Cyclic Nucleotide Phosphodiesterase Type 5 Activity Limits Blood Flow to Hypoperfused Myocardium During Exercise

Jay H. Traverse, MD; Ying Jie Chen, MD, PhD; Ruisheng Du, PhD; Robert J. Bache, MD

Background—Nitric oxide (NO) causes vasodilation by stimulation of guanylate cyclase in vascular smooth muscle to produce cGMP. The resultant vasodilator effect is regulated by a family of cGMP phosphodiesterases (PDEs). Sildenafil, a selective inhibitor of PDE5 used for treatment of erectile dysfunction, has been found to cause relaxation of isolated epicardial coronary artery segments. The present study examined the effects of sildenafil on coronary blood flow and hemodynamics during exercise in normal and ischemic heart.

Methods and Results—In chronically instrumented normal dogs, sildenafil 2 mg/kg PO caused a slight but significant increase in left anterior descending (LAD) coronary blood flow during resting conditions, with a nonsignificant trend toward increased coronary flow during treadmill exercise. Exercise in the presence of LAD stenosis that decreased distal coronary pressure to 57±2 mm Hg reduced LAD flow during exercise from 69±8 to 41±7 mL/min (P<0.05), with hypoperfusion most severe in the subendocardium. At the same distal coronary pressure, sildenafil increased LAD flow in the ischemic region to 50±11 mL/min (P<0.05). Increase in ischemic region blood flow produced by sildenafil was uniform across the LV wall, given that no change occurred in the transmural distribution of perfusion.

Conclusions—Inhibition of PDE5 with sildenafil caused vasodilation of coronary resistance vessels with an increase of blood flow into an ischemic myocardial region during exercise in the presence of coronary artery stenosis. (Circulation. 2000;102:2997-3002.)

Key Words: sildenafil ■ stenosis ■ cGMP ■ exercise ■ blood flow ■ ischemia

Nitric oxide (NO) produced by the vascular endothelium plays a significant role in regulation of vasomotor tone in coronary circulation. NO causes vasodilation by stimulating guanylate cyclase in vascular smooth muscle to generate cGMP, which activates a cGMP-dependent protein kinase. Levels of cGMP in vascular smooth muscle are tightly regulated by several cyclic nucleotide phosphodiesterase enzymes (PDEs) that catalyze cGMP degradation and terminate this second messenger signal. Sildenafil (Viagra, Pfizer) is a highly selective inhibitor of PDE5 that recently has been approved for treatment of erectile dysfunction. Sildenafil potentiates the activity of cGMP in the corpus cavernosum, thereby augmenting vasodilator activity of neuronally mediated NO production. Sildenafil has also been demonstrated to increase cGMP levels and cause smooth muscle relaxation in isolated segments of epicardial coronary artery. However, the effect of sildenafil on coronary resistance vessels has not been studied. Consequently, we undertook the present study to determine whether selective inhibition of PDE5 with sildenafil results in changes of coronary blood flow or myocardial oxygen consumption (MV\textsubscript{O}_2) at rest or during treadmill exercise in chronically instrumented dogs. Because of the strong association between erectile dysfunction and coronary artery disease, we also examined the effect of sildenafil on regions of myocardium that became ischemic during exercise in the presence of flow-limiting coronary artery stenosis.

Methods

Studies were performed in 12 adult mongrel dogs that weighed 25 to 30 kg each and were trained to run on a treadmill. All studies were performed in accordance with the “Position of the American Heart Association on Research Animal Use” adopted by the association in November 1984 and were approved by the Animal Care Committee of the University of Minnesota.

Surgical Instrumentation

Animals were premedicated with acepromazine (10 mg IM), anesthetized with sodium pentobarbital (30 mg/kg IV), intubated, and ventilated with room air supplemented with oxygen. A left thoracotomy was performed in the 5th intercostal space. A heparin-filled polyvinylchloride catheter, 3.0 mm OD, was introduced into the internal thoracic artery and advanced into the ascending aorta. The pericardium was opened and a second catheter placed into the left atrium through the appendage. A similar catheter was introduced into...
Myocardial blood flow was measured with 15-

Measurement of Regional Myocardial Blood Flow

treadmill. Fifteen minutes later, resting hemodynamics were rec-
cording instruments were connected, each dog was placed on the

direct-writing recorder (Coulbourne Instruments Inc). After all re-
iastolic pressure (LVEDP). LAD coronary blood flow was measured

distal to the occluder for measurement of coronary pressure.6 A
coronary artery (LAD) was dissected free, and a Doppler velocity

Experimental Protocol

After they recovered from surgery, animals were returned to the
laboratory for study. Aortic, left ventricular, and coronary pressures
were measured with pressure transducers at mid-chest level (Spec-
tramed Inc, model TNF-R). The fluid-filled catheter in the LV was
used to calibrate the Konigsberg micromanometer. LV pressure was
recorded both at normal and high gain for measurement of end-di-
astolic pressure (LVEDP). LAD coronary blood flow was measured
with the Doppler velocity probe. Data were recorded on an 8-channel
direct-writing recorder (Coulbourne Instruments Inc). After all re-
cordings instruments were connected, each dog was placed on the

treadmill. Fifteen minutes later, resting hemodynamics were rec-
corded and 3 cm³ of blood was withdrawn from the aortic and
coronary venous catheters and placed on ice for blood gas analysis
(n = 12). Exercise was then begun at 6.4 km/h with a 10% grade.

Data were recorded by use of Wilcoxon signed-rank test.
P < 0.05. Sildenafil did not significantly change aortic
pressure, which was 80% of external diameter of the artery. Rate-pressure product
was calculated as heart rate multiplied by LV systolic pressure.

Data are expressed as mean ± SEM. Individual comparisons of
significant differences between control and sildenafil groups were
performed by use of Wilcoxon signed-rank test. P < 0.05 was
required for statistical significance.

Results

Normal Coronary Artery Inflow

Hemodynamics

Hemodynamic data at rest and during exercise under control
conditions and after administration of sildenafil are shown in
Table 1. Resting heart rate during control conditions was
110 ± 8 bpm, mean aortic pressure was 109 ± 4 mm Hg, and
LVEDP was 7 ± 2 mm Hg. After administration of sildenafil,
resting arterial pressure and LVEDP were unchanged, but
there was a significant increase in heart rate to 124 ± 8 bpm
(P < 0.05). Exercise caused significant increases in aortic
pressure, LV systolic pressure, and LVEDP compared with
rest (P < 0.05). Sildenafil did not significantly change aortic
or LV systolic or diastolic pressures during exercise, but heart
rate was significantly higher than during control exercise.

Coronary Blood Flow

Coronary blood flow during rest and exercise at baseline and
after sildenafil are shown in Figure 1. Under baseline condi-

| TABLE 1. Hemodynamic Measurements at Rest and During Treadmill Exercise During Control Conditions and After Sildenafil (2 mg/kg) |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Mean Aortic Pressure, mm Hg | Heart Rate, bpm | LV Pressure, mm Hg | LVEDP, mm Hg | LAD Coronary Pressure, mm Hg | Rate Pressure Product \(×10^{-3}, \) mm Hg×bpm |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| **CON** | **SIL** | **CON** | **SIL** | **CON** | **SIL** | **CON** | **SIL** |
| **Rest** | **109±4** | 105±3 | 110±8 | 124±8* | 132±5 | 124±4 | 7±2 | 7±1 | 105±5 | 99±4 | 14.5±1.2 | 15.3±1.2 |
| **Exercise** | **124±7†** | 121±5† | 198±9† | 208±6† | 154±7† | 150±6† | 12±2‡ | 8±3 | 109±6 | 108±4 | 30.7±2.1† | 31.6±1.7† |

**CON** indicates control; **SIL**, sildenafil.

*P < 0.05 vs CON; †P < 0.01 vs rest; ‡P < 0.07 vs rest.


gamma spectrophotometer (Packard Instrument Co) at window
settings that corresponded to the peak energies of each radionuclide.

Measurement of MV\(\text{O}_2\)

Blood samples from aorta and coronary vein at rest and during
exercise were analyzed for oxygen content with a blood gas analyzer
(Instrumentation Laboratory, model 113). Blood oxygen content
(volume percentage) was calculated as (hemoglobin × 0.0136 × \(\text{O}_2\)
saturation) + (\(\text{PO}_{2}\) × 0.0031)

MV\(\text{O}_2\) was calculated as myocardial blood flow multiplied by the
arteriovenous difference in oxygen content.

Determination of Plasma Sildenafil Level

At the conclusion of exercise, 3 mL of blood was withdrawn from
the aortic catheter and immediately centrifuged at 3000 rpm for 10
minutes at 4°C. Plasma was frozen at −70°C for determination of
sildenafil concentration by use of high-performance liquid chromato-
ography with mass spectrometric detection.8

Data Analysis

Heart rate and pressures were measured from strip chart recordings.
LAD coronary blood flow was calculated from the coronary Doppler
flow shift with the equation \(q = 2.5 \times d^2 \times f\) where \(q\) is coronary blood
flow (in milliliters per minute), \(d\) is the internal diameter (ID) of the
vessel (in millimeters), and \(f\) is Doppler frequency shift (in kило-
hertz).9 On the basis of our previous observations, ID was taken to be
80% of external diameter of the artery. Rate-pressure product
was calculated as heart rate multiplied by LV systolic pressure.

Data are expressed as mean ± SEM. Individual comparisons of
significant differences between control and sildenafil groups were
performed by use of Wilcoxon signed-rank test. P < 0.05 was
required for statistical significance.

the left ventricle (LV) at the apical dimple. A solid-state microma-
nometer (Konigsberg Instruments Inc, model P5) was also intro-
duced into the LV at the apex. The proximal left anterior descending

coronary artery (LAD) was dissected free, and a Doppler velocity

probe (Craig Hartley; 2.5- to 3.5-mm ID) was placed around the
vessel. Immediately distal to the velocity probe, a hydraulic occluder
(3.0-mm OD) was placed around the artery. A heparin-filled
silicone-rubber catheter (0.3-mm ID) was then placed into the LAD
distal to the occluder for measurement of coronary pressure.4 A
fourth catheter was advanced into the coronary sinus through the
right atrial appendage until the tip was positioned within 1 cm of the
anterointerventricular vein to allow selective sampling of venous
effluent of the myocardium perfused by LAD. The pericardium
was loosely closed and catheters tunneled subcutaneously to exit at the
base of the neck. The thoracotomy was closed in layers and the chest
vacuated of air. Catheters were protected with a nylon vest and were
flushed daily with heparinized saline.

**Measurement of Regional Myocardial Blood Flow**

Myocardial blood flow was measured with 15-\(\mu\)m diameter micro-
spheres labeled with \(^{141}\text{Ce}, ^{51}\text{Cr}, ^{85}\text{Sr}, ^{95}\text{Nb},\) or \(^{46}\text{Sc}\) (NEN Co) as
previously described.7 After completion of the exercise studies,
animals were euthanatized with an overdose of pentobarbital and the
heart removed and fixed in 10% buffered formalin. LV was sec-
tioned into 4 equal layers from epicardium to endocardium, weighed, and placed into vials for counting in a
tions, resting LAD coronary blood was 42±5 mL/min and increased during exercise to 69±8 mL/min (P<0.01). After sildenafil, resting coronary blood flow increased to 50±8 mL/min (P<0.05 versus control rest). During exercise after sildenafil, coronary flow increased to 78±14 mL/min, which tended to be greater than during control exercise (P=0.10).

Myocardial Oxygen Consumption

MV$_{\dot{O}_2}$ was computed in 7 animals at rest and during exercise before and after sildenafil (Figure 1). During control conditions, coronary venous oxygen tension was 23 mm Hg at rest and decreased significantly to 18±2 mm Hg during exercise. Sildenafil tended to increase coronary venous oxygen tension during resting conditions to 28±7 mm Hg, but this was not significant. After sildenafil, exercise caused a significant decrease of coronary venous Po$_2$ to 18±2 mm Hg, which was identical to that observed during control exercise.

Coronary Stenosis

Hemodynamics

Hemodynamics during exercise in the presence of coronary stenosis before and after sildenafil are shown in Table 2. During control exercise, mean aortic pressure was 123±6 mm Hg and heart rate was 207±7 bpm; these values were not significantly different during exercise after sildenafil. Inflation of the occluder during control exercise decreased distal coronary pressure in the LAD to 57±2 mm Hg. Application of stenosis resulted in a significant increase in LVEDP, to 17±3 mm Hg (P<0.05 versus control exercise). Distal coronary pressure in the LAD was identically decreased during exercise with sildenafil.

Regional Myocardial Blood Flow

Myocardial blood flow was measured with microspheres during exercise in the presence of coronary stenosis in 7 dogs. During control exercise, mean blood flow in the normal zone was 2.42±0.38 mL · min$^{-1}$ · g$^{-1}$, whereas subendocardial/epicardial (ENDO/EPI) flow ratio was 1.34±0.08. Blood flow in the anterior region perfused by the stenotic LAD was decreased to 1.00±0.19 mL · min$^{-1}$ · g$^{-1}$ (P<0.01), whereas END0/EPI flow ratio was decreased to 0.38±0.07. Sildenafil caused a significant increase in blood flow to the region perfused by the LAD, to 1.11±0.18 mL · min$^{-1}$ · g$^{-1}$ (P<0.05), with no change in the END0/EPI flow ratio (0.36±0.05; Figure 2). Normal-zone myocardial blood flow tended to increase to 2.85±0.32 mL · min$^{-1}$ · g$^{-1}$ after sildenafil, although this change was not significant. Normal-zone ENDO/EPI ratio was unchanged (1.29±0.11) after sildenafil.

Plasma Sildenafil Levels

Plasma sildenafil levels ranged from 248 to 779 nmol/L (mean, 520±86 nmol/L). Because sildenafil is 84% bound to plasma protein in the dog, this represents a mean plasma-free sildenafil concentration of 66.4±12.4 nmol/L.

TABLE 2. Hemodynamic Measurements During Treadmill Exercise in Presence of LAD Stenosis During Control Conditions and After Sildenafil (2 mg/kg)

<table>
<thead>
<tr>
<th></th>
<th>Mean Aortic Pressure, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>LVEDP, mm Hg</th>
<th>LAD Coronary Pressure, mm Hg</th>
<th>Rate Pressure Product × $^{-1}$, mm Hg·bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise + stenosis</td>
<td>123±6</td>
<td>207±7</td>
<td>17±3*</td>
<td>57±2</td>
<td>30.3±2.5</td>
</tr>
<tr>
<td>Exercise + stenosis + SIL</td>
<td>118±5</td>
<td>214±6</td>
<td>14±4*</td>
<td>57±3</td>
<td>32.0±2.0</td>
</tr>
</tbody>
</table>

*P<0.05 vs control exercise. SIL indicates sildenafil.
Discussion

In the present study, sildenafil caused modest vasodilation of coronary resistance vessels during resting conditions, with a nonsignificant trend toward an increase in coronary blood flow during treadmill exercise in the normal heart. These effects occurred with no change in MVO$_2$, which indicates that sildenafil exerted a weak primary vasodilator influence on coronary resistance vessels. In a myocardial region that became ischemic during exercise in the presence of coronary artery stenosis, sildenafil caused a significant increase in coronary blood flow. This increase in blood flow occurred with no change in distal coronary pressure, which suggests that sildenafil caused vasodilation of resistance vessels in the ischemic myocardial region. Implications of these findings are discussed below.

Systemic Hemodynamics

In healthy men, sildenafil caused a small decrease in resting blood pressure with no change in heart rate.$^{10}$ In men with stable angina pectoris who underwent pulmonary artery catheterization, intravenous sildenafil 40 mg produced a 27% decrease in resting pulmonary artery pressure and a 7% decrease in cardiac output. In the present study, a nonsignificant trend was present toward a decrease in resting mean aortic and LV systolic pressure 1 hour after sildenafil. In contrast to the patient studies, we observed a modest increase in heart rate after sildenafil. This probably represented a reflex response to the decrease in systemic vascular resistance, although a direct chronotropic effect of PDE inhibition cannot be excluded. Interestingly, sildenafil did not alter the increase in aortic and LV systolic pressures in response to exercise. During exercise with coronary stenosis, a significant increase occurred in LVEDP compared with control exercise consistent with the development of myocardial ischemia. Sildenafil tended to blunt the increase in LVEDP during exercise in the presence of stenosis, although this difference was not significant.

Effect of PDE5 Inhibition on Normal Coronary Circulation

PDE5 inhibition would be expected to augment the effects of endogenous NO on coronary circulation. Previous studies with PDE5 inhibitors zaprinast and E4021 demonstrated an increase in cGMP levels in isolated coronary arteries and a dose-dependent increase in epicardial artery diameter in awake swine.$^{5,11}$ Conversely, competitive inhibition of NO synthesis with monomethyl-l-arginine caused a decrease in coronary artery diameter but did not decrease coronary blood flow.$^{12}$ These findings support the concept that in the normal heart, NO contributes to tonic vasodilation of coronary arteries. Using intravital microscopy, Jones et al$^{13}$ observed that inhibition of endogenous NO production with N-nitro-l-arginine methyl ester caused constriction of small coronary resistance arteries (100 to 400 $\mu$m) in open-chest dogs, but this was offset by vasodilation of the arterioles (<100 $\mu$m) and resulted in no significant change in blood flow. This suggests that metabolic vasodilator adjustments at the level of the coronary arterioles are able to counter vasoconstriction of resistance arteries that occurs when endogenous NO production is blocked, thereby maintaining coronary blood flow appropriate to the metabolic demands of the myocardium.

Although NO production is not critical for maintenance of coronary blood flow, infusion of authentic NO or administration of NO donors can cause increases in coronary flow,$^{14}$ which indicates that microcirculation is responsive to NO (and hence cGMP) and that NO can override compensatory metabolic vasoregulation. Furthermore, Kuo et al$^{15}$ observed that NO can exert vasodilator activity at the levels of both resistance arteries (100 to 400 $\mu$m) that are not under metabolic control and arterioles (<100 $\mu$m), which are under metabolic control, which suggests that a PDE inhibitor such as sildenafil might have the potential to interfere with metabolic vasoregulation and cause an inappropriate increase in coronary blood flow. In fact, in the present study, sildenafil exerted only a very weak vasodilator effect on the coronary resistance vessels during resting conditions. The minimal resistance vessel–dilating effect of sildenafil suggests that this agent would have little likelihood to cause coronary steal in patients with occlusive coronary artery disease.

The increased cardiac work during exercise is accompanied by an increase in coronary blood flow that results in increased endothelial shear, which would be expected to cause vasodilation by an NO-dependent mechanism. However, inhibitors of NO synthesis do not decrease coronary blood flow during exercise, which demonstrates that NO is not obligatory for coronary resistance vessel dilation during exercise in normal heart.$^{16}$ In the present study, PDE5 inhibition with sildenafil resulted in a nonsignificant trend toward increased coronary blood flow during exercise with a tendency toward increased subendocardial flow. Although we did not assess contractility, there was no change in coronary venous PO$_2$ or MVO$_2$, which suggests that this dose of sildenafil had negligible effects on PDE3, which degrades cAMP, and is consistent with previous observations in isolated dog trabecular muscle, in which sildenafil had no effect on contractility.$^{17}$ The minimal effect of sildenafil on blood flow in the normally perfused region may be the result of alternative pathways for degradation of cGMP. In addition to PDE5, 4 other PDE isoenzymes have been identified in vascular smooth muscle, of which the main cGMP-hydrolyzing activity in coronary vascular smooth muscle is accomplished by PDE1 and PDE5. IC$_{50}$ of sildenafil for inhibition of human PDE1 and PDE5 is 280 and 3.5 nmol/L,
respective, which indicates high selectivity for PDE5. Mean plasma-free sildenafil concentration of 66.4 ± 12.4 nmol/L in the present study would have provided a high degree of blockade of PDE5 with relatively little inhibition of PDE1. The modest effect of sildenafil on coronary flow may have occurred because sildenafil principally inhibits PDE5, with much less effect on PDE1, which provides an alternative pathway for degradation of cGMP.

PDE5 Inhibition During Exercise With Myocardial Ischemia
In the present study, PDE5 inhibition with sildenafil significantly increased blood flow to the hyperperfused myocardial region subserved by a stenotic coronary artery. Ischemia produced by the stenosis was sufficient to cause a significant increase in LVEDP. Coronary pressure distal to the stenosis was identical before and after sildenafil, so that the increase in myocardial blood flow was the result of an effect of sildenafil at the level of the coronary microvasculature. An increase in blood flow could be the result of either decreased extravascular forces acting on the intramural coronary vessels or secondary to vasodilation of the coronary microvessels. LVEDP tended to be lower after sildenafil; a decrease in diastolic intracavitary pressure would reduce extravascular forces that impede blood flow in the microcirculation. In contrast, a trend existed toward increased heart rate after sildenafil; increase in heart rate would increase extravascular forces opposing coronary blood flow and might cause a decrease in myocardial perfusion. However, neither the change in LVEDP nor the change in heart rate were significant. Consequently, findings support a vasodilator effect of sildenafil on the coronary microvessels.

Myocardial ischemia results in metabolic arteriolar vasodilation. Nevertheless, some degree of vasodilator reserve persists in the coronary resistance vessels during exercise-induced myocardial ischemia, in part because of adrenergic vasoconstrictor tone that can compete with metabolic vasodilation. Thus, blockade of α-adrenergic receptors results in an increase in coronary blood flow during exercise-induced ischemia. In addition, sympathetic activation of α- and β-adrenergic receptors on the coronary endothelium can cause release of NO during exercise. Increased adrenergic activity during exercise-induced ischemia possibly could augment endothelial NO production, thereby amplifying the effect of PDE5 inhibition during ischemia. The finding in the present study that sildenafil increased blood flow in the ischemic myocardial region is analogous to previous reports that nitroglycerin or other NO donors can increase blood flow to a myocardial region that becomes ischemic during exercise in the presence of coronary stenosis. Some evidence exists that NO has increased importance in the presence of myocardial ischemia. Thus, in dogs in which a flow-limiting coronary stenosis resulted in myocardial ischemia during treadmill exercise, inhibition of NO synthase with LNNA worsened myocardial hypoperfusion but did not decrease blood flow in normally perfused myocardium. Although several phosphodiesterases occur in vascular smooth muscle that can catalyze cGMP, the high specificity of sildenafil for PDE5 suggests that the increase in blood flow in the present study was mediated by inhibition of this enzyme. Evidence also suggests that NO can cause vasodilation by pathways independent of cGMP, but these mechanisms would not contribute to the present findings.

In agreement with previous reports, stenosis resulted in marked redistribution of blood flow away from deeper myocardial layers, with hypoperfusion most severe in subendocardium. Increase of blood flow into the ischemic myocardial region produced by sildenafil was transmurally uniform. This is different from studies in which nitroglycerin and other NO donors resulted in a preferential increase in blood flow to the subendocardium, possibly because of a vasodilator effect on the penetrating arteries that conduct blood from the epicardial arteries to the subendocardial microvasculature. This difference between the effect of NO donors and sildenafil on the transmural distribution of blood flow in the ischemic region suggests that PDE5 activity may not be significantly involved in cGMP degradation in the penetrating coronary arteries.

Conclusions
PDE5 inhibition by sildenafil caused a modest vasodilator effect on coronary resistance vessels in normal heart. However, when coronary stenosis resulted in myocardial hypoperfusion during exercise, sildenafil caused a significant increase in blood flow to the ischemic region at the same distal coronary pressure, as a result of vasodilation of the resistance vessels. These findings suggest that PDE5 contributes to regulation of coronary blood flow in the normal heart and that the NO-mediated activity of PDE5 is enhanced in the presence of myocardial ischemia.

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References


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