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Editorial Commentary

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The emergence and success of PDE5 inhibitors as effective therapy for erectile dysfunction (ED) is remarkable, considering the intent behind the development of the original compound. Initially designed as an antianginal agent, it quickly became apparent that the first PDE5 inhibitor on the market, sildenafil, displayed erectogenesis as a side effect, and the drug was soon recognized as a potential revolutionary treatment for ED. Subsequent research led to the understanding of the biological and pharmacological roles of NO, cGMP, one specific PDE subtype, PDE5, and PDE5 inhibitors in the regulation of vascular smooth muscle relaxation and vasodilation, and, thereby, penile erection. This exceptional bench-to-bedside transition of PDE5 inhibitors as therapeutic agents for ED has finally allowed realization of 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, replacing non-specific methylxanthine PDE inhibitors such as theophylline in the treatment of disease, in this case, ED.

Despite intensive efforts to develop other PDE inhibitors as therapeutic agents, and despite impressive preclinical data with some PDE inhibitors, only agents targeting PDE5 (including sildenafil, vardenafil (Levitra®), and tadalafil (Cialis®)) have completely fulfilled the dual promises of serving as effective therapeutic agents that selectively inhibit specific PDE families. PDE4 inhibitors, such as cilomilast and rofumilast, have proven to be potent anti-inflammatory agents in many preclinical studies and model systems, and are in Phase III clinical trials as potential therapeutic agents for asthma and chronic obstructive pulmonary disease. PDE3 inhibitors, which enhance myocardial contractility and smooth muscle relaxation and inhibit platelet aggregation, failed in clinical trials of the long-term treatment of cardiac failure. However, milrinone is used for acute and short-term treatment of adult patients hospitalized with refractory and decompensated cardiac failure, and cilostazol has been approved for the treatment of intermittent claudication.

Why is PDE5 inhibition safe and efficacious? The corpus cavernosum is relatively enriched in PDE5, and sildenafil, vardenafil, and tadalafil are potent inhibitors that can be administered orally at concentrations sufficient to inhibit PDE5, with minimal serious side effects related to inhibition of other PDEs or non-PDE targets. For example, transient, mild visual disturbances associated with sildenafil are presumably related to inhibition of the photoreceptor PDE6, which hydrolyzes cGMP and is almost exclusively expressed in the retina. In addition, PDE5 inhibitors are taken as needed, during periods of sexual stimulation/activity. Patients also use them under pharmacologically optimal conditions, where these agents work most effectively in conjunction with active cyclases; in other words, PDE5 inhibitors act in the penis in the presence of augmented local production of cGMP via NO-induced activation of guanylyl cyclase. However, this latter effect is also the basis for one major contraindication of PDE5 inhibitor therapy—concurrent treatment with nitroglycerine or other nitrates, which can result in severe systemic hypotension and death. In sum, PDE5 inhibitor therapy has been very successful because its therapeutic use to treat ED combines both pharmacological specificity as well as rather precise biological targeting. PDE5 inhibitors selectively inhibit a specific therapeutic target (ie, PDE5) in a specific, localized environment relatively enriched in the therapeutic target (ie, the corpus cavernosum) in the context of a circumscribed, temporally and spatially limited, and activated biological process (ie, NO-induced elevation of cGMP in the corpus cavernosum, with consequent effects on vasodilation and penile erection during periods of sexual activity).

The story, of course, is not complete. At this closed symposium, sponsored by Bayer and GlaxoSmithKline, various aspects of the biology and pharmacology of NO/cGMP signaling and PDE5 were discussed, with special emphasis on newer PDE5 inhibitors and potential new therapeutics focused on NO signaling, especially endothelial and neuronal NO synthases. With respect to the latter, Dr Tom Lue discussed a possible role for downregulation of neuronal NO synthase in the pathogenesis of ED secondary to injury suffered during pelvic surgery for cancer of the prostate, bladder, and rectum. Dr Lue presented data suggest-

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These comments were written by Vincent Manganiello, MD, PhD in his private capacity. The views expressed in these comments do not necessarily represent the views of NIH, DHHS or the United States government

ing that in rat models of neurogenic and vasculogenic ED, intracavernous injection of angiogenic and neurotrophic growth factors upregulated neuronal NO synthases (NOS) and facilitated recovery from ED. Such information suggests further study of the potential use of growth factors to enhance recovery of erectile function following radical pelvic surgery.

Dr Arthur Burnett discussed different signaling pathways involved in the activation of neuronal (n) and endothelial (e) NOS, and their distinct and integrated roles in the regulation of penile erection, with nNOS involved in initiation and eNOS involved as the prime facilitator for maximal and sustained erectile response. Understanding mechanisms involved in the regulation of NOS and NO offers potential novel therapeutic targets.

Dr Ching-Shwun Lin discussed his work relating to regulation of gene expression of different PDE5 isoforms (PDE5A1, 5A2 and 5A3), and their differential tissue distributions. Of particular interest were studies describing the presence, in the single PDE5A gene, of a common promoter that directed transcription of all three PDE5A isoforms, as well as an intronic promoter specific for PDE5A2 expression. Both promoters were activated by cAMP and cGMP. Although these results might imply the possibility of tachyphylaxis resulting from chronic sildenafil therapy (or the use of other PDE5 inhibitors), the concentration of sildenafil required to induce PDE5 expression in cell cultures was much higher than that required to produce a clinical response. Dr Lin also discussed studies in rodents suggesting that hypoxia/anoxia downregulated PDE5 expression, suggesting a possible link between recurrent priapism and reduced PDE5 expression.

Dr Jackie Corbin summarized structure/function studies important for understanding how the properties of PDE5 both allow efficient hydrolysis of cGMP as well as potentiate the actions of PDE5 inhibitors. All 11 PDE gene families encode proteins that exhibit a common structural organization, with a conserved catalytic domain in C-terminal portions and divergent regulatory modules and domains in N-terminal portions of the PDE molecules. In the regulatory domains of PDEs 2, 5, and 6 are found homologous, so-called GAF-domains, which contain allosteric sites that bind cGMP with high affinity. In PDE5, binding of cGMP to GAF domains induces conformational changes, that increase affinity of the catalytic site for cGMP and activate PDE5; binding of cGMP to the catalytic site, in turn, increases cGMP-binding to GAF domains. cGMP-binding to GAF domains also allows phosphorylation of PDE5 by cGMP-dependent protein kinase (PKG), which increases affinity of GAF domains for cGMP and stimulates enzyme catalytic activity. Thus, elevation of intracellular cGMP provides negative feedback control and enhances its own destruction via direct, cGMP-induced allosteric activation of PDE5 and indirect activation due to phosphorylation by PKG. On the other hand, inhibition of PDE5 by PDE5 inhibitors can increase cGMP, which binds to GAF domains; this, in turn, increases binding of inhibitors to the catalytic site, thus providing positive feedback with respect to the potentiation of cGMP accumulation by PDE5 inhibitors. These interactions between catalytic sites and noncatalytic, allosteric GMP-binding sites have important implications for efficacy, potency, and pharmacokinetics of PDE5 inhibitors. In this regard, it will be important to elucidate: (1) the functional consequences of the virtual 'sequestration' of cGMP at high-affinity binding sites in PDE5, with respect to availability of cGMP to PKG and other cGMP binding proteins in tissues enriched in PDE5; (2) how binding of cGMP to allosteric sites and how phosphorylation of PDE5 alter inhibitor binding at the catalytic site (and *vice versa*); and (3) how cGMP-induced, high-affinity binding of inhibitors to PDE5 catalytic sites regulates the duration of effects of inhibitors and their clearance from cells and tissues.

Dr Hengming Ke described attempts to gain insight into the binding of sildenafil (and, by inference, other PDE5 inhibitors) to the catalytic site of PDE5 by modeling interactions of PDE4 and PDE5 inhibitors within the three-dimensional crystal structure of the catalytic pocket of PDE4. As might be expected from conservation of the catalytic domain among all PDEs, sildenafil readily 'fit' into the catalytic site of PDE4, but specific PDE4 residues, not found in PDE5, blocked critical hydrophobic interactions between sildenafil and the PDE4 catalytic site. This type of information, gathered by modeling drug interactions with three-dimensional crystal structures, can perhaps provide insight into relationships between specific residues and/or conformational changes and inhibitor selectivity, and thus contribute tools for the design of novel subtype-selective inhibitors.

Dr Rick Cote summarized findings that, of the 11 PDE families, PDE5 shares the greatest similarities with PDE6 over any other family in terms of both amino-acid sequences and biochemical and pharmacological properties. Both bind Zn^{2+} with high affinity at the catalytic site and prefer cGMP as substrate, although the catalytic efficiency of PDE6 far exceeds that of PDE5. Like PDE5, PDE6 contains GAF domains that bind cGMP with high affinity. In contrast to PDE5, which is phosphorylated/activated by PKG, activation of PDE6 involves displacement of its inhibitory subunit, PDE6γ, from the PDE6 catalytic site by the activated heterotrimeric G protein, transducin. PDE6 is virtually specifically expressed in the retina, where it undergoes post-translational carboxymethylation and isoprenylation of the C-terminal portion of its catalytic subunits. This post-translational modification is unique for PDE6 among the 11 PDE gene families and is responsible for the association of PDE6 with photoreceptor membranes.

Perhaps most important for this symposium and for future development of specific PDE5 inhibitors is the similarity in catalytic sites of PDEs 5 and 6 with respect to their interactions with existing inhibitors; for example, sildenafil inhibits PDE6 with only an eight- to 10-fold lower potency than PDE5. This most likely accounts for transient visual disturbances associated with sildenafil therapy. A more detailed understanding of the molecular differences between, and the architecture of, PDE5 and PDE6 catalytic sites will be required for the rational design of more specific drugs that will more completely discriminate between PDE5 and PDE6.

In considering the side effects of PDE5 inhibitors, it is important to consider not only those that occur due to interaction of the drugs with other PDEs (eg, PDE6) and/or non-PDE targets, but also those that might arise because of perturbations of cGMP-signaling pathways. As discussed by Dr Donald Maurice, some effects of PDE5 inhibitors may reflect 'crosstalk' between PDE5 and other PDEs, especially PDE2 and PDE3, which result from increased concentrations of cGMP produced via inhibition of PDE5. On the other hand, in target tissues enriched in PDE5, such as platelets and vascular smooth muscle myocytes, consideration must also be given to the presence and phenotypic modulation of other PDEs, which also regulate cellular concentrations of cAMP and cGMP and thereby might indirectly modulate activity and/or expression of PDE5 and, consequently, effects of PDE5 inhibitors.

Thus, and as pointed out by various participants at the symposium, in the face of increasing understanding of PDE5 biology and experience with PDE5 inhibitors, we may discover adverse effects that arise from widespread and chronic use of these drugs. These effects could perhaps be related to downstream effects of cGMP on signaling and metabolic pathways, to 'cross-talk' between PDE5 and other PDEs, as well as to downstream modulatory effects on expression of genes, possibly including upregulation of PDE5 itself. Such considerations may limit the application of PDE5 inhibitors. On the other hand, information from research studies similar to those discussed at this symposium and newer, more effective PDE inhibitors and other novel therapeutic agents (targeting NO/cGMP signaling systems and pathways, and/or especially eNOS and nNOS) may improve the treatment of sexual dysfunction (in both men and women) and also expand our repertoire of treatable diseases. For example, PDE5 inhibitors potentially may prove to be successful in the treatment of other disease states such as pulmonary hypertension. Other PDE-specific inhibitors may ultimately be useful for the treatment of asthma (PDE3, PDE4), congestive heart failure (PDE3, PDE4), osteoporosis (PDE4), and certain inflammatory diseases (PDE4).

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