Therapeutic Potential of Phosphodiesterase 5 Inhibition for Cardiovascular Disease

Thorsten Reffelmann, MD; Robert A. Kloner, MD, PhD

Several families of phosphodiesterases (PDE), the enzymes catalyzing hydrolysis of cyclic (c) nucleoside monophosphates, namely, 3′5′-cAMP (cAMP) and 3′5′-cGMP (cGMP), have been identified and characterized in recent years. Since selective pharmacological inhibitors of isoform 5 (a cGMP-specific PDE), such as sildenafil, tadalafil, or vardenafil, have become available, the physiological function and interaction of different PDE isoforms, their tissue distribution, and the therapeutic potential of PDE 5 inhibition have attracted increasing interest.

The discovery in 1989 of sildenafil, a highly selective inhibitor of PDE 5, was the result of extensive research on chemical agents targeting PDE 5 that might potentially be useful in the treatment of coronary heart disease. Initial clinical studies on sildenafil in the early 1990s were not promising with respect to its antiangiotal potential. However, a remarkable side effect was reported by a number of volunteers participating in these investigations; sildenafil seemed to enhance penile erections, which soon thereafter became the main focus of further studies.

PDE 5 is found in high concentration in smooth muscle cells of the corpora cavernosa. Relaxation of smooth muscle cells of penile arteries, arterioles, and sinusoids in response to sexual stimulation results in an increase in blood volume within the rigid tunica albuginea and compression of draining venules, and hence a penile erection. Relaxation of the arterial smooth muscles occurs after stimulation of the enzyme guanylate cyclase by nitric oxide released from nonadrenergic-noncholinergic nerves and endothelial cells, with subsequent formation of cGMP (Figure 1). cGMP activates a cGMP-dependent protein kinase, which leads to phosphorylation of ion channels with the final consequence of a reduced cytosolic calcium concentration. Thus, sildenafil inhibiting the breakdown of cGMP was found to be effective in a high percentage of male patients suffering from erectile dysfunction.

However, as PDE 5 is expressed in various other tissues, such as the arterial vasculature, including pulmonary and coronary arteries, venous vasculature, skeletal muscles, visceral and tracheobronchial muscles, and platelets, effects in patients are complex. In this review, we try to outline the effects of PDE 5 inhibition in addition to its efficacy in erectile dysfunction. Potential implications, and whether some of these effects are therapeutically exploitable, will be discussed. Because of limited information on newer agents, the main conclusions will be drawn from results obtained with sildenafil treatment.

Selectivity of PDE 5 Inhibitors and Tissue Distribution of PDE Isoforms

To date, at least 11 isoforms of PDE have been discovered, and the differential distribution of PDE isoforms in various tissues as well as the selectivity of pharmacological agents is the basis for potential tissue-specific effects of PDE inhibitors. Information regarding the functional aspects of some isoforms, especially isoforms 7 to 11, is still fragmentary, and the following discussion will focus on PDE 1 to 6.

Sildenafil is highly selective for the cGMP-hydrolyzing isoform 5 with a half-maximal inhibition (IC₅₀) of PDE 5 activity at a concentration of 3.5 nmol/L, followed by IC₅₀ values of 34 to 38 nmol/L for PDE 6 (cGMP-hydrolyzing PDE in the retina) and 280 nmol/L for PDE 1 (cAMP- and cGMP-hydrolyzing PDE isoform) (Figure 1). The cAMP-hydrolyzing PDE 3 and 4 and the cAMP- and cGMP-hydrolyzing isoform 2, as well as PDE 7 to 11, are inhibited by sildenafil with an IC₅₀ of >2600 nmol/L (Table).

For vardenafil, lower absolute concentrations are required to inhibit PDE 5; however, relative selectivity for PDE 5 is similar to that of sildenafil (Table).

Tadalafil, the third available selective inhibitor of PDE 5, is characterized by 2 potentially important differences, as follows. (1) At concentrations effective in inhibiting PDE 5, tadalafil has lower inhibitory effects on PDE 6, which might be important with respect to ophthalmologic side effects (see below). (2) Compared with PDE 11 (cGMP- and cAMP-hydrolyzing isoform), tadalafil is only 5-fold selective for PDE 5. Thus, PDE 11 inhibition might occur at clinically relevant doses of tadalafil. Whether and how this translates into side effects is not yet known.

Wallis et al measured PDE isoenzyme activities by fast protein liquid chromatography, identifying the isoenzymes on the basis of salt concentration for elution, substrate specificity (cAMP and cGMP), and antibodies against PDE isoenzymes.

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239
Tissue from human corpus cavernosum contained high activities of PDE 2, 3, and 5, whereas human cardiac ventricles mainly contained PDE 1, smaller peaks of PDE 2 and 3, but not PDE 5. Human saphenous veins contained PDE 1, 4, and 5, whereas PDE 1 to 5 were demonstrated in human mesenteric arteries. Human platelets demonstrated PDE 3 and 5 expression, and sildenafil enhanced antiaggregatory effects of a nitric oxide donor. Furthermore, they demonstrated accumulation of cGMP in isolated sections of canine coronary arteries after incubation with sildenafil. In the human pulmonary circulation, the isoforms 1, 3, 4, and 5 seem to be involved in regulating pulmonary resistance.

A peculiarity of the cAMP-hydrolyzing PDE 3 is that it can be inhibited by cGMP. Therefore, in tissues containing both PDE 3 and 5, sildenafil could indirectly increase cAMP concentrations via inhibition of PDE 3 by cGMP (Figure 1). In isolated dog trabeculae carnaeae stimulated by isoprenaline, sildenafil did not exhibit effects on contractility, in contrast to the selective PDE 3 inhibitor milrinone, which argued against a significant PDE 5 activity in canine cardiomyocytes. However, Senzaki et al reported evidence for PDE 5A expression in canine cardiomyocytes, as assessed by immunohistochemistry, and also a modulatory effect of PDE 5 inhibition on β-adrenergic signaling in the myocardium. In tissue obtained from human auricles, Stief et al demonstrated increased cAMP (and cGMP) concentrations in response to sildenafil, which could be the result of such a cross-talk between the 2 isoforms PDE 5 and 3 or of the partial inhibition of PDE 1 by sildenafil.

Direct and Indirect Effects of PDE 5 Inhibition on the Myocardium

Soon after the approval of sildenafil for the treatment of erectile dysfunction, several reports of adverse cardiac events in patients on sildenafil raised concerns about its safety in cardiovascular disorders. Most of the risk factors for coronary artery disease are highly prevalent in patients with erectile dysfunction. In addition, sexual intercourse is associated with a slightly increased risk of a cardiac event. Therefore, potentially harmful effects of sildenafil on the heart need to be considered very carefully. Nonetheless, in randomized trials, no increase in cardiovascular events was demonstrated, and a recent report from the U.S. Food and Drug Administration did not find a higher incidence of cardiac events than what would have been expected for the patient population. Recent analyses of adverse events in phase 3 trials investigating tadalafil and vardenafil reported a similar profile and incidence of side effects.

Several theoretical concepts could explain a potential for reduced tolerance of the heart to ischemia or a lowered threshold for arrhythmia. Increased sarcoplasmatic concentrations of cAMP, occurring either as a result of partial PDE 1 inhibition by sildenafil or the cross-talk between PDE 5 and 3 discussed above, could increase myocardial contractility. Sugiyama et al reported an inhibitory effect of sildenafil on the cAMP-hydrolyzing activity of canine and bovine cardiac ventricular membrane preparations, which—if similar in the human heart—would support this concept. Also, Senzaki et al reported evidence for PDE 5A expression in canine cardiomyocytes, which is still controversial with respect to human cardiomyocytes. Recently, a lower threshold for ventricular tachycardias was demonstrated in a pacing model of isolated swine right ventricles treated with a high dose of sildenafil combined with a nitric oxide donor. On the other hand, cGMP-elevating interventions may also exhibit negative inotropic effects that could antagonize effects of elevated cAMP. In a rabbit in vivo model of ischemia/reperfusion, we did not find a higher incidence of arrhythmia or increased

### Tissue Distribution of Different PDE Isoforms and Selectivity of Sildenafil, Vardenafil, and Tadalafil

<table>
<thead>
<tr>
<th>PDE Isoform</th>
<th>Main Tissue Distribution*</th>
<th>IC50, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vascular smooth muscle, cardiomyocyte, brain</td>
<td>280–281</td>
</tr>
<tr>
<td>2</td>
<td>Vascular smooth muscle, cardiomyocyte, brain, corpus cavernosum</td>
<td>&gt;30 000</td>
</tr>
<tr>
<td>3</td>
<td>Vascular smooth muscle, cardiomyocyte, corpus cavernosum, platelets</td>
<td>16 200</td>
</tr>
<tr>
<td>4</td>
<td>Vascular smooth muscle, cardiomyocyte</td>
<td>7680</td>
</tr>
<tr>
<td>5</td>
<td>Corpus cavernosum, vascular smooth muscle, skeletal muscle, platelets</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>Retina</td>
<td>34–38</td>
</tr>
<tr>
<td>7–10</td>
<td>Various</td>
<td>≥2610</td>
</tr>
<tr>
<td>11</td>
<td>Skeletal muscle, heart, vascular smooth muscle</td>
<td>2730</td>
</tr>
</tbody>
</table>

*Mainly human tissues.
Adapted from references 2 and 8.
contractility with sildenafil treatment (Reffelmann and Kloner, unpublished observations, 2003).

Phillips et al.26 reported pronounced sympathetic activation after 100 mg of sildenafil in healthy volunteers. Given the presence of PDE 5 in parts of the brain, they interpreted their findings as a potential central nervous effect.1 Such an increased sympathetic stimulation could also lower the threshold for arrhythmia or the tolerance to ischemia, or limit the additional compensatory adrenergic activation in response to any blood pressure–lowering intervention.26

Geelen et al.27 reported that sildenafil blocks the rapid component of the inward rectifier potassium channel and might prolong cardiac action potential. This could result in a proarhythmic tendency or, via higher Ca++ transients, increased myocardial oxygen consumption. But the observed effects required high doses of sildenafil, probably above the therapeutic level.28 Other investigators observed action potential shortening effects of sildenafil.29 In our rabbit in vivo model, 1.45 mg/kg sildenafil intravenously did not lengthen QT/QTc duration during ischemia or promote ventricular arrhythmia (Reffelmann and Kloner, unpublished observation, 2003).

As observed by Piccirillo et al, sildenafil treatment in patients with chronic heart failure was not accompanied by lengthening of QT/QTc duration, but a reduction of vagal, and an increase of sympathetic, heart rate modulation seemed to follow sildenafil treatment, probably as a result of the slight blood pressure reduction.30

Interestingly, a recent publication by Ockaili et al.31 reported a pronounced infarct size-reducing effect of 0.7 mg/kg sildenafil in an in vivo rabbit model of coronary occlusion. As this effect could be blocked by 5-hydroxydecanoate, an inhibitor of mitochondrial ATP–sensitive potassium channels, the authors suggested that cardioprotection by sildenafil occurred by activation of these potassium channels, mimicking ischemic preconditioning. However, the exact mechanisms and potential clinical implications require further investigations.

In summary, several experimental studies suggested potential direct and indirect effects of PDE 5 inhibition on the myocardium; however, to date there is no clear evidence in support of either detrimental or beneficial cardiac effects of PDE 5 inhibition in clinical circumstances.

Effects of PDE 5 Inhibition on Systemic Arterial Blood Pressure

Blood pressure–lowering effects of PDE 5 inhibitors are dependent on the degree of activation of the nitric oxide–guanylate cyclase pathway. Therefore, combination with any agent acting as a nitric oxide donor is contraindicated in the treatment with PDE 5 inhibitors, as the accumulation of cGMP can lead to life-threatening hypotension.32 Recent guidelines have recommended a 24-hour time interval between the administration of any nitric oxide donor and sildenafil, or vice versa.22

Except when used in conjunction with nitric oxide donors, sildenafil, tadalaafil, and vardenafil21 result in only mild reduction of arterial blood pressure. Zusman et al.15 reported a non–dose-dependent reduction of diastolic and systolic blood pressure after sildenafil by 7 to 10 mm Hg without changes in heart rate. In hypertensive patients, effects of sildenafil on blood pressure were also small,33 and several studies demonstrated that the combination with a wide range of antihypertensive drugs (with the exclusion of nitric oxide donors), such as calcium-channel blocker, β-blocker, angiotensin-converting enzyme inhibitors, or α1-antagonists, is well tolerated.34

Effects of PDE 5 Inhibition on Coronary Circulation

Wallis et al.2 demonstrated accumulation of cGMP in canine coronary arterial segments after incubation with sildenafil, indicating that breakdown of cGMP depends at least in part on PDE 5 activity in coronary arteries. But PDE 1 to 4 were also identified in vascular smooth muscle cells.2 In bovine and porcine coronary arteries, 73% to 80% of the total cGMP-hydrolyzing activity was attributed to PDE 1.35 Thus, it is likely that with sildenafil treatment, PDE 1 activity still allows a significant breakdown of cGMP. Chen et al.36 investigated the effects of 2 mg/kg oral sildenafil on nonischemic coronary flow in a chronically instrumented dog model. Given the small increase of myocardial blood flow at rest and during exercise with sildenafil, they concluded that resistance vessel dilation in response to PDE 5 inhibition contributes little to the increase of myocardial blood flow during exercise or reactive hyperemia.36

Concerns about the safety of sildenafil in patients with ischemic heart disease led to the hypothesis that a coronary steal phenomenon, shifting blood flow from the ischemic myocardium to the nonischemic tissue, could be responsible for some of the observed cardiac events. However, animal models did not support such a concept.37,38 Herrmann et al.39 measured coronary flow before and after oral sildenafil by means of an intracoronary Doppler wire in patients with coronary artery disease. Sildenafil did not alter coronary flow at rest in stenosed arteries and arteries without angiographically high-grade stenoses. Importantly, it increased coronary flow reserve, measured after intracoronary adenosine, to a similar extent in stenosed and nonstenosed arteries (by 13%). Using a crossover design, Arruda-Olson et al.40 did not find any difference in anginal symptoms, maximal exercise duration, and extent of exercise-induced ischemia between patients treated with sildenafil and those treated with placebo. Similarly, recent investigations demonstrated that the ability to exercise among patients with coronary artery disease is not impaired with vardenafil treatment41 or with tadalaafil treatment.42 After vardenafil treatment, time to ischemic threshold during exercise-tolerance testing seemed to be slightly but significantly prolonged in these investigations.41 A recent study described a small beneficial effect on ST-segment depression during exercise testing in patients with known coronary artery disease also after sildenafil treatment.43

These studies demonstrate that coronary hemodynamics and circulation are not unfavorably altered after treatment with sildenafil and other PDE 5-inhibitors; indeed, coronary blood supply may experience a slight improvement. Nevertheless, sildenafil is not a classical antianginal agent.
However, an interesting field, which might have some clinical impact, is related to endothelial dysfunction. With dysfunctional endothelium, the release of nitric oxide by the endothelium is significantly compromised, which can result in abnormal vasomotion during exercise, pacing, or exposure to cold. Application of acetylcholine—in healthy arteries a powerful vasodilator because of the release of nitric oxide from the endothelium—can lead to paradoxical vasoconstriction, as constrictor effects of muscarinic receptors on vascular smooth muscles predominate over endothelial nitric oxide release. The study by Halcox et al demonstrated that especially coronary arterial segments that contracted after application of acetylcholine, denoting endothelial dysfunction, dilated after application of acetylcholine combined with sildenafil treatment, suggesting that the inhibition of cGMP breakdown by sildenafil could at least in part compensate for reduced nitric oxide–related cGMP production due to endothelial dysfunction. Katz et al demonstrated a significant increase in endothelium-dependent flow-mediated vasodilation of the brachial artery in patients with congestive heart failure after 25 or 50 mg oral sildenafil. These findings warrant further studies to determine whether sildenafil might be beneficial in certain subgroups of patients with endothelial dysfunction. It seems worthwhile to examine whether, for instance, patients with vasospastic angina or diffuse coronary microvessel disease, both conditions that were shown to be accompanied by significant endothelial dysfunction, benefit from pharmacological inhibition of PDE 5.

**Effects of PDE 5 Inhibition on Pulmonary Vasculature**

In human pulmonary circulation, the isoforms 1, 3, 4, and 5 of PDE seem to be involved in regulating pulmonary resistance. Thus, the blood pressure–lowering effects of sildenafil in the pulmonary circulation are of special interest. Zhao et al demonstrated that 100 mg of oral sildenafil markedly reduced the rise in arterial pulmonary pressure in response to breathing 11% inspiratory oxygen in healthy volunteers, which paralleled their findings in a mouse model of hypoxia-induced pulmonary hypertension. In a lamb model of acute pulmonary hypertension, inhalation of nebulized sildenafil reduced pulmonary artery pressures significantly and had a synergistic effect with inhalation of nitric oxide, whereas pulmonary right-to-left shunt volume did not increase after sildenafil. However, a recent study performed in normal pigs described an increase in right-to-left shunt volume and subsequently a decrease of systemic arterial oxygen tension after sildenafil, along with reduced pulmonary arterial pressure after sildenafil.

These experimental data suggest that at least in certain conditions with increased pulmonary arterial pressure, PDE 5 inhibition might be of benefit, and the possibility of additional inhalative application of other agents used to lower pulmonary artery pressure might provide a unique approach to achieve site-specific, additive, or even overadditive effects.

Michelakis et al investigated the effect of oral sildenafil in patients with severe primary or secondary pulmonary hypertension who were evaluated for potential heart-lung transplantation. They reported a reduction of pulmonary vascular resistance that was similar in extent to that observed after inhaled nitric oxide. In addition, sildenafil slightly increased cardiac index and decreased pulmonary capillary wedge pressures. The combination of inhaled nitric oxide and sildenafil seemed to be effective in a synergistic manner. Wilkens et al reported additive effects of inhaled iloprost with 25 mg oral sildenafil in lowering pulmonary artery pressure without major adverse events in a series of patients with primary pulmonary hypertension. Comparison of inhaled nitric oxide in combination with either intravenous epoprostenol or with oral sildenafil in patients with pulmonary hypertension due to fibrotic lung disease revealed a marked reduction of pulmonary arterial pressure by both treatments; however, a decreased ratio of pulmonary to systemic vascular resistance was only measured in patients who received nitric oxide and sildenafil. Importantly, the ventilation/perfusion mismatch, and subsequently the right-to-left shunt, deteriorated with epoprostenol/nitric oxide, but the ventilation/perfusion mismatch was unaltered with a sildenafil/nitric oxide combination, which was accompanied by an even slight reduction of right-to-left shunting. If achievable on a long-term basis, these effects on the pulmonary circulation might favorably influence symptoms, similar to the improved exercise capacity in patients with congestive heart failure, as reported by Bocchi et al.

Besides classical primary pulmonary hypertension and pulmonary hypertension due to cardiac disease, some benefit might also exist in pulmonary hypertension of relatively rare etiology, or postoperative pulmonary hypertension, and difficult weaning problems in mechanical respiration. The weaning of inhaled nitric oxide, which is often followed by a rebound phenomenon, might especially be a target for PDE 5 inhibition. However, these sporadic reports are based on small numbers of patients and need further investigations.

**Sildenafil: Main Side Effects, Situations Requiring Special Caution, and Some Other Diseases to Think About**

The most commonly reported side effects of sildenafil can be attributed to vasodilation, such as flushing, nasal congestion, headache (16%), dizziness and hypotension, or relaxation of the lower esophageal sphincter resulting in dyspepsia and reflux-related symptoms. Some cases of esophageal ulcers were reported in patients on sildenafil, and interestingly, sildenafil seemed to lower esophageal sphincter tone even in idiopathic achalasia. Visual abnormalities such as blurred vision or increased light perception might be related to partial vision or increased light perception might be related to partial inhibition of PDE 6 in the cones and rods of the retina and were reported to be transient. However, several case reports describe a temporal association between sildenafil use and nonarteritic anterior ischemic optic neuropathy, which requires further attention. In addition, transient myalgias are described by some patients.

The presence of PDE 3 and 5 in platelets is consistent with in vitro observations that sildenafil potentiates inhibitory effects of nitric oxide donors on ADP-dependent platelet aggregation. In healthy subjects receiving sildenafil alone or a combination with aspirin or warfarin, effects on bleeding...
time or prothrombin time were reported to be minimal.\textsuperscript{22} However, a recent study demonstrated a transient prolongation of the bleeding time 1 hour after 100 mg oral sildenafil, as well as an inhibition of collagen-induced platelet aggregation.\textsuperscript{62} In patients, especially cardiovascular patients being treated with multiple drugs, more complex situations may be present than in healthy subjects. Case reports of intracerebral hemorrhage, gastric variceal bleeding, epistaxis, and hemorrhoidal bleeding associated with sildenafil treatment suggest that the effect of PDE 5 inhibition in platelets might not be absolutely negligible.

In this context, it is important to mention that potential interactions between sildenafil and other medication frequently used in cardiovascular patients, eg, ticlopidine, clopidogrel, or diprydamole, need to be considered in some cases. The oral bioavailability of sildenafil amounts to 41\%\textsuperscript{63} because of a hepatic first-pass effect. It is metabolized by the cytochrome P450 3A4 pathway, which is known to be inhibited by a number of (in part commonly prescribed) drugs (for a list, see ref 22) and also by certain dietary components. Thus, concurrent use of sildenafil and drugs inhibiting this pathway, as well as severe renal impairment or hepatic dysfunction, can lead to higher plasma levels.\textsuperscript{64,22} Presumably, some of the described side effects can be attributed to supratherapeutic plasma levels of sildenafil.

The pharmacological basis of neurological symptoms temporarily associated with sildenafil is poorly understood, even if PDE 5 is expressed to a high degree in parts of the brain; Gilad et al reported 2 cases of tonic-clonic seizures after sildenafil.\textsuperscript{65} Interestingly, 1 of their patients had an additional epileptic seizure when he took sildenafil again 3 months later.\textsuperscript{65} Other case reports include transient ischemic attacks, strokes, and transient global amnesia after sildenafil.

Especially for the neurological patient, an additional therapeutic application of sildenafil is potentially conceivable for vasospasm related to subarachnoidal hemorrhage. In a dog model of subarachnoidal hemorrhage, Inoha et al\textsuperscript{66} reported increased expression of PDE 5 in the basilar artery 7 days after hemorrhage, which temporarily paralleled the occurrence of vasospasm. Furthermore, they reported partial relaxation of the spastic basilar artery after PDE 5 inhibition.\textsuperscript{66}

### Summary

Pharmacological inhibition of PDE 5 exhibits differential effects in different organs according to its expression in various tissues. Therapeutic doses of sildenafil, used in the treatment of male erectile dysfunction, exhibit slight blood pressure–lowering effects and do not appear to compromise coronary blood flow in coronary artery disease. However, the combination of sildenafil with any agent serving as a nitric oxide donor is contraindicated because of potentially life-threatening hypotension. Despite theoretical concerns of promoting cardiac arrhythmia and reducing myocardial tolerance to ischemia, the risk for a cardiac event with sildenafil treatment is not higher than what would be expected for the population of patients treated for erectile dysfunction, characterized by several coronary risk factors. In patients with pulmonary hypertension of various etiologies, treatment with sildenafil is promising and may provide the unique opportunity of improving the efficacy of inhalative agents, leading to site-specific, additive, or overadditive effects. In addition, further investigations of the effect of PDE 5 inhibition in diseases associated with endothelial dysfunction seem to be worthwhile, as PDE 5 inhibition could favorably influence abnormal vasomotion caused by compromised endothelial nitric oxide release. Neurological, ophthalmological, or gastrointestinal side effects require further investigations, even if their incidence might be low and a causal connection has not yet been proven.

### References


**KEY WORDS:** cardiovascular diseases | vasospasm | pulmonary heart disease | inhibitors | nitric oxide
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