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The Effect of Sildenafil on Human Vascular Function, Platelet Activation, and Myocardial Ischemia

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OBJECTIVES We studied the effects of sildenafil, a phosphodiesterase 5 inhibitor, on coronary and peripheral vascular function, platelet activation, and myocardial ischemia.

BACKGROUND Nitric oxide vasodilates and inhibits platelet activation by generating cyclic guanosine 5′-monophosphate, which is metabolized by phosphodiesterase type 5.

METHODS The effect of oral sildenafil on resting coronary vascular tone, endothelium-dependent and -independent function and platelet activation was measured in 24 patients. An additional 24 patients with coronary artery disease (CAD) and ischemia during exercise, and 12 control subjects received either 100 mg of sildenafil, 10 mg of isosorbide dinitrate (ISDN) or placebo during exercise on three separate days in a randomized, double-blind manner. Flow-mediated dilation of the brachial artery was measured, and CAD patients underwent treadmill exercise testing.

RESULTS Sildenafil (100 mg) vasodilated epicardial coronary arteries (+6.9 ± 1.3%, $p < 0.0001$). Coronary epicardial and microvascular responses with acetylcholine and cold-pressor testing improved, with a greater enhancement in patients with CAD and endothelial dysfunction. Verapamil responses were unchanged. Both resting and adenosine diphosphate-stimulated platelet IIb/IIIa receptor activation was inhibited by sildenafil ($p < 0.05$). Brachial arteries dilated in response to sildenafil in controls. Peak flow-mediated dilation was similar, but the duration of hyperemia was prolonged after sildenafil administration ($p < 0.001$). Compared with placebo, ISDN improved myocardial ischemia during exercise ($p < 0.05$), whereas the effect of sildenafil was intermediate between the two.

CONCLUSIONS Sildenafil dilates epicardial coronary arteries, improves endothelial dysfunction and inhibits platelet activation in patients with CAD. It has an intermediate effect on myocardial ischemia compared with ISDN and placebo. (J Am Coll Cardiol 2002;40:1232–40) © 2002 by the American College of Cardiology Foundation

Endothelial nitric oxide (NO) modulates vascular tone at rest, facilitates vasodilation during stress and inhibits platelet aggregation by activating intracellular guanylate cyclase, which in turn generates cyclic guanosine 5′-monophosphate (cGMP) (1–7). Vascular smooth muscle cGMP levels are regulated by the activity of phosphodiesterase type 5 (PDE5), and sildenafil citrate (Viagra Pfizer, Inc., New York, New York), a highly selective antagonist of PDE5, enhances the effect of NO in experimental models (8). Because endothelial dysfunction is associated with vascular inflammation, platelet activation and rapid progression of atherosclerosis and its adverse events, strategies that enhance NO bioavailability may positively impact outcomes in patients with coronary artery disease (CAD). We hypothesized that PDE5 inhibition with sildenafil would abrogate coronary and peripheral vascular endothelial dysfunction in patients with CAD, inhibit platelet activation and ameliorate myocardial ischemia during stress.

METHODS

Protocols were approved by the Institutional Review Board and informed written consent obtained.

Study 1. PATIENTS. We studied 24 patients with either CAD (n = 15) or with angiographically normal coronary arteries (NCA, n = 9) undergoing cardiac catheterization for investigation of chest pain and/or abnormal noninvasive cardiac investigations. CAD was defined as the presence of plaquing (n = 2) or significant stenosis >50% in one (n = 7) or two (n = 6) epicardial coronary arteries. Mean age of the patients was 54 ± 2 years, and mean cholesterol level was 191 ± 8 mg/dl. Twenty-two were male,11 had hypertension, 6 were smokers and 6 were diabetic. Fifteen were on statin therapy, and those with recent acute coronary syndromes were excluded. Aspirin and angiotensin-converting enzyme inhibitors were withheld for at least seven days, and other cardiac medications were discontinued for ≥5 half-lives before the study. A low nitrate diet was

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Abbreviations and Acronyms

ACH = acetylcholine
CAD = coronary atherosclerosis
cGMP = cyclic guanosine monophosphate
CPT = cold-pressor testing
CVR = coronary vascular resistance
D = diameter
FMD = flow-mediated dilation
ISDN = isosorbide dinitrate
PDE5 = phosphodiesterase type V
NCA = normal coronary arteries
NO = nitric oxide

maintained for 24 h before the study, and nitrovasodilators were withheld for 24 h before and after the procedure.

PROTOCOL. After diagnostic coronary angiography, a 3F infusion catheter was introduced into a coronary artery without significant stenosis (<20%) and blood flow velocity was measured using a 0.014-inch wire equipped with a Doppler crystal at its tip (FloWire, EndoSonics, Rancho Cordova, California). Coronary blood flow was derived from the velocity and diameter measurements using the formula ($\pi \times$ average peak velocity $\times$ 0.25 $\times$ diameter^2) as described previously (9). Coronary vascular resistance (CVR) was derived as mean arterial blood pressure divided by coronary blood flow (2).

After baseline measurements, cold-pressor testing (CPT) was performed by immersing the hand in ice-cold water for 90 to 120 s. After recovery, endothelium-dependent coronary vasomotion was estimated using 2-min infusions of acetylcholine (ACH) at 1.5 and 15 μg/min (estimated intracoronary concentrations of 10^{-7} mol/l and 10^{-6} mol/l, respectively), and endothelium-independent function was determined using intracoronary verapamil at 300 μg/min for 2 min. Patients were given 100 mg of sildenafil orally, and after 45 min, when its peak hemodynamic effects occur, CPT was repeated and ACH and verapamil were readministered (10).

Systemic, pulmonary and coronary vascular hemodynamics were recorded and coronary angiography was performed after each intervention. Epicardial diameter was measured in segments free of overlap, severe tapering or bifurcations using quantitative angiography (CAAS II, Pie Medical, Maastricht, Netherlands; 3 segments were analyzed in 12, and 2 segments in 12 patients) by an investigator who had no knowledge of the study sequence. In addition, to investigate the effect of sildenafil on stenotic atherosclerotic segments, we measured minimum lumen diameter in segments ranging from 20% to 40% stenosis (n = 6) that were present in epicardial arteries neighboring the study vessel. Central venous blood was obtained at baseline and 45 min after sildenafil for platelet flow cytometry in 16 subjects (10 with CAD).

REPRODUCIBILITY STUDIES. Reproducibility of coronary vascular changes with these interventions were assessed in six patients in whom ACH and verapamil infusions and CPT were repeated after 45 min. Epicardial diameter responses with ACH 15 μg/min (2.13 $\pm$ 0.2 to 2.24 $\pm$ 0.2 mm, r = 0.97), verapamil 300 μg/min (2.45 $\pm$ 0.2 to 2.47 $\pm$ 0.2 mm, r = 0.99) and CPT (1.93 $\pm$ 0.2 to 1.92 $\pm$ 0.2 mm, r = 0.96) were reproducible after 45 min. Similarly, CVR responses with ACH (r = 0.91), verapamil (r = 0.99) and CPT (r = 0.76) were also reproducible.

PLATELET FLOW CYTOMETRY. Immediately after withdrawal, aliquots of 450 μl citrated venous whole blood were mixed with 50 μl of either phosphate-buffered saline, 200 μmol/l ADP solution (Bio/Data Corp., Horsham, Pennsylvania) or 100 μmol/l TRAP (Biosearch, New Brunswick, New Jersey). Two 5-μl aliquots were added to tubes containing saturating concentrations of either FITC-labeled PAC-1, PE-labeled CD-62 and Per-CP–labeled CD-61(Becton Dickinson Immunocytometry Systems, San Jose, California) with RGDS (Sigma, St. Louis, Missouri) as a competitive inhibitor of PAC-1 binding or FITC-labeled PAC-1, PE-labeled mouse anti-human IgG_1 (Beckman Coulter, Inc., Fullerton, California.) and Per-CP–labeled CD-61. Samples were fixed in paraformaldehyde solution and analyzed within 24 h on a FACScan flow cytometer (Becton Dickinson). The percentage of platelets positive for PAC-1 (representing activated IIb/IIIa receptor) or CD-62 (representing surface expression of P-selectin, a marker of α-granule release) were calculated from 10,000 events positive for the platelet-specific antibody CD-61 that bound PAC-1-FITC or CD-62-PE antibodies with a fluorescent intensity greater than a threshold set at 1% from the respective negative control sample.

Study 2. PATIENTS. Twelve healthy volunteers, eight male, aged 32 $\pm$ 2 years and 24 patients with asymptomatic or mildly symptomatic stable CAD who developed $\geq$1 mm ST-segment depression on exercise were studied after discontinuation of all medications for at least five half-lives. Six patients with CAD had single, nine had two-vessel and nine had three-vessel disease. All patients had exercise-induced reversible myocardial perfusion defects. Mean age was 65 $\pm$ 2 years, 22 were male, 12 were hypertensive, 2 were smokers, 9 were diabetic and 21 were on statin therapy, with a mean cholesterol level of 187 $\pm$ 9 mg/dl.

PROTOCOL. The study was performed on 3 separate days when subjects received either 100 mg of sildenafil, 10 mg of isosorbide dinitrate (ISDN) or placebo in a double-blind, randomized, placebo-controlled manner. Each morning after an overnight fast, subjects underwent vascular reactivity studies before and 45 min after the study drug. CAD patients also underwent treadmill exercise testing 1 h after receiving the study medication.

FLOW-MEDIATED VASODILATION (FMD). Two-dimensional images of the brachial artery were obtained above the antecubital crease using a 12.5-MHz linear array ultrasound transducer (ATL HDI 5000 cv, Andover, Massachusetts). Diameter (D) was measured at baseline and after 60 s of

Table 1. Hemodynamic Effects

	Baseline	45 Min Post-Sildenafil
Study 1		
Pulmonary arterial pressure (mm Hg)	17.0 ± 1.0	14.7 ± 1.0‡
Pulmonary wedge pressure (mm Hg)	9.4 ± 0.8	7.6 ± 0.8†
Pulmonary vascular resistance (dynes.s.cm^5)	119.2 ± 8.8	109.6 ± 11.2
Cardiac output (l/min)	5.2 ± 0.3	5.4 ± 0.3
Mean arterial pressure (mm Hg)	103 ± 2	101 ± 3
Systemic vascular resistance (dynes.s.cm^5)	1,680 ± 88	1,552 ± 80
Heart rate (beats/min)	69 ± 2	70 ± 2

	Controls			Patients		
	Placebo	Sildenafil	Isosorbide	Placebo	Sildenafil	Isosorbide
Study 2						
HR (beats/min)	57 ± 3	62 ± 4*	60 ± 3	61 ± 2	63 ± 2	69 ± 3§
SBP (mm Hg)	110 ± 4	109 ± 3	104 ± 6	141 ± 4	129 ± 4*	120 ± 4*§
DBP (mm Hg)	67 ± 2	62 ± 2*	62 ± 2*	81 ± 2	72 ± 2*	70 ± 2*
MBP (mm Hg)	81 ± 3	77 ± 2*	76 ± 3*	101 ± 3	91 ± 2*	89 ± 2*§

†$p < 0.01$, ‡$p < 0.001$ vs. baseline. *$p < 0.05$ vs. placebo, §$p < 0.05$ vs. sildenafil.
DBP = diastolic blood pressure; HR = heart rate; MBP = mean systemic blood pressure; SBP = systolic blood pressure.

hyperemia in all patients and after 80 s, 2 min, 2.5 min, 3 min, 3.5 min and 4 min in 16 subjects (eight patients and eight controls) (11). Measurements were repeated 45 min after the study drug. Analysis was performed in a blinded manner by a single observer. The intra-observer correlation for repeated measurements of D in our laboratory is 0.98 (mean 3.64 ± 0.24 and 3.60 ± 0.2 mm).

TREADMILL EXERCISE TESTING. Patients with CAD underwent symptom-limited, maximal treadmill exercise, using the National Institutes of Health Combined protocol, immediately after completion of the vascular reactivity study (12). Heart rate, blood pressure and 12-lead electrocardiogram (ECG) were recorded every 30 s during the exercise period. All patients underwent exercise testing on at least two previous occasions to ensure reproducibility and familiarity.

STATISTICAL ANALYSIS. Means were compared by paired or unpaired Student *t* test. Dose–response curves with ACH were compared by repeated measures analysis of variance (ANOVA, SAS, Version 6.12; Cary, North Carolina: SAS Institute,1996). If the *F* value was significant, Bonferroni multiple comparison tests were performed. The effect of sildenafil on FMD from the 60-s to 4-min time points was studied by the repeated measures ANOVA model, including patients, medication (sildenafil and pre-sildenafil), presence or absence of CAD and time as main effects and incorporating the two factor interactions between them. Data are expressed as mean ± SEM, and all p values are two-tailed. Multiple stepwise regression analysis was performed to establish the most parsimonious model for the relationship between the magnitude of change in epicardial diameter with sildenafil and the covariates age, gender, race, presence of CAD, hypertension, diabetes, current cigarette use, total cholesterol, low-density and high-density lipoprotein and triglyceride levels.

RESULTS

Study 1: systemic and pulmonary hemodynamic effects. Supine mean pulmonary arterial and wedge pressures fell by 13 ± 3% and 18 ± 6%, respectively, after sildenafil but systemic and pulmonary vascular resistance, mean supine blood pressure, heart rate and cardiac output were unchanged (Table 1).

Resting coronary vascular tone. EPICARDIAL CIRCULATION. Significant epicardial vasodilation occurred 30 min after sildenafil, reaching a maximum of 6.9 ± 1.3% after 45 min ($p < 0.0001$) (Fig. 1). Vasodilation was similar in patients with and without CAD (+5.6 ± 1.1%, $p < 0.0001$ and +7.4 ± 1.8%, $p < 0.001$, respectively) and in mid- and distal epicardial segments. In comparison, verapamil produced 8.4 ± 1.1% vasodilation before sildenafil. Stenotic segments of coronary arteries dilated by 13.3% in response to sildenafil, from 1.41 ± 0.12 to 1.57 ± 0.57 mm, $p = 0.03$. Increasing age, but not the presence of CAD, was an independent predictor of the epicardial response to sildenafil, ($p < 0.05$ by multivariate analysis).

MICROVASCULAR CIRCULATION. Coronary blood flow ($p = 0.15$) and CVR ($p = 0.26$) remained unchanged after sildenafil in all groups (Table 2).

Coronary vascular endothelial function. EPICARDIAL CIRCULATION. Epicardial coronary diameter with ACH was greater after sildenafil was administered ($p = 0.001$, ANOVA) but not after verapamil ($p = 0.17$) (Table 2). The response was separately assessed in epicardial segments that initially constricted (denoting endothelial dysfunction) and those that dilated (normal endothelial function) with ACH. Segments with constriction but not those that initially dilated with ACH improved after sildenafil (Fig. 2). Similarly, patients with CAD had tended to exhibit an improvement in their epicardial coronary responses (from −1.3 ±

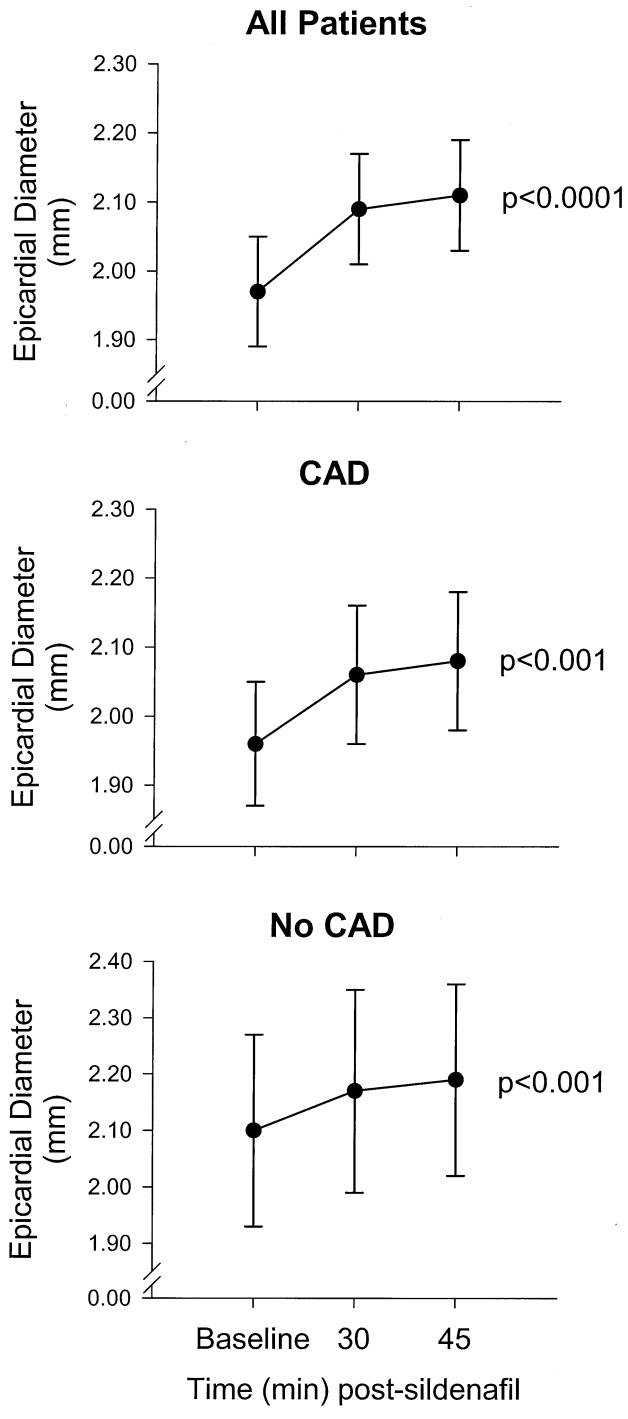

Figure 1. Effect of sildenafil (100 mg PO) on epicardial coronary diameter in all patients and the subsets with and without coronary atherosclerosis (CAD).

2.8% to 0.4 ± 3% with 15 μg/min of ACH, p = 0.07), but this was not observed in those with NCA (p = 0.3).

To assess endothelial function with ACH independent of the baseline dilator effect of sildenafil, we calculated the ratio of epicardial diameter with ACH/verapamil before and after sildenafil was administered. There was a significant improvement in the epicardial ACH/verapamil ratio after sildenafil administration (p < 0.0001 by ANOVA).

MICROVASCULAR CIRCULATION. ACH and verapamil infusions at baseline produced progressive microvascular dilation (Table 2). After sildenafil administration, CVR with ACH was lower (p = 0.03, ANOVA) but not with verapamil (p = 0.9), indicating selective improvement in microvascular endothelium-dependent function (Table 2, Fig. 3). In patients with CAD, ACH-mediated microvascular dilation was enhanced (p = 0.013, ANOVA for percentage of change in CVR), whereas it remained unchanged in those with NCA (Fig. 3).

Coronary vascular response to CPT epicardial circulation. The heart rate during CPT was similar (77 ± 8 before vs. 78 ± 3 beats/min after sildenafil) but increase in blood pressure was lower after sildenafil (132 ± 4 before vs. 122 ± 4 mm Hg after, p = 0.04). Coronary epicardial diameter during CPT was greater after sildenafil (from 1.92 ± 0.1 mm pre- to 2.04 ± 0.1 mm, p < 0.001). This dilation was observed in patients with and without CAD (p ≤ 0.01 in both). To assess the response to CPT independent of the baseline dilator effect of sildenafil, we calculated the epicardial diameter ratio of CPT/verapamil before and after sildenafil. There was a significant improvement in the CPT/verapamil ratio after sildenafil (p < 0.001) (Fig. 4).

MICROVASCULAR CIRCULATION. There was no coronary microvascular dilation observed during CPT at baseline (5.5 ± 3.6% change in CVR). After sildenafil, there was significant microvascular vasodilation with CPT (−2.3 ± 3% fall in CVR, p = 0.02) (Fig. 4).

Platelet flow cytometry. The percent of platelets positive for PAC-1 expression in the unstimulated (7.7 ± 2% to 4.4 ± 1%, p = 0.04) and ADP-stimulated samples (85 ± 3% to 67 ± 5%, p = 0.008) were significantly lower after sildenafil but remained unchanged with TRAP stimulation (Fig. 5). Sildenafil therapy did not significantly alter the percentage of platelets positive for CD-62 with and without ADP or TRAP (data not shown).

Study 2: resting hemodynamics. CONTROLS. Mean and diastolic blood pressure were lower with both ISDN and sildenafil compared with placebo (Table 1).

CAD PATIENTS. Heart rate increased with ISDN compared with both sildenafil and placebo. Systolic and diastolic blood pressures fell with ISDN and sildenafil, with the effect of ISDN being greater (Table 1).

Treadmill exercise. ISCHEMIC THRESHOLD. Compared with placebo, ISDN improved time-to and rate-pressure product at 1 mm of ST depression (both p < 0.05). Sildenafil had an intermediate effect with no significant difference compared with either ISDN or placebo (Table 3).

PEAK EXERCISE. There was no difference in total exercise time or rate-pressure product at peak exercise between the three treatment periods. However, ST depression at peak exercise was lower with ISDN compared with placebo (Table 3). Sildenafil had an intermediate effect on this

Table 2. Coronary Vascular Effects of Sildenafil

	Baseline	S	ACH 1	ACH1 + S	ACH2	ACH2 + S	Verapamil	Verapamil + S
Coronary blood flow (ml/min)	39.5 ± 5	41.8 ± 5	48.9 ± 6	57.8 ± 8†	91.9 ± 12	94.4 ± 14	72.8 ± 12	71 ± 12
Coronary vascular resistance	3.5 ± 0.4	3.4 ± 0.4	3.5 ± 0.4	2.3 ± 0.3*	1.6 ± 0.2	1.5 ± 0.2*	2.0 ± 0.4	2.0 ± 0.5
Diameter (mm)	1.99 ± 0.08	2.12 ± 0.08‡	1.97 ± 0.08	2.10 ± 0.09‡	1.95 ± 0.08	2.1 ± 0.09‡	2.2 ± 1	2.2 ± 0.1

Coronary blood flow, vascular resistance, and diameter were significantly different pre- vs. post-sildenafil (S) by ANOVA ($p < 0.05$). *$p \leq 0.05$, †$p < 0.01$, ‡$p < 0.001$ pre- vs. post-sildenafil (S). Vascular resistance is (mm Hg/ml/min).
ACH = acetylcholine (ACH1 = 1.5 and ACH2 = 15 μg/min dose); S = sildenafil.

parameter (Table 3). Rate-pressure product at each stage of exercise was similar in the three study groups. No significant arrhythmia or hemodynamic disturbance was observed.

CHEST PAIN. Twelve patients (50%) experienced chest pain during treadmill exercise when administered placebo, six (25%) when administered ISDN and seven (29%) when administered sildenafil (p = NS between groups).

Resting brachial artery diameter. CONTROLS. Brachial arteries dilated from 3.39 ± 0.23 to 3.54 ± 0.22 mm (4.8 ± 1.6%, $p < 0.01$) after sildenafil and to 4.18 ± 0.21 mm (23 ± 2%, $p < 0.001$) after ISDN.

CAD PATIENTS. ISDN dilated the brachial artery by 18 ± 2%, $p < 0.001$ (from 3.67 ± 0.11 to 4.38 ± 0.11 mm), but D was unchanged (3.8 ± 0.1 mm) after sildenafil (+1.6 ± 0.9%, p = 0.12 vs. baseline and p = 0.06 vs. controls). Brachial artery dilation was greater with ISDN compared to sildenafil in both groups. The D remained unchanged with placebo.

EFFECT ON FMD PEAK. FMD at 60 s was unchanged by placebo and ISDN in both groups. Peak FMD was unchanged by sildenafil in both controls (+8.7 ± 1.8% before vs. +8.4 ± 1.7% after, p = NS) and in CAD patients (+6.8 ± 1.0% before vs. +6.2 ± 0.9% after, p = NS).

Hyperemic vasodilation of the brachial artery persisted for a significantly longer duration after sildenafil compared to pre-sildenafil; by ANOVA, a significant interaction with time was noted, with higher D after sildenafil between 2.5 and 4 min of hyperemia ($p < 0.05$) (Fig. 6). Similar effects were noted in subjects with and without CAD.

DISCUSSION

We have investigated the effects of sildenafil on human coronary and peripheral circulation, on platelet function, and on myocardial ischemia in patients with CAD with the hypothesis that increased NO-mediated cGMP availability will improve vascular and platelet function and ameliorate myocardial ischemia. We demonstrated that 100 mg of oral sildenafil produces epicardial coronary arterial vasodilation in patients with CAD and in those with NCA who exhibit risk factors. This effect was observed in smooth and mildly atherosclerotic segments of the coronary arteries. Brachial arterial vasodilation was observed with sildenafil in healthy controls but not in patients with CAD. Sildenafil also selectively improved endothelium-dependent coronary vascular responses and vasomotion during physiologic stress without affecting endothelium-independent responses. This effect was greater in patients with CAD and those with endothelial dysfunction. Consistent with these findings, prolongation of peripheral vasodilation was observed during hyperemia.

The impact of sildenafil on patients' ischemic threshold during exercise was modest, with an effect that was inter-

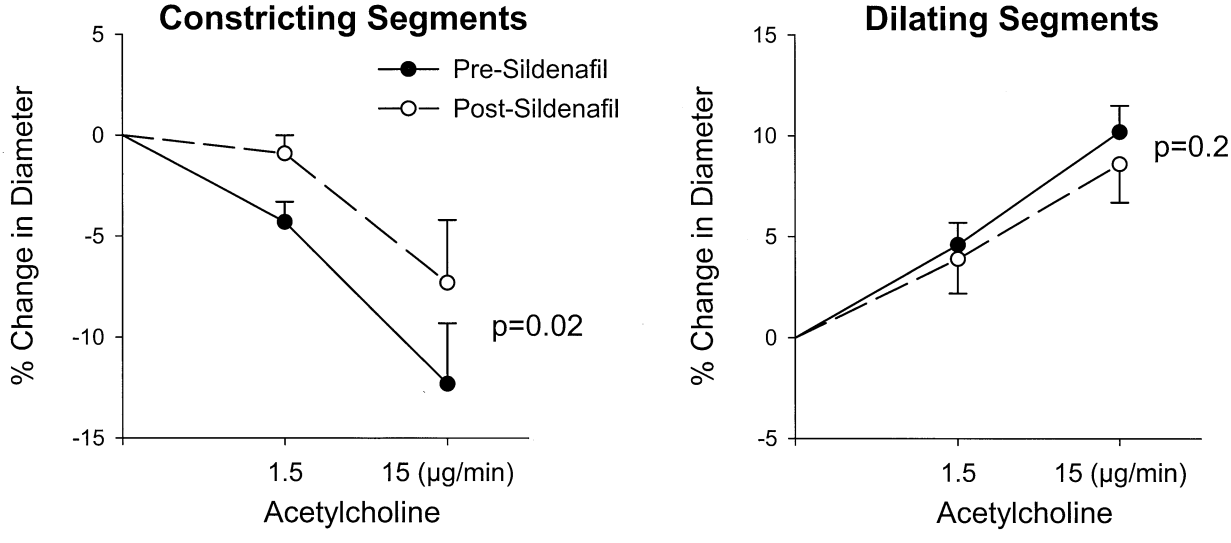

Figure 2. Effect of sildenafil on percent of change in epicardial coronary artery diameter in segments that were initially constricted with acetylcholine (denoting endothelial dysfunction) and those that were initially dilated with acetylcholine (preserved endothelial function).

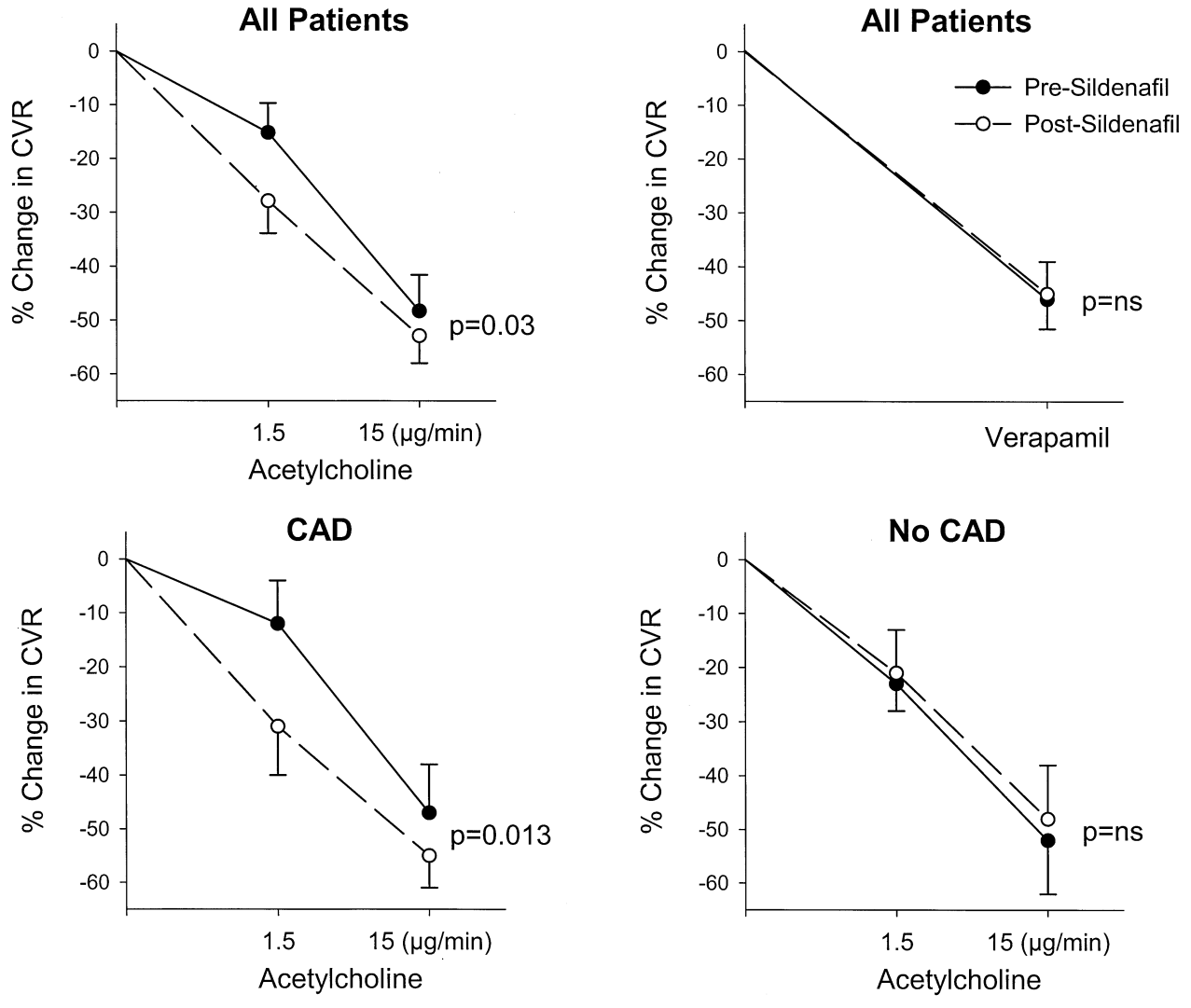

Figure 3. Effect of sildenafil on microvascular vasodilation (percent of change in coronary vascular resistance [CVR] as a result of acetylcholine and verapamil in all patients and in response to acetylcholine in patients with and without angiographic coronary atherosclerosis (CAD).

mediate between placebo and a 10 mg of oral ISDN. No patient experienced significant exacerbation of exercise-induced ischemia or cardiac arrhythmia with sildenafil.

Coronary, pulmonary, and systemic effects. A small fall in supine pulmonary and seated systemic arterial pressures confirmed the previously reported mild vasodilator activity of sildenafil (10,13). The smaller fall in arterial pressure observed with sildenafil in the catheterization study may be explained by the subjects' supine position rather than time to peak onset of the drug effect because significant pulmonary and epicardial coronary vasodilation were observed. The effects of sildenafil on blood pressure and heart rate were less compared with 10 mg of ISDN in patients with CAD. To our knowledge, this is the first demonstration of human epicardial coronary vasodilation by sildenafil in patients with and without CAD in vivo and confirms previous observations in porcine coronary arteries (14). The lack of epicardial vasodilation observed in a previous study may have been due to differences in quantitative angiographic techniques or to the confounding effects of concomitant cardiac medications (13). The magnitude of epicardial dilation with sildenafil was approximately a third of that observed with nitroglycerin (15) and similar to that observed with verapamil. The apparent greater percent of dilation of mildly stenotic segments was not significantly different from smooth segments. Peripheral conduit arterial vasodilation with sildenafil was less marked than in the coronary arteries, which may be due to differences in patient selection. However, it is likely that the vasodilator response to PDE5 antagonism is proportional to basal production of NO and differences in basal NO activity between these circulations may provide an explanation (3,4,16,17). Similarly, the lack of vasodilation of the brachial arteries in CAD patients compared to controls may have been due to differences in age and/or tonic NO activity.

Endothelium-dependent vascular function. Acetylcholine vasoconstricts vessels with dysfunctional endothelium because the constrictor effect of smooth muscle muscarinic

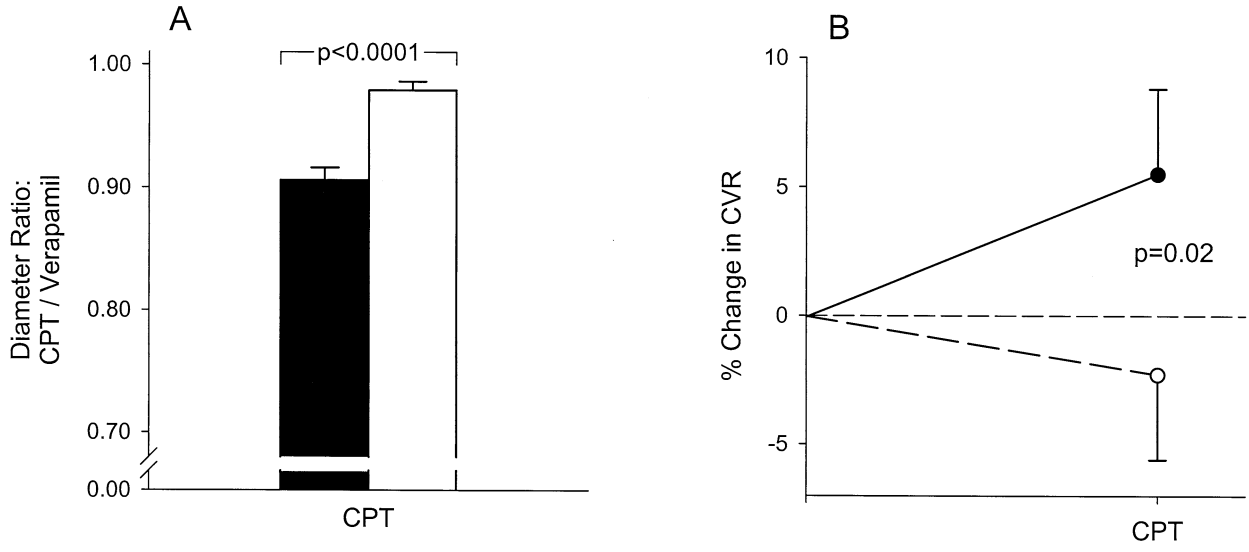

Figure 4. (A) Effect of sildenafil on epicardial coronary artery diameter ratio cold-pressor testing (CPT)/verapamil. **(B)** Effect of sildenafil on microvascular vasodilation (reduction in coronary vascular resistance) during CPT. **Black bars** = pre-sildenafil; **white bars** = post-sildenafil.

receptor activation predominates over the dilating effects of endothelium-derived relaxing factors, in particular NO (2,18,19). We observed selective improvement in ACH responses independent of the response to verapamil, denoting improvement in endothelial function, which was consistent with findings in canine experiments (20). A greater enhancement of endothelial function occurred in patients with CAD and those with more severe endothelial dysfunction. Because inhibition of NO synthase abolishes PDE5 antagonist-mediated vasodilation, potentiation of cGMP almost certainly explains our observations (21,22).

An important consequence of endothelial dysfunction is the resulting abnormal vasomotion during physiologic stresses such as exercise, pacing or exposure to cold, believed to be due to reduced shear-mediated NO release (3,23–26). Improvement in coronary vasomotion during CPT after sildenafil extends observations we made during pharmacologic testing. They are also consistent with previously reported improvement in coronary flow reserve with adenosine in subjects with CAD and enhancement of FMD in heart failure after sildenafil treatment (13,27).

Our findings in the brachial artery confirmed observations made in the coronary circulation. FMD, a largely NO-dependent phenomenon, was prolonged by sildenafil, without affecting maximal vasodilation (4).

Exertional myocardial ischemia. As expected, indices of ischemia improved after ISDN. A modest improvement was observed after sildenafil, but their magnitude was not

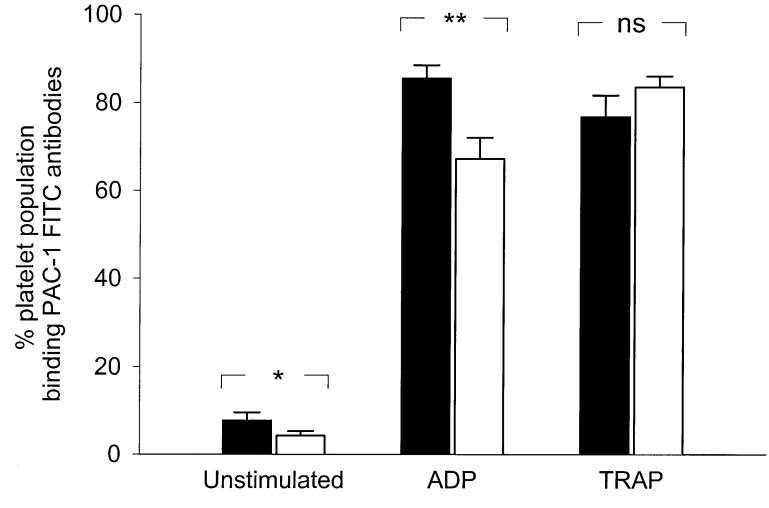

Figure 5. Effect of sildenafil on surface expression of activated platelet glycoprotein IIb/IIIa receptors illustrated by the percentage of platelets binding FITC-conjugated PAC-1 antibodies. ADP = adenosine diphosphate; TRAP = thrombin receptor activator peptide. **Black bars** = pre-sildenafil; **white bars** = post-sildenafil. *$p < 0.05$, **$p < 0.01$, NS = not significant.

Table 3. Study 2: Effects of Placebo, Sildenafil, and Isosorbide Dinitrate on Exercise Parameters

	Placebo	Sildenafil	Isosorbide
Exercise duration to ischemic threshold (s)	538 ± 63	563 ± 62	598 ± 68*
Peak exercise duration (s)	687 ± 50	692 ± 57	710 ± 53
Heart rate (beats/min)	140 ± 3	142 ± 4	139 ± 3
Systolic blood pressure (mm Hg)	164 ± 5	159 ± 5	165 ± 5
Diastolic blood pressure (mm Hg)	78 ± 2	73 ± 3	71 ± 3
RPP at ischemic threshold ($\times 10^3$ mm Hg.beats/min)	19.0 ± 0.8	20.6 ± 0.8	21.9 ± 0.8*
RPP at peak exercise ($\times 10^3$ mm Hg.beats/min)	22.8 ± 0.8	22.6 ± 0.9	22.9 ± 0.8
Maximum ST depression (mm)	−1.77 ± 0.20	−1.55 ± 0.16	−1.52 ± 0.18*

Ischemic threshold is at onset of 1-mm ST segment depression. *$p < 0.05$ vs. placebo.
RPP = rate-pressure product.

significantly different compared to either placebo or ISDN, a finding that is consistent with the lower vasodilator potency of sildenafil compared to nitroglycerin on resting vascular tone (15).

Platelet activation. We have previously reported that endogenous NO importantly contributes to platelet passivation in vivo in the human coronary and peripheral circulations (6,7). Here, we examined the effect of sildenafil on two markers of platelet activation that reflect activated platelet glycoprotein IIb/IIIa receptor (PAC-1 antibody binding) and surface expression of P-selectin (CD-62 antibody binding) a marker of alpha-granule release (28,29). We observed reduced binding of PAC-1 in the unstimulated and ADP-stimulated whole blood whereas CD-62 binding was not significantly altered by sildenafil, suggesting increased threshold for activation of the IIb/IIIa receptor without an effect on platelet degranulation (30,31). This is also consistent with potentiation of the anti-aggregatory action of NO donors by sildenafil reported previously (29,32). Whether the observed anti-platelet effect results in a clinically relevant benefit requires further investigation.

Study limitations. We examined the effect of a single dose of sildenafil; however, its action after multiple dosages may vary. Study 1 was not placebo-controlled because of the invasive nature of the protocol, but the analysis was blinded. The trend toward an improvement in exercise-induced ischemia with sildenafil compared with placebo is consistent with our observation of improved physiologic coronary vasomotion and also its known beneficial effect on coronary flow reserve (13). However, this may only reach statistical significance in a larger study population. It may not be reasonable to extend our findings to very symptomatic or unstable patients with CAD, and interactions with the sympathetic nervous system or other cardiac medications were also not investigated (33).

Conclusions. Sildenafil dilates epicardial coronary arteries, improves endothelial dysfunction, and improves physiologic coronary vasomotion, suggesting that PDE5 antagonism has a potential role in the treatment of patients with vascular endothelial dysfunction. Furthermore, sildenafil attenuates activation of the platelet IIb/IIIa receptor, and its anti-ischemic effect was intermediate between ISDN and placebo. Further studies are required to determine the clinical impact of these observations.

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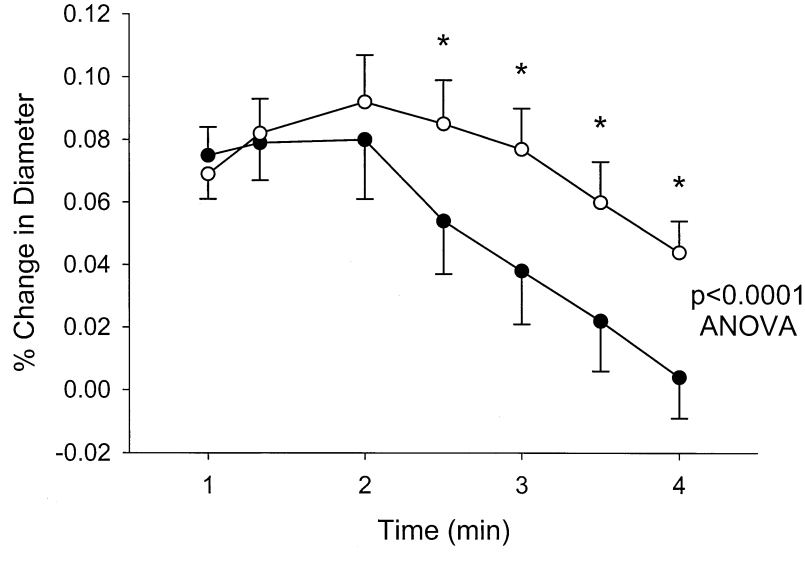

Figure 6. Flow-mediated vasodilation (percent of change in diameter of the brachial artery compared with baseline) is shown during hyperemia, before and after sildenafil. **Filled circles** = pre-sildenafil; **open circles** = post-sildenafil.*$p < 0.05$ pre- vs. post-sildenafil.

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