity of the catalytic site for sildenafil, thus providing positive feedback in terms of sildenafil potentiating accumulation of cGMP (Corbin et al., 2003). This latter effect of sildenafil, and presumably of analogous PDE5 inhibitors, may have important pharmacokinetic and therapeutic implications. The ability of sildenafil, with K_i/K_d values $\ll K_m$ for cGMP, to indirectly up-regulate its binding to the catalytic site of PDE5 suggests that, in tissues enriched in PDE5, PDE5 inhibitors may be sequestered/concentrated bound to PDE5. The duration of the effects of these drugs may depend not only on their inherent chemical properties but also on their dissociation from PDE5, which could be regulated more by the drugs K_d values than competition with cGMP substrate.

cGMP signaling can mediate relaxation of smooth muscle, including trabecular smooth muscle of the corpus cavernosum (Francis et al., 2001; Corbin and Francis, 2002; Corbin et al., 2002). Upon sexual stimulation, neuronal impulses augment release of NO from nonadrenergic, noncholinergic neurons and endothelial cells in the corpus cavernosum. NO activates soluble guanylyl cyclase and increases cGMP, inducing relaxation of trabecular smooth muscle, arterial vasodilation, increased blood flow, occlusion of venous outflow, and penile erection. PDE5 is relatively abundant in the corpus cavernosum and apparently controls the cGMP pool that regulates vascular dilation and, consequently, tumescence; specific PDE5 inhibitors enhance effects of NO on accumulation of cGMP and penile erection. A number of factors (especially disease states characterized by impaired endothelial function and NO signaling) can disrupt this complex biological network and thus cause erectile dysfunction. Formation and accumulation of cGMP seem to be critical in this process, because oral administration of the PDE5 inhibitor sildenafil (Viagra) improves penile erection with minimal risk of side effects and adverse events in many men with erectile dysfunction (Corbin and Francis, 2002; Corbin et al., 2002).

Despite intensive efforts to develop other PDE inhibitors

as therapeutic agents, and despite impressive preclinical data with some PDE inhibitors, only sildenafil (Viagra) has fulfilled the promise of PDEs as therapeutic targets in major disease processes. PDE4 inhibitors, such as cilomilast and roflumilast, potent anti-inflammatory agents in many preclinical studies and model systems, remain in phase III clinical trials as potential therapeutic agents for asthma and chronic obstructive pulmonary disease. PDE3 inhibitors, which enhance myocardial contractility and inhibit platelet aggregation, failed in clinical trials of long-term treatment of cardiac failure, although milrinone is used for emergent and short-term treatment of patients hospitalized for refractory cardiac failure, and cilostazol has been approved for the treatment of intermittent claudication.

Why has Viagra proven safe and efficacious? Sildenafil is a potent inhibitor that can be administered orally, when needed, at concentrations sufficient to inhibit PDE5, with minimal serious side effects related to inhibition of other PDEs or non-PDE targets (Corbin and Francis, 2002; Corbin et al., 2002). Transient, mild visual disturbances, for example, are presumably related to inhibition of PDE6, which is almost exclusively expressed in the retina. Viagra acts in pharmacologically optimal conditions (i.e., PDE inhibitors work most effectively in conjunction with active cyclases) in the penis in the presence of augmented local production of cGMP via NO-induced activation of guanyl cyclase. This latter effect, however, is also the basis for one major contraindication of sildenafil therapy: concurrent treatment with nitroglycerine or other nitrates (Corbin and Francis, 2002; Corbin et al., 2002). Combination of nitrates and sildenafil can result in severe systemic hypotension and death. In sum, Viagra therapy combines pharmacological specificity with specific biological targeting. PDE5 inhibitors inhibit a specific target in a specific, localized environment relatively enriched in the therapeutic target (i.e., PDE5 of the corpus cavernosum) in the context of a circumscribed, temporally and spatially limited, and activated biological process (i.e., NO-induced elevation of cGMP in the penis with consequent effects on vasodilation and erection during periods of sexual activity) (Francis et al., 2001; Corbin and Francis, 2002; Corbin et al., 2002).

The story, of course, is not complete. With increased understanding of PDE5 biology and additional experience with PDE5 inhibitors, we may discover adverse effects arising from long-term or repeated use of these drugs, perhaps related to previously unrecognized downstream effects of cGMP on signaling and metabolic pathways, on "cross-talk" between PDE5 and other PDEs, such as PDE2 and PDE3, as well as on gene expression, including, perhaps, up-regulation of PDE5 itself. Will the presence of individual genetic backgrounds and specific modifier genes teach us limits in the applications of PDE5 inhibitors as well as fundamental new insights into NO/cGMP signaling pathways and vascular smooth muscle physiology? Given the central role of NO, will focus on erectile dysfunction catalyze research and therapeutics related to the biology of NO signaling and, especially, of neuronal and endothelial NO synthases? Perhaps a more fundamental question relates to whether the current use of PDE5 inhibitors has established a new paradigm for PDE inhibitors. As discussed above, in the treatment of erectile dysfunction, Viagra targets a specific, temporally and spatially circumscribed, and activated biological process. Will

successful therapeutic PDE inhibitors depend not only on the development of more potent “family-specific” inhibitors but also, as in the case of sildenafil, on rather precise biological targeting as well as pharmacological specificity? Will successful therapeutics require drugs that target individual PDE family-isoforms in specific cells or subcellular compartments/microdomains in the context of specific, activated cAMP/cGMP signaling pathways?

On the other hand, with increased understanding of these signaling pathways and PDE5 biology, new PDE5 inhibitors and other therapeutic agents may improve treatment of erectile dysfunction and also expand our repertoire of treatable diseases. For example, not only have PDE5 inhibitors proven successful in the treatment of erectile dysfunction but they are also exhibiting promise as vasodilators in the treatment of pulmonary hypertension (Ghofrani et al., 2002).

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