

Molecular Biology and Pharmacology of PDE-5-Inhibitor Therapy for Erectile Dysfunction

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In light of newly emerging selective phosphodiesterase type 5 (PDE-5) inhibitors, it is necessary to understand the basic biochemical and pharmacological principles of PDE-5 inhibitor action (Corbin and Francis, 1999).

Coffee was discovered about 2000 years ago in Ethiopia by goat herders. The active ingredient in coffee is caffeine. In 1960, the first mechanism discovered for caffeine was PDE inhibition, although this drug has effects on other non-PDE proteins that cause some of its symptoms. Thus, caffeine was the first known PDE inhibitor. It was later shown that at least 11 mammalian PDE families exist, and that caffeine inhibits most of them. Moreover, caffeine is not a very potent inhibitor of these PDEs. For decades, nonselective natural PDE inhibitors such as caffeine and theophylline, as well as thousands of synthetic PDE inhibitors, have been used to investigate physiological effects of cyclic nucleotides and PDE activity. Many have also been tested for therapeutic effects. As a group, many of these inhibitors lack potency and specificity because they block PDE catalytic activity in several PDEs, including PDE-5.

Major research efforts have led to the production and development of compounds that are more selective and potent in inhibiting particular PDEs (Corbin and Francis, 2002). Sildenafil (Viagra) is the first commercialized compound in this class. This class has been recently joined by vardenafil (Levitra) and tadalafil (Cialis). Vardenafil has a similar structure as sildenafil, but tadalafil structure is different (Figure 1). As will be discussed below, vardenafil is more potent than sildenafil *in vitro* to inhibit PDE-5, and this difference is explained by very slight differences (see arrows) in molecular structures of these 2 compounds. Part of the ring structure of sildenafil or vardenafil is similar to that of caffeine. This same ring structure is similar to a ring structure in cyclic guanosine monophosphate (cGMP). This is important because these drugs are competitive inhibitors of cGMP for PDE-5. Even though the tadalafil structure differs significantly

from those of the other 2 inhibitors, its molecular mechanism of action is believed to be similar.

The normal pathway for penile erection (Figure 2) may not work properly if the cGMP level in corpus cavernosum smooth muscle cells is not elevated sufficiently or if relaxation of smooth muscle in this tissue is deficient (Jeremy et al, 1997; Corbin and Francis, 1999). PDE-5 inhibitors enhance erectile function during sexual stimulation by penetrating into smooth muscle cells and inhibiting PDE-5. This results in decreased degradation of cGMP, which maintains sufficient cellular levels of cGMP in both corpus cavernosum and the vessels supplying it. This increases relaxation of the smooth muscle, which dilates the corporeal sinusoids resulting in increased blood flow, allowing an erection to occur. Sildenafil or one of the other PDE-5 inhibitors foster accumulation of the cell cGMP by competitively inhibiting PDE-5, which triggers penile erection. PDE-5 inhibitors do not increase the nitric oxide level, but they potentiate the nitric oxide effect to stimulate erection. Without sexual arousal, this effect activates the nerve-nitric oxide pathway, these inhibitors are ineffective.

PDE-5 was discovered in our laboratory. A cartoon of the enzyme structure is shown in Figure 3. Each of the 2 subunits of PDE-5 has a catalytic domain and a regulatory domain. The catalytic domain, but not the regulatory domain, is the target of PDE-5 inhibitors. The catalytic domain contains a binding site for cGMP. When cGMP occupies this site, the catalytic machinery (paired black structures), which is located very near the catalytic binding site, is brought into close proximity and breaks the cyclic phosphate bond of cGMP to form linear 5'-GMP. Sildenafil or other PDE-5 inhibitors can also occupy the catalytic site, thus blocking access to cGMP. In fact, sildenafil occupies the site about 1000 times more avidly than does the natural substrate, cGMP. However, the PDE-5 inhibitors are not broken down by the catalytic machinery. Occupation of the catalytic site by these inhibitors competitively inhibits cGMP breakdown because cGMP cannot bind to gain access to the catalytic machinery. Inhibition of cGMP breakdown leads to elevation of cGMP in smooth muscle cells of the penile corpus cavernosum, resulting in relaxation of the muscle and penile erection.

Although the catalytic domain of PDE-5 is the direct target of PDE-5 inhibitors, certain features of the regulatory domain impact the PDE-5 inhibitor actions on the

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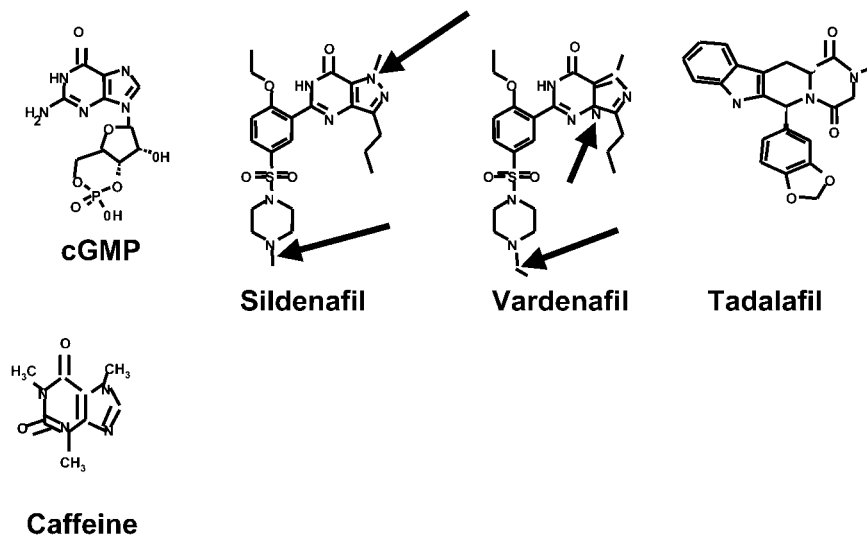


Figure 1. Molecular structures of sildenafil, vardenafil, and tadalafil as compared with those for caffeine and cGMP. Arrows denote differences between structures of sildenafil and vardenafil.

enzyme (Corbin et al, 2000; Corbin et al, 2003). This domain contains allosteric cGMP-binding sites as well as a phosphorylation site for regulation of the enzyme. When cGMP binds to the allosteric sites, the cGMP is not degraded as it is in the catalytic site, but enzyme functions are activated by the binding reaction. In this case, cGMP binding is stimulatory for PDE-5 inhibitor binding at the catalytic site. This means that when cGMP is elevated in smooth muscle cells after a patient takes Viagra or other PDE-5 inhibitor, this should stimulate the catalytic site to bind more of the inhibitor. That is, the PDE-5 inhibitor stimulates its own efficacy. For example, were it not for this built-in enzyme mechanism, a 200-mg dose rather than a 100-mg dose of a particular PDE-5 inhibitor might be required to induce penile erection in a particular patient. In addition to cGMP binding to the allosteric sites, phosphorylation of PDE-5 by cGMP-dependent protein kinase could also affect PDE-5 inhibitor action.

Assuming all other factors are equal, the higher the affinity (potency) of a PDE-5 inhibitor for PDE-5, the lower the expected dose of the inhibitor that will be needed (Corbin and Francis, 2002). This concept of potency can be assessed by measuring the concentration of a particular PDE-5 inhibitor in vitro that inhibits PDE-5 activity by 50% and is known as the IC_{50} . Highly potent drugs are expected to have affinities (IC_{50} values) in the nanomolar range. However, as discussed below, factors such as pharmacokinetics have strong impacts on the dose required. Higher potency does not mean that a PDE-5 inhibitor has a greater clinical effect, but that less of it is needed for the desired effect. For the PDE-5 inhibitors, less vardenafil is required than sildenafil or tadalafil to achieve the same degree of in vitro PDE-5 inhibition. Var-

denafil is therefore more potent than are sildenafil and tadalafil, although not necessarily more efficacious. On the basis of in vitro potencies, it would be predicted that a lower dosage of vardenafil than sildenafil would be required to cause penile erection in men. This appears to be the case because 5- to 20-mg doses of vardenafil (Levitra) are used compared with 25- to 100-mg doses of sildenafil (Viagra).

There are methods in addition to classical IC_{50} to measure potency of a drug. In our laboratory, we have recently measured the strength of binding of radiolabeled PDE-5 inhibitors to PDE-5 (Corbin et al, 2003). As compared with IC_{50} , measurement of binding is a more direct method of determining potency (Figure 4). The K_D of a PDE-5 inhibitor obtained by this approach should approach the IC_{50} of the same PDE-5 inhibitor. In fact, our results show that the K_D for sildenafil, vardenafil, or tadalafil approximates the IC_{50} for each compound. The finding that the value of K_D approaches the value of IC_{50} for each inhibitor suggests that these compounds do not bind to an appreciable extent to sites on PDE-5 other than the catalytic domain. The approach of determining K_D not only provides a new tool to measure PDE inhibitor potency but may also reveal new properties of PDE-5 and PDE-5 inhibitors which were not possible to study previously.

On the basis of comparative IC_{50} values, the potency of sildenafil is about 1 million times higher than that of caffeine (Corbin and Francis, 1999). Other experimental PDE inhibitors have intermediate potencies. According to literature values, the in vitro biochemical potencies of the 3 new commercial PDE-5 inhibitors are within the same range of each other, albeit vardenafil is slightly more potent than the other 2 inhibitors (Corbin and Francis,

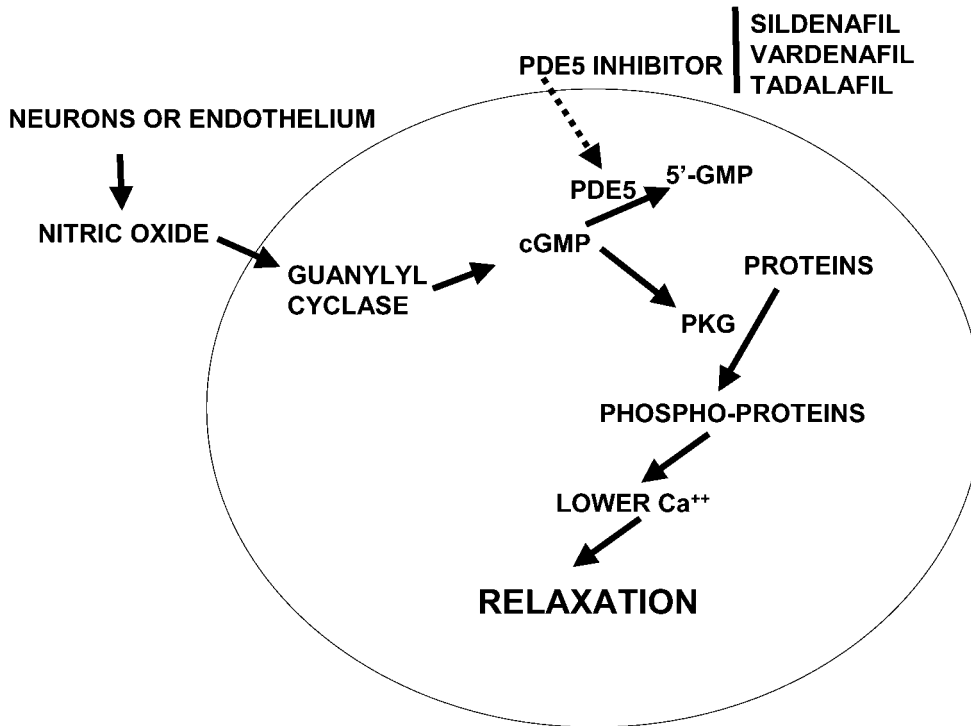


Figure 2. Regulation of penile corpus cavernosum smooth muscle relaxation and effect of PDE-5 inhibitors.

2002). The in vitro potency of a PDE-5 inhibitor is not the same as efficacy. As discussed below, efficacy is based on the actual in vivo (clinical) effects of the inhibitor.

The biochemical selectivity of an inhibitor for PDE-5

is a key factor in determining its side-effect profile (Corbin and Francis, 2002). Once a large enough separation exists between the affinity (IC_{50}) of the inhibitor for PDE-5 and its affinity for nontarget PDEs (or other proteins), the less likely it is that it can achieve sufficient plasma

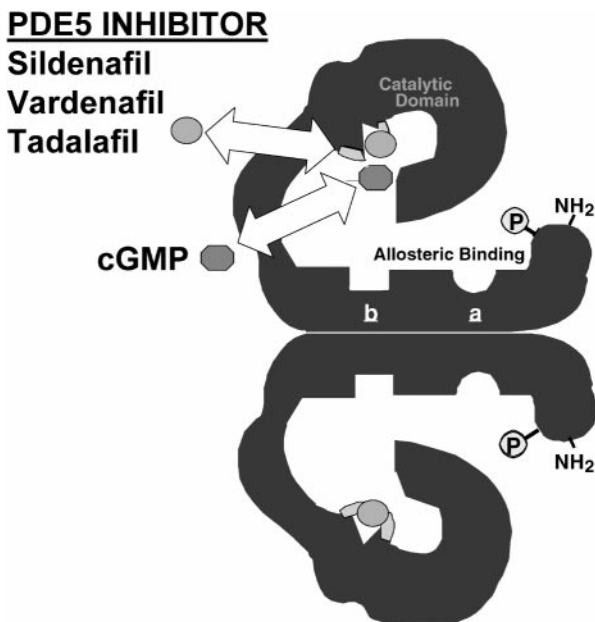


Figure 3. Cartoon of PDE-5 molecular structure showing molecular effect of PDE-5 inhibitor on the catalytic domain. P indicates phosphate; NH₂, amino terminus.

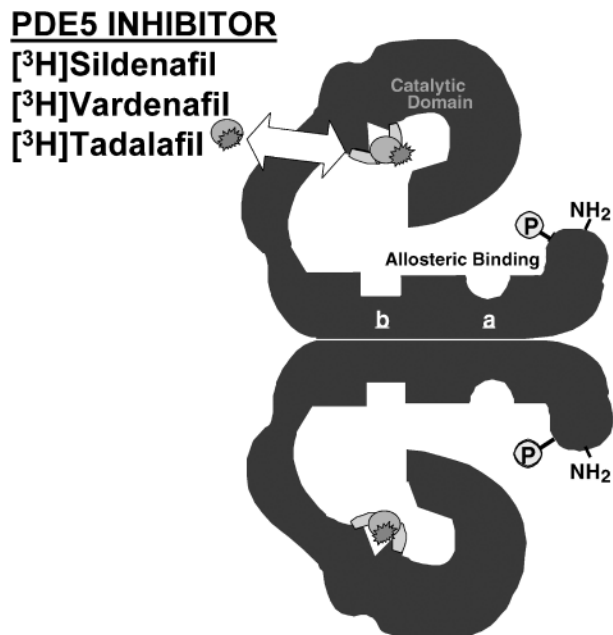


Figure 4. Cartoon of PDE-5 molecular structure showing binding of radiolabeled PDE5 inhibitor to the catalytic domain. P indicates phosphate; NH₂, amino terminus.

concentrations to activate the nontarget site at therapeutic doses. For PDE-5 inhibitors, selectivity is usually expressed in terms of potency (IC_{50}) to inhibit PDE-5 as opposed to inhibiting any others in the PDE family. The selectivity is computed by dividing the IC_{50} s of the 2 compounds that are compared. The mammalian PDEs are composed of 11 families of enzymes that catalyze the termination of second messenger activity in cells by breaking the phosphodiester bond of either cyclic adenosine monophosphate or cGMP. Eleven distinct families have been identified (PDE-1 to PDE-11) that are known or implicated in a broad range of cellular functions. PDE-5 is cGMP-specific and is present in relatively high concentrations in the smooth muscle of corpora cavernosum of the penis. Sildenafil and vardenafil cross-react slightly with PDE-6 (ie, their IC_{50} s for PDE-5 are only 4- to 10-fold lower than those for PDE-6). This may explain the complaint of some patients that sildenafil causes visual disturbances. Tadalafil cross-reacts with PDE-11 to some extent, but the consequences of this effect are unknown. None of the 3 PDE-5 inhibitors cross-reacts to a large extent with any of the other PDEs except for PDE-6 and PDE-11 (ie, the IC_{50} s of these compounds for PDE-5 are more than 1000 times lower than those for most of the other PDEs). Except for visual disturbances, the other reported side effects of PDE-5 inhibitors (headaches, flushing, slight lowering of blood pressure, etc) are likely caused by PDE-5 inhibition in smooth muscle tissues outside the penile corpus cavernosum.

In addition to biochemical properties discussed above, pharmacokinetic properties of PDE-5 inhibitors (ingestion, movement in the circulation, tissue uptake, elimination) have great impact on efficacy (Corbin and Francis, 2002). There are several common pharmacokinetic parameters that can be measured and quantified that describe bodily distribution of a PDE-5 inhibitor. The bioavail-

ability, maximum plasma concentration (C_{max}), the time (T_{max}) required for attaining C_{max} , and time ($t_{1/2}$) required for elimination of one half of the inhibitor from plasma are all important factors. Sildenafil, vardenafil, and tadalafil have broadly similar C_{max} and T_{max} , but the $t_{1/2}$ of tadalafil is considerably longer than that of the other 2 PDE-5 inhibitors. The extended $t_{1/2}$ of tadalafil could provide a longer therapeutic effect, which may be preferred for spontaneous sexual activity but could expose the patient to greater risk of side effects.

The rate of exit of a PDE-5 inhibitor from smooth muscle cells should also be considered because this could affect its duration of action. Disappearance of the inhibitor from plasma may imply, but does not prove, its disappearance from the cells in which it produces its effects. Because the inhibitor binds tightly to PDE-5 in these cells, this could significantly retard its exit from these cells and prolong effects of PDE-5 inhibitors in patients. Studies of clearance of PDE-5 inhibitors from plasma are documented, but studies of clearance of these inhibitors from smooth muscle cells are rare.

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