JACC Vol. 14, No. 5
November 1, 1989:1181–90

Coronary Vasomotion in Response to Sympathetic Stimulation in Humans: Importance of the Functional Integrity of the Endothelium

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The coronary vasomotor response to the cold pressor test was studied with use of quantitative coronary angiography in 32 patients without evidence of coronary artery disease and 55 patients with such disease; in a subset of 22 patients (9 with normal coronary arteries and 13 with coronary artery disease), the effects of the cold pressor test were compared with the effects of the endothelium-dependent vasodilator acetylcholine with simultaneous intracoronary Doppler flow velocity measurements to assess the influence of endothelial dysfunction. The cold pressor test induced vasodilation of 8.9 ± 5.7% in all 77 analyzed vessel segments of the group with normal arteries ($p < 0.01$). In contrast, in patients with coronary artery disease, the 52 analyzed stenotic segments were constricted by −12.1 ± 9.5% ($p < 0.01$), the 57 analyzed vessel segments with luminal irregularities were constricted by −8.9 ± 5.2% ($p < 0.01$) and 40 (85%) of 47 angiographically normal segments also were constricted by −7.0 ± 4.9% ($p < 0.05$).

Preserved vasodilating capability was demonstrated by intracoronary nitroglycerin in all analyzed segments. In nine patients with normal coronary arteries, the analyzed vessel segments were dilated in response to both the cold pressor test and intracoronary acetylcholine by 10.9 ± 5.4% and 13.4 ± 4.7%, respectively. In contrast, in all 13 patients with coronary artery disease, vasoconstriction of identical vessel segments by −9.1 ± 3.75% and −23 ± 10.4%, respectively, was observed after both the cold pressor test and intracoronary acetylcholine. Intracoronary propranolol did not significantly affect either the vasodilative response in 11 normal coronary arteries (11.3 ± 4.4% before and 8.6 ± 4.3% after beta-blockade) or the vasoconstrictor response in 8 atherosclerotic coronary arteries (−11.4 ± 4.6% before and −14.6 ± 5.3% after beta-blockade).

The dilation of normal and the constriction of atherosclerotic coronary arteries with cold pressor testing exactly mirror the response to the endothelium-dependent dilator acetylcholine. Endothelial dysfunction in coronary atherosclerosis resulted in a loss of normal dilator function and permitted vasoconstrictor responses to sympathetic stimulation. Thus, coronary vasomotion of large epicardial arteries in response to sympathetic stimulation by the cold pressor test in humans is intimately related to the integrity of endothelial function.

(J Am Coll Cardiol 1989;14:1181–90)

In vitro studies (1) have demonstrated the important role of the endothelium in modulating the responsiveness of the underlying vascular smooth muscle. Furchgott and Zawadski (2) showed that the ability of acetylcholine to dilate blood vessels depended on an intact endothelial cell layer. The discovery that the endothelium plays a critical role in mediating vasodilation to a variety of stimuli (3–5) prompted the widely accepted hypothesis that damaged endothelial cells may predispose blood vessels to episodes of spasm (6). However, relatively little attention has been paid to the role of the endothelium in mediating vasomotion by adrenergic mechanisms. Recent experimental studies (7) in intact dogs have demonstrated that the vasodilator response to epinephrine (a mixed alpha- and beta-adrenergic agonist) is reversed to a vasoconstrictor response after removal of the endothelium, thereby indicating that the endothelium mediates the dilation in response to adrenergic stimulation in large canine iliac arteries.

Sympathetic stimulation by the cold pressor test in humans was recently shown (8) to dilate normal coronary arteries but constrict atherosclerotic coronary arteries in vivo, thus suggesting that normal vasodilation is replaced by paradoxic vasoconstriction in the presence of atherosclero-

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Manuscript received March 20, 1989; revised manuscript received May 10, 1989, accepted May 17, 1989.

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0735-1097/89/$3.50

sis. On the basis of these results, it was hypothesized that endothelial dysfunction present in atherosclerosis may result in a loss of dilator function and permit constrictor responses to sympathetic stimulation.

The present study was designed to assess the importance of the functional integrity of the endothelium in modulating coronary vasomotion in response to cold pressor testing. For this purpose, the effects of cold pressor test-induced sympathetic stimulation on coronary vasomotor tone were determined in a large series of patients with no evidence of coronary artery disease or with an early stage of coronary artery disease indicated by minor irregularities without hemodynamically significant stenosis. To specifically evaluate the role of intact endothelial function in mediating the vasomotor response to sympathetic stimulation, cold pressor test-induced vasomotion was compared with the effects of endothelium-dependent vasodilator acetylcholine.

Methods

Classification of patients. Eighty-seven patients undergoing routine diagnostic cardiac catheterization were studied. These patients were classified into two groups on the basis of their history and the presence or absence of atherosclerosis on the diagnostic angiogram.

Group I: normal control group. Thirty-two patients with angiographically normal coronary arteries served as control subjects. Patients with a history of arterial hypertension (defined as systolic blood pressure >150 mm Hg and diastolic blood pressure >90 mm Hg) or the presence of left ventricular hypertrophy, diabetes mellitus and hypercholesterolemia were excluded from the normal control group. Total serum cholesterol values in these patients ranged from 148 to 213 mg/100 ml (mean ± SD 179 ± 14) and triglyceride levels ranged from 111 to 147 mg/100 ml (mean 131 ± 8). The majority of these 32 patients were studied because of atypical chest pain; 3 patients (all of them women) had an abnormal thallium exercise test and 4 had intermittent left bundle branch block as the cause for referral for diagnostic angiography. The mean age of these patients was 45.2 years; there were 17 women and 15 men. All subjects had angiographically normal, smooth coronary arteries without luminal irregularities and no evidence of segmental wall motion abnormalities on left ventricular cineangiography.

Group II: patients with coronary artery disease. Fifty-five patients were classified as having coronary artery disease. Seventeen of these patients had luminal irregularities in at least one major coronary artery, with no focal stenosis >50% diameter narrowing on diagnostic coronary angiography. Mean total serum cholesterol level in these patients was 231 ± 35 mg/100 ml (range 154 to 316); high density lipoprotein (HDL) cholesterol was 50 ± 12 mg/100 ml (range 29 to 81, n = 11), and triglycerides were 179 ± 32 mg/100 ml (range 122 to 288). Twenty-six patients had one vessel disease (defined as >50% diameter narrowing), eight patients had two vessel disease and four patients had three vessel disease. Total serum cholesterol in these patients with at least one coronary lesion with >50% diameter narrowing was 246 ± 39 mg/100 ml (range 158 to 335), HDL cholesterol was 45 ± 21 mg/100 ml (range 23 to 89) and triglycerides were 183 ± 42 mg/100 ml (range 122 to 279). Ten subjects were women, 45 were men; their mean age was 54.3 years. Patients with unstable angina, recent myocardial infarction or clinical evidence of heart failure were excluded.

Written informed consent was obtained from all patients before the studies. Study protocols were approved by the Ethical Committee of the University of Freiburg.

Study design. Vasoactive therapy was discontinued at least 24 h before cardiac catheterization. No patient received a beta-adrenergic blocking agent within 48 h before the study. Diagnostic coronary angiography was performed by a standard percutaneous femoral approach using the Judkins technique. The cold pressor test was performed ≥5 min after the control angiogram. For cold pressor testing, the patient's hand and forearm were immersed in ice water for 90 s. Heart rate and aortic pressure were continuously measured, and serial hand injections of nonionic contrast material (Ultravist, Schering AG) into the left coronary artery were performed at control and at the end of the cold pressor test (immediately after removal of the forearm from ice water). After a 5 min recovery period, 0.3 mg of nitroglycerin was injected into the left coronary artery to assess the vasodilatory capability of the coronary arteries, and a final angiogram was obtained.

Studies with acetylcholine. In 9 subjects from the normal control group and 13 patients with coronary artery disease, the vasomotor effects of the cold pressor test and acetylcholine were compared. Of the 13 patients with coronary artery disease, 3 had >50% luminal narrowing of the left anterior descending artery, 5 had luminal irregularities with no focal stenosis >50% and 4 had an angiographically completely normal left anterior descending artery, but evidence of atherosclerosis elsewhere in the coronary system.

Ten minutes after cold pressor testing, acetylcholine was selectively infused into the left anterior descending artery through a Doppler catheter. Increasing dosages were used to achieve estimated final blood concentrations in the coronary bed of 10^{-8}, 10^{-7} and 10^{-6} *M* (assuming a blood flow of 80 ml/min) at an infusion rate of 1 ml/min, lasting 3 min for each concentration. For this purpose, an additional 5,000 U of heparin was given intravenously, and an 8F guiding catheter (Schneider) was introduced into the left main coronary artery. A 3F Monorail-Doppler catheter (Schneider) with a 20 MHz pulsed Doppler crystal was advanced into the left anterior descending artery by means of a 0.014 in (0.036 cm) guidewire. The Doppler catheter was carefully positioned to obtain a stable flow velocity signal. Before the Doppler catheter was introduced into the guiding catheter, flow

velocity recordings were referenced to zero and calibrated. Continuous phasic and mean coronary flow velocity measurements and serial contrast injections were performed during control study, at the end of cold pressor testing, at recontrol study, at the end of each acetylcholine infusion period and after intracoronary nitroglycerin, which was injected 5 min after the end of the acetylcholine infusion.

Studies with intracoronary beta-blockade. To evaluate the role of beta-adrenergic receptors in mediating the coronary vasomotor response, 11 subjects from the normal control group and 8 with coronary artery disease underwent two serial cold pressor tests before and after the intracoronary administration of propranolol. Right atrial pacing at 10 beats/min above the intrinsic heart rate was performed throughout the study in the initial four subjects. However, transient termination of atrial pacing in these patients did not reveal any changes in heart rate or aortic blood pressure after the administration of intracoronary propranolol compared with the hemodynamic values before propranolol. Therefore, atrial pacing was not performed in the remaining 15 patients undergoing the beta-blockade study. In nine of these patients (four with normal coronary arteries and five with coronary artery disease), left anterior descending artery blood flow velocity was continuously measured by the Doppler catheter, as described. After the initial cold pressor test, 1 mg of propranolol was infused into the left main coronary artery over 2 min, and a second cold pressor test was performed. Coronary angiograms were obtained under control conditions, at the end of the initial cold pressor test, at recontrol study after a 5 min recovery period, 2 min after the intracoronary infusion of 1 mg of propranolol, at the end of the second cold pressor test under beta-blockade and after intracoronary nitroglycerin, which was injected 5 min after the second cold pressor test.

Quantitative coronary angiography. Coronary angiography was performed with use of a simultaneous biplane multidirectional isocentric radiographic system (Siemens Bicor). Coronary arteries under study were positioned near the isocenter, and special care was taken to avoid overlapping of coronary segments. Biplane cineangiograms were recorded at a rate of 25 frames/s. The study angiograms were examined by three investigators, and coronary arteries were classified as smooth, irregular or stenotic by a consensus decision before analysis. Measurements of coronary segments were made in several vessels, but only one segment per vessel was analyzed. Coronary segments were measured at the site of stenosis in stenotic vessels, at the site of luminal irregularities in minimally diseased vessels and in a well defined region of the angiographically smooth-appearing vessels. For quantitative analysis, end-diastolic cine frames were videodigitized and stored in the image analysis system (Mipron I, Kontron electronics) in a 512 × 512 matrix with an eight bit gray scale. With use of the 12 cm field of view, the resulting pixel density was 7.3 pixels/mm. The geometric resolution of the X-ray imaging chain was >4 line pairs/mm.

Automatic vessel segment contour detection was performed by a geometric edge differentiation technique similar to the method described by Reiber et al. (9). In brief, after interactive delineation of a centerline within the vessel segment to be measured, the computer automatically generates a number of scanlines perpendicular to the centerline. The first and second derivative function of the densograms along each scanline are then computed, and the contour point is defined as 60% of the distance between the extrema of the first and second derivatives. With use of the detected contour points, the computer then automatically generates a refined centerline of the vessel segment, and the edge detection algorithm is repeated. Each individual scanline is smoothed by a second order polynomial fit, and smoothing of the contour is obtained by averaging three neighboring scanlines. Calculation of the exact radiologic magnification factor of the measured segment is used to scale the data from pixels to millimeters, as previously described (10).

This technique was previously validated in phantom studies. The accuracy and precision of quantitative angiographic measurements were determined from the analysis of cine films of a Plexiglas block with precision-drilled models of coronary arteries with diameters of 1, 2, 3 and 3.5 mm filled with contrast medium and filmed under 10 cm of water. The accuracy of the contour detection technique was defined by the average difference of the computed results with the true values, and the precision was defined by the pooled standard deviations of the differences. These phantom measurements revealed an accuracy of 3.5 ± 1.7% (mean ± 1 SD, n = 80 measurements) and a precision of 2.4 ± 1.1%. The reproducibility of the measurements (repeated analysis of the cineangiograms by one analyst) revealed a coefficient of variation of 1.4 ± 0.6%. Interobserver variability associated with the computer analysis was 1.7 ± 0.7% (coefficient of variation).

Six to 8 mm segments from angiographically normal vessels and vessels with luminal irregularities were measured. A series of diameter measurements was obtained for each scanline for the length of the arterial segment, displayed in graphic form showing diameter versus segment length and the mean diameter value was calculated. Tapered vessel segments were not evaluated. In stenotic segments, the narrowest portion of the stenosis was measured. Whenever possible, measurements were performed in both views of the biplane images. In both normal and irregular segments, the diameter values of both views were averaged; in stenotic segments, the narrowest diameter from one of the two views was chosen. Only single plane analysis was performed for those coronary segments demonstrating overlap with other parts of the coronary tree in one view.

Reproducibility of serial measurements. Anatomic landmarks were used to reproduce the regions of interest to be

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measured spatially in repeated measurements to assess serial changes. The reproducibility of serial measurements of the same coronary segment during repeated contrast injection was assessed in the present study. For this purpose, in 10 of the patients receiving selective infusion of acetylcholine into the left anterior descending artery, a segment in the midportion of the left circumflex artery was measured at control study and at the end of each of the three acetylcholine infusion periods. Thus, the coefficients of variation comprise not only the natural variability of the diameter of a coronary segment measured at sequential time points without interposed interventions (because acetylcholine was infused only into the left anterior descending artery, not into the circumflex artery), but also the intraobserver variability associated with repeated measurements of the same coronary segment in serial cineangiographic recordings. In addition, potential contrast material effects on the diameters of coronary arteries caused by serial injections would have been detected in this way. The mean coefficient of variation was 2.2 ± 1.1% (n = 40 measurements). Moreover, there were no significant directional changes in the measured diameters after serial injections of contrast material. Thus, a 5 min recovery period after the injection of 5 to 8 ml of nonionic contrast material into the left coronary system proved to be sufficient for avoiding any effects of the contrast material itself on the diameter of epicardial coronary arteries during serial studies.

Estimation of coronary blood flow. For rough estimation of directional changes in coronary blood flow, the mean Doppler-derived blood flow velocity was multiplied by the computed cross-sectional area of the vessel at the site of the Doppler crystal. The cross-sectional area was calculated from biplane views, assuming an elliptical shape of the vessel. Because the injection of contrast material into the coronary circulation resulted in the typical biphasic response of coronary blood flow velocity with an initial decrease followed by an increase in flow velocity as a result of the hyperemic effects of the contrast material, the mean blood flow velocity immediately before the contrast injection was used for estimation of coronary blood flow.

Statistical analysis. All data are expressed as mean values ± SD. Statistical comparisons were made by analysis of variance for repeated measures followed by the Student-Newman-Keuls test. Changes in hemodynamics and vessel diameters during the cold pressor test before and after infusion of propranolol were analyzed by Student's paired *t* test. Statistical significance was assumed if a null hypothesis could be rejected at the 0.05 probability level.

Results

Systemic hemodynamics during sympathetic stimulation (Table 1). Sympathetic stimulation by the cold pressor test increased heart rate and systolic blood pressure in all patients in both groups. The mean increase in the heart rate-systolic pressure product was 40.7 ± 13.5% in the group with normal coronary arteries and 43.2 ± 20.8% in the group with coronary artery disease. Thus, the systemic hemodynamics in response to the cold pressor test did not demonstrate any significant differences between groups I and II.

Table 1. Systemic Hemodynamics During Cold Pressor Stimulation

	Control	CPT	NTG
Group I (control subjects [n = 32])			
Heart rate (beats/min)	81.1 ± 9.8	88.0 ± 10.8*	83.2 ± 9.1
Systolic BP (mm Hg)	119 ± 15.8	153.9 ± 25.6*	114.1 ± 17.3
Rate-pressure product	9.67 ± 1.9	13.6 ± 2.9*	9.53 ± 2.6
Group II (patients with CAD [n = 55])			
Heart rate (beats/min)	72.5 ± 13.8	81.1 ± 15.3*	75.3 ± 14.8
Systolic BP (mm Hg)	133.2 ± 24.3	166.3 ± 27.8*	127.1 ± 27.3
Rate-pressure product	9.69 ± 3.1	13.7 ± 4.2*	9.48 ± 3.9

*$p < 0.01$ cold pressor test (CPT) vs. control. Data are expressed as mean values ± SD. BP = blood pressure; CAD = coronary artery disease; NTG = nitroglycerin; Rate-pressure product = heart rate × systolic blood pressure × 10^3; SBP = systolic blood pressure.

Response of Epicardial Coronary Arteries to Cold Pressor Test (Fig. 1)

Group I: Subjects with normal coronary arteries (Fig. 1A). Seventy-seven segments of angiographically normal coronary arteries were analyzed in the 32 subjects. All 77 segments dilated by 8.9 ± 5.7% in response to the cold pressor test. The mean vessel diameter increased from 2.73 ± 0.14 mm at control study to 2.97 ± 0.12 mm ($p < 0.01$) at the end of cold pressor testing. The vasodilating capability of all segments was demonstrated by further dilation in response to intracoronary nitroglycerin, which increased vessel diameter to 3.39 ± 0.17 mm ($p < 0.01$), reflecting an increase of 24.4 ± 12.8% compared with control vessel diameter.

Group II: patients with coronary artery disease (Fig. 1B). In the 55 patients with coronary artery disease, a total of 52 stenotic segments were analyzed. All 52 stenotic segments were constricted by −12.1 ± 9.5% in response to sympathetic stimulation. The mean minimal stenosis diameter decreased from 1.23 ± 0.11 mm at control study to 1.08 ± 0.13 mm at the end of cold pressor testing ($p < 0.01$). Intracoronary nitroglycerin resulted in an increase in minimal stenosis diameter to 1.40 ± 0.14 mm ($p < 0.01$), reflecting an increase of 14.3 ± 12.9% compared with control vessel diameter.

A total of 57 segments of coronary arteries with minor luminal irregularities were analyzed in the 55 group II patients (Fig. 1C). All 57 irregular segments were constricted by −8.9 ± 5.2% in response to cold pressor testing.

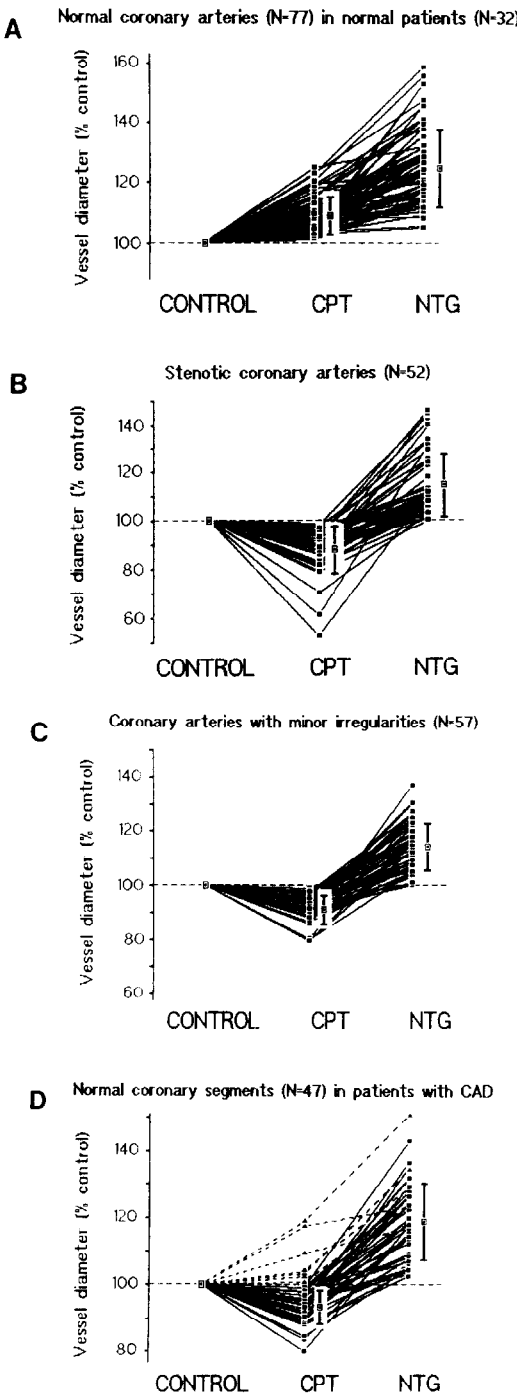


Figure 1. Responses of all analyzed coronary artery segments to cold pressor testing (CPT) and intracoronary nitroglycerin (NTG) in all patients. **A,** Normal coronary segments in 32 patients. **B,** Stenotic coronary segments in 38 patients with coronary artery disease (>50% diameter narrowing). **C,** Coronary segments with minor luminal irregularities in 35 patients with coronary artery disease (17 patients with luminal irregularities [<50% diameter narrowing] only, 14 patients with one vessel disease and 4 patients with two vessel disease but luminal irregularities in other vessels). **D,** Normal coronary segments in 31 patients with coronary artery disease (CAD) in other vessels (14 patients with luminal irregularities, 13 patients with one vessel disease and 4 patients with two vessel disease).

The mean vessel diameter decreased from 1.89 ± 0.18 mm at control study to 1.72 ± 0.19 at the end of cold pressor testing ($p < 0.01$). Intracoronary nitroglycerin increased the mean vessel diameter to 2.16 ± 0.2 mm ($p < 0.01$), reflecting an increase of 14.2 ± 8.6% compared with control values and demonstrating the preserved vasodilative capability of these irregular segments.

A total of 47 segments of angiographically normal coronary arteries were analyzed in the 55 group II patients with evidence of coronary artery disease elsewhere in the coronary system (Fig. 1D). Only 7 (15%) of the 47 segments were dilated in response to sympathetic stimulation, whereas the majority of these angiographically normal segments demonstrated a paradoxic vasoconstrictor response to the cold pressor test despite a completely normal angiographic appearance. The mean vessel diameter decreased from 2.13 ± 0.19 mm at control study to 1.98 ± 0.21 mm ($p < 0.05$) at the end of cold pressor testing, reflecting a decrease of 7.0 ± 4.9%. Intracoronary nitroglycerin increased the mean vessel diameter to 2.52 ± 0.22 mm ($p < 0.01$), reflecting an increase of 18.6 ± 11.4% compared with control values and demonstrating the vasodilative potential of all measured segments.

Comparison of Responses to Cold Pressor Test and Intracoronary Acetylcholine

Systemic hemodynamics and intracoronary blood flow (Table 2). Heart rate and systolic blood pressure increased in response to the cold pressor test both in the 9 subjects with normal coronary vessels and the 12 patients with coronary artery disease. The heart rate-systolic blood pressure product increased similarly by 39.2 ± 11.9% in those with normal coronary arteries and by 41.3 ± 15.8% in the group with coronary artery disease. The intracoronary infusion of acetylcholine had no effect on systemic hemodynamics at the concentrations given in both groups.

During the cold pressor test, intracoronary blood flow velocity within the left anterior descending artery increased by 42.3 ± 12.7% in subjects with normal coronary arteries and by 59.1 ± 21.3% in patients with coronary artery disease (p = NS).

Comparative responses of epicardial coronary arteries to cold pressor test and acetylcholine (Fig. 2). In the group with normal coronary arteries, all nine left anterior descending artery segments were dilated in response to sympathetic stimulation, from 3.1 ± 0.23 mm at control study to 3.43 ± 0.25 mm at the end of the cold pressor test ($p < 0.01$), reflecting an increase of 10.9 ± 5.4% (Fig. 2A). After baseline values had returned, intracoronary acetylcholine caused an increase in diameter of the identical coronary artery segments from 3.16 ± 0.24 mm at recontrol study to 3.58 ± 0.27 mm at the maximal concentration of 10^{-6} *M* ($p < 0.01$), reflecting an increase of 13.3 ± 4.7%. Further

Table 2. Systemic Hemodynamics in Response to Cold Pressor Test and Acetylcholine

	Control	CPT	Recontrol	ACh_{max}	NTG
Normal subjects (n = 9)					
Heart rate (beats/min)	79.2 ± 7.3	87.3 ± 8.9*	79.9 ± 8.1	78.1 ± 9.9	80.2 ± 10.1
Systolic BP (mm Hg)	125 ± 17.1	157.3 ± 23.4*	128.7 ± 19.9	126.2 ± 18.7	122.8 ± 20.1
Rate-pressure product	9.85 ± 1.9	13.7 ± 3.1*	10.1 ± 2.3	9.9 ± 2.4	9.8 ± 3.6
Patients with CAD (n = 13)					
Heart rate (beats/min)	74.1 ± 11.3	83.2 ± 13.8*	75.7 ± 12.1	74.6 ± 11.4	76.1 ± 12.7
Systolic BP (mm Hg)	135.7 ± 21.5	161.8 ± 31.2*	138.81 ± 28.2	136.6 ± 27.8	132.9 ± 30.1
Rate-pressure product	10.0 ± 2.9	13.5 ± 4.6*	10.5 ± 3.4	10.2 ± 3.6	10.1 ± 4.2

*$p < 0.01$ cold pressor test vs. control. Data are expressed as mean values ± SD. ACh_{max} = maximal concentration of acetylcholine; other abbreviations as in Table 1.

vasodilation was obtained by intracoronary nitroglycerin, which increased vessel diameter to 3.81 ± 0.38 mm ($p < 0.01$), corresponding to an increase of 22.7 ± 6.3% compared with recontrol values in the study.

In contrast, in all patients with coronary artery disease, including the four patients whose angiograms revealed an apparently normal left anterior descending artery with atherosclerosis in other coronary vessels, the analyzed vessel segment of the left anterior descending artery was constricted in response to sympathetic stimulation. The mean diameter decreased from 2.95 ± 0.26 mm at control study to 2.68 ± 0.28 mm at the end of cold pressor testing ($p < 0.01$), reflecting a decrease of 9.1 ± 3.8% (Fig. 2B). After baseline values returned, intracoronary acetylcholine caused a reduction in diameter of the identical coronary segments from 2.94 ± 0.28 mm at recontrol study to 2.25 ± 0.36 mm ($p < 0.01$) at the maximal concentration of 10^{-6} *M* (complete, spontaneously reversible occlusion of the left anterior descending artery was encountered in two patients at the maximal acetylcholine concentration of 10^{-6} *M*; therefore, the vessel diameters at the 10^{-7} *M* concentration were used in these two patients), reflecting a decrease of 23.2 ± 10.4%. The vasodilative capability of all analyzed segments was demonstrated by the response to intracoronary nitroglycerin, which increased vessel diameter in all segments to 3.31 ± 0.39 mm ($p < 0.01$), reflecting an increase of 12.5 ± 6.3% compared with recontrol values. Thus, the response to sympathetic stimulation of the cold pressor test exactly mimicked the effects of the endothelium-dependent vasodilator acetylcholine.

Cold pressor test-induced directional changes in coronary blood flow were estimated with use of the Doppler-derived mean coronary blood flow velocity and the computed vessel cross-sectional area as a rough estimate of blood flow within the left anterior descending artery. In all nine subjects with normal coronary arteries, mean left anterior descending artery blood flow increased by 66 ± 22% during sympathetic stimulation. Similarly, in the 13 patients with coronary artery disease, blood flow increased by 38 ± 31% (p = NS versus group I) in response to the cold pressor test.

Responses to Cold Pressor Test Before and After Beta-Adrenergic Blockade

Systemic hemodynamics and intracoronary blood flow velocities (Table 3). After the administration of intracoronary propranolol, there was essentially no change in systemic hemodynamics. A similar pressor response to the cold pressor test (both before and after beta-blockade) was observed, with an increase in the heart rate-systolic blood pressure product of 40 ± 19% before and 39 ± 20% after

Figure 2. Responses in identical left anterior descending coronary artery segments to cold pressor testing (CPT), intracoronary acetylcholine infusion and after nitroglycerin (NTG). **A,** 9 patients with normal coronary arteries. **B,** 13 patients with coronary artery disease. ACh_{max} = maximally achieved intracoronary acetylcholine concentration (10^{-6} *M*); C_1 = control; C_2 = recontrol.

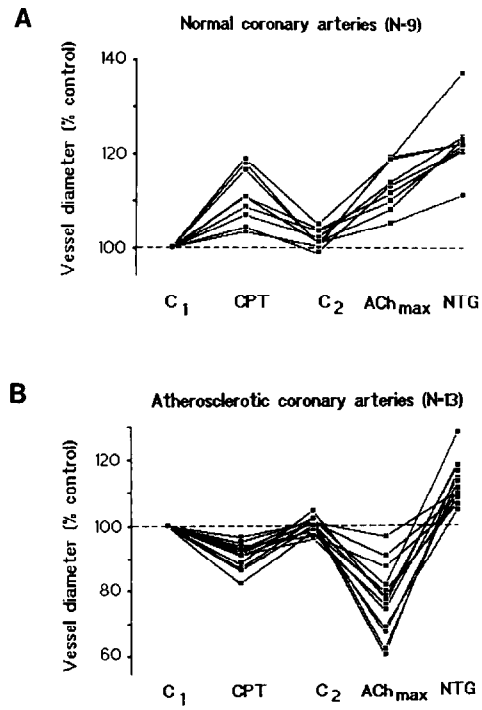


Table 3. Systemic Hemodynamics in Response to Cold Pressor Test Before and After Beta-Blockade

	Control	Pre-Beta CPT	Recontrol	Beta-blockade	Post-Beta CPT	NTG
Normal subjects (n = 11)						
Heart rate (beats/min)	78.4 ± 9.9	86.4 ± 10.6*	80.1 ± 10.1	77.9 ± 9.7	84.6 ± 11.1*	80.4 ± 11.3
Systolic BP (mm Hg)	126 ± 15.7	161 ± 23*	129 ± 17.2	124 ± 21	157 ± 25.6*	121 ± 21.1
Rate-pressure product	9.8 ± 2.0	13.9 ± 2.6*	10.1 ± 2.2	9.7 ± 1.9	13.4 ± 2.4*	9.6 ± 2.3
Patients with CAD (n = 8)						
Heart rate (beats/min)	70.1 ± 7.2	82.2 ± 8.8*	70.3 ± 8.3	71.2 ± 5.7	83.6 ± 7.9*	72.1 ± 9.1
Systolic BP (mm Hg)	147 ± 13.7	182 ± 30.1*	142 ± 17.9	140 ± 18.2	173 ± 21.5*	143 ± 25.1
Rate-pressure product	10.2 ± 1.7	14.9 ± 2.5*	9.8 ± 1.9	10.1 ± 2.1	14.5 ± 2.65*	9.8 ± 2.8

*$p < 0.01$ cold pressor test vs. control. Data are expressed as mean ± SD. Pre-Beta CPT = cold pressor test before beta-adrenergic blockade; Post-Beta CPT = cold pressor test after beta-adrenergic blockade; other abbreviations as in Table 1.

beta-blockade in the group with normal coronary arteries, and of 48 ± 24% before and 42 ± 25% after beta-blockade in the patients with coronary artery disease.

Mean intracoronary blood flow did not significantly change after intracoronary beta-blockade in the patients studied by Doppler ultrasound (four with normal coronary arteries and five with coronary artery disease). Moreover, there was a similar increase in coronary blood flow of 57 ± 17% and 51 ± 21%, respectively, during the pre- and postbeta-blockade cold pressor test.

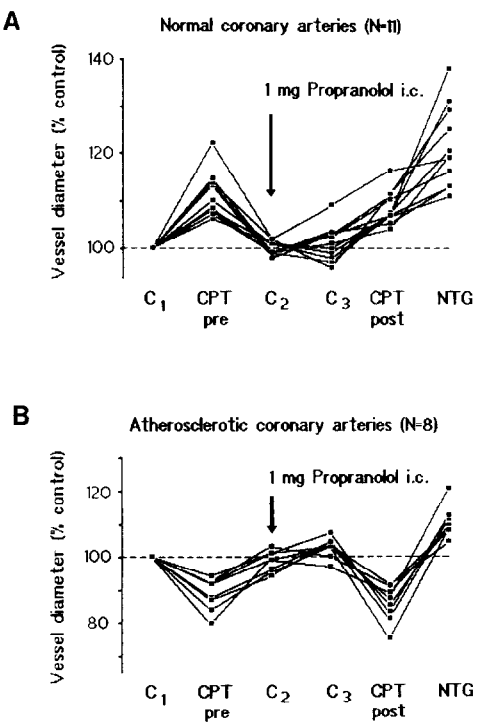
Responses of epicardial coronary arteries to cold pressor test after beta-blockade (Fig. 3). In the group with normal coronary arteries (Fig. 3A), all 11 left anterior descending artery segments were dilated by 11.3 ± 4.4% (from a mean control diameter of 3.09 ± 0.17 mm to 3.40 ± 0.26 mm) ($p < 0.01$), in response to the first cold pressor test. After baseline values returned, intracoronary propranolol did not induce any significant changes in vessel diameter. All identical coronary segments were again dilated by 8.6 ± 4.3% (from 3.16 ± 0.21 mm to 3.37 ± 0.20 mm) ($p < 0.01$) in response to the second cold pressor test during beta-blockade. In no instance did we observe a reversal of a vasodilative response before beta-blockade to a vasoconstrictor response after beta-blockade.

In the eight patients with coronary artery disease (Fig. 3B), all measured vessel segments were constricted by 11.4 ± 4.6% and 14.6 ± 5.3%, respectively, during both the pre- and post-beta-blockade cold pressor testing. Cold pressor test-induced vasoconstrictor responses were not significantly different before and after beta-blockade. Thus, intracoronary beta-blockade leaving alpha-adrenergic mechanisms unopposed did not significantly affect the vasomotor response to sympathetic stimulation in normal or atherosclerotic coronary arteries.

Discussion

Our results indicate that the normal response to sympathetic stimulation by cold pressor testing in humans is coronary vasodilation in patients with normal coronary arteries, but paradoxic vasoconstriction in atherosclerotic coronary arteries even at a very early stage of coronary artery disease. Coronary vasomotion in response to sympathetic stimulation exactly mirrored the effects of the endothelium-dependent vasodilator acetylcholine. Endothelial dysfunction in coronary atherosclerosis resulted in a loss of the normal dilator function and permitted vasoconstrictor

Figure 3. Responses of coronary artery segments to cold pressor testing (CPT) before and after beta-adrenergic blockade by intracoronary (i.c.) propranolol (**arrow**) and after nitroglycerin (NTG). **A,** 11 patients with normal coronary arteries. **B,** 8 patients with coronary artery disease. C_1 = control; C_2 = recontrol; C_3 = control after propranolol; CPT_{pre} and CPT_{post} = before and after cold pressor testing after propranolol.



responses to sympathetic stimulation. These results suggest that coronary vasomotion in response to cold pressor testing in humans is intimately related to the integrity of endothelial function.

Response to sympathetic stimulation. Vasodilation of normal coronary arteries and the paradoxic vasoconstriction of atherosclerotic coronary arteries in response to sympathetic stimulation by cold pressor testing confirm the observations recently reported by Nabel et al. (8). However, the paradoxic vasoconstrictor response of angiographically "normal"-appearing coronary arteries in patients with evidence of atherosclerosis elsewhere in the coronary system contrasts with their report (8) of a vasodilative response in angiographically smooth regions, even in coronary vessels with stenosis and luminal irregularities in other segments.

Intraoperative echocardiographic studies (11) have demonstrated that in vivo coronary artery atherosclerosis is far more extensive than that predicted by coronary angiography. Coronary artery segments without any angiographic narrowing in patients with angiographic evidence of coronary artery disease elsewhere in the coronary system demonstrated substantial intimal atherosclerosis by high frequency echocardiography. Thus, no or apparently trivial angiographic narrowing can mask severe coronary atherosclerotic disease.

Thus, it is very likely that the angiographically normal-appearing coronary arteries demonstrating a vasoconstrictor response to sympathetic stimulation in our study are, in fact, in a very early stage of atherosclerosis or may have mainly diffuse intimal thickening. This conclusion is particularly substantiated by our demonstration (12) of a vasoconstrictor response to acetylcholine, indicating the presence of impaired endothelial functional integrity in angiographically normal-appearing epicardial coronary artery segments in patients with coronary artery disease.

Endothelium-mediated vasomotion. Young and Vatner (7) recently demonstrated the pivotal role of the endothelium in mediating vasomotion of canine iliac arteries in response to sympathetic stimulation by epinephrine, a mixed alpha- and beta-adrenergic agonist. The vasodilator response to epinephrine in normal arteries was reversed to a vasoconstrictor response after removal of the endothelium. In addition, endothelial integrity played an important role in protecting against alpha-adrenergic vasoconstriction of large canine iliac arteries.

Our present results extend these experimental observations to the human coronary artery system in vivo. The response of human coronary arteries to sympathetic stimulation by the cold pressor test exactly mimicked the response to the endothelium-dependent vasodilator acetylcholine. Acetylcholine has been demonstrated (13) to be a very sensitive indicator of endothelial integrity in porcine coronary arteries, and may also induce paradoxic vasoconstriction early in the course of coronary atherosclerosis in humans, thereby indicating a defect in endothelial vasodilator function (12,14). Thus, the results of our study strongly suggest that unimpaired, intact endothelial function is a major determinant of vasodilation of human coronary arteries in vivo in response to sympathetic stimulation by cold pressor testing.

Mechanisms of endothelium-mediated vasomotion. The mechanism by which the functional integrity of the endothelium mediates coronary vasodilation in response to sympathetic stimulation in humans remains to be determined. Recent experimental findings (15) indicate that increases in shear stress on endothelial cells inhibit the adrenergic neurogenic vasoconstriction of rabbit carotid arteries by augmenting the release of endothelial cell-derived vasodilators. Coronary blood flow increased significantly during the cold pressor test in both groups of patients in our study. Thus, it is conceivable that metabolic vasodilation of small arterioles resulted in increased flow throughout the vascular bed and led to flow-mediated release of endothelium-derived relaxing factor (16,17). We have recently demonstrated (18) that there is considerable flow-mediated vasodilation of normal human coronary arteries in vivo.

Moreover, recent experimental observations suggest that acetylcholine generates an endothelium-derived hyperpolarizing factor (19,20), which is different from endothelial-derived relaxing factor, believed to be nitric oxide (21). Similarly, a potassium-selective ionic current activated by shear stress has been shown to cause endothelial cell hyperpolarization (22). It is conceivable that the observed vasodilation in response to sympathetic stimulation is mediated by increased shear stress due to increased coronary blood flow and driving pressure during the cold pressor test causing endothelial cell hyperpolarization.

Thus, although the underlying mechanisms of endothelium-mediated vasodilation might be different for acetylcholine and sympathetic stimulation, the endothelium appears to be the common mediator for vasodilation in response to both stimuli.

Role of interaction between platelets and epithelium. Consequently, several potential mechanisms may explain the reversal of the vasodilator response of normal coronary arteries to the vasoconstrictor response of human coronary vessels with impaired endothelial function during sympathetic stimulation. By mediating vasodilation in response to cold pressor test-induced increases in driving pressure and flow, possibly through the signal of shear stress on the endothelium, endothelium-derived relaxing factor (or factors) would provide a flow-related dilator feedback to oppose the constrictor feedback of the myogenic response to increased intraluminal pressure. If the endothelium is dysfunctional because of disease, sympathetic vasoconstriction might be augmented. In addition, if in intact vessels the endothelium releases endothelial cell-derived vasodilators, which oppose the action of all vasoconstrictors, endothelial

dysfunction would then cause a nonspecific enhancement of the response to all vasoconstrictor stimuli. Therefore, it is conceivable that interactions between platelets and the diseased endothelium may enhance the constrictor responsiveness of the vessel. This could occur by direct sympathetic stimulation of platelet alpha-adrenergic receptors to release thromboxane, serotonin or histamine (all of which are potent vasoconstrictors) or by platelet aggregation and activation at the site of endothelial injury. Numerous experimental studies (4,6,23,24) have demonstrated that the endothelium prevents the vasoconstriction induced by aggregating platelets.

Adrenergic receptor-mediated vasomotion. Stimulation of beta-adrenergic receptors in the intact conscious animal has been shown (25) to dilate large coronary arteries independent of blood flow-mediated vasodilation. In the present study, cold pressor stimulation still elicited coronary vasodilation after intracoronary beta-blockade in patients with normal coronary arteries, indicating that beta-adrenergic receptor stimulation is not the major determinant of the coronary vasodilator response to sympathetic stimulation. In addition, coronary blood flow increased in all patients with normal coronary arteries in response to cold pressor testing in the presence of beta-blockade. Thus, unopposed alpha-adrenergic constriction during sympathetic stimulation either does not mediate vasoconstriction after beta-adrenergic blockade or, more likely, is offset by a metabolically or flow-dependent endothelium-mediated mechanism, or both. Consistent with these findings are those of Gaglione et al. (26), who demonstrated that intracoronary propranolol does not modify the significant vasodilative response of normal coronary arteries to dynamic exercise in humans. These results are also in agreement with numerous experimental studies (27–29) demonstrating that, in normal coronary arteries, sympathetic stimulation does not reduce coronary blood flow after beta-adrenergic blockade, but alpha-receptor–mediated coronary vasoconstriction competes with local metabolic and flow-dependent vasodilation secondary to increased metabolic demands. Moreover, it was recently shown (30) that norepinephrine-induced relaxation of porcine coronary microvessels is mediated by both beta-adrenergic receptors and release of endothelium-derived relaxing factor (or factors), whereas only minimal alpha-adrenergic–mediated constriction of porcine coronary microvessels was noted.

A dose of 1 mg of propranolol was chosen on the basis of previous studies (8,26) using intracoronary propranolol for local beta-adrenergic blockade. This dose delivers a drug concentration to the left coronary artery in excess of that known to produce beta-blockade in the myocardium (31). Moreover, 1 mg of intracoronary propranolol represents approximately 10% of the full systemic dose (0.1 mg/kg body weight) and has no secondary effects on global hemodynamic determinants of myocardial oxygen consumption (26).

Experimental studies (32) of isolated vessels demonstrated alpha$_2$-adrenoceptor (present on the endothelial cells)-mediated endothelium-dependent vasorelaxation. Although the weak vasoconstriction in response to selective alpha$_2$-adrenergic stimulation in intact vessels (7,33) did not support these findings, this mechanism cannot be ruled out in our patients.

Comparison with previous studies. Our results appear to differ from two previously published observations (34,35) in humans that propranolol produces a small but significant decrease in coronary flow. Kern et al. (35) observed a small decrease in coronary flow after intravenous injection of propranolol during cold pressor testing and concluded that beta-adrenergic blockade potentiated coronary artery vasoconstriction, possibly mediated by unopposed alpha-adrenergic vasomotor tone, although coronary artery diameter was not measured. However, the reported decrease in coronary blood flow after intravenous beta-adrenergic blockade can easily be explained by secondary effects on myocardial oxygen consumption, such as a decrease in heart rate and contractility (27), and might not be related to changes in epicardial coronary vasomotor tone. Indeed, preliminary data from Bortone et al. (36) demonstrate opposite effects of intracoronary and intravenous beta-blockade on the diameter of large epicardial coronary arteries in humans. In addition, in the study by Kern et al. (35), potentiation of coronary artery vasoconstriction in response to cold pressor testing after beta-adrenergic blockade was observed only in the group of patients with significant coronary artery disease, whereas no increase in coronary vascular resistance was found in patients with normal coronary arteries.

Conclusions. The functional integrity of the endothelium appears to be the major determinant of coronary vasomotion in response to the cold pressor test. Endothelial dysfunction in coronary atherosclerosis results in a loss of the normal dilator function and permits vasoconstrictor responses to sympathetic stimulation in human coronary arteries in vivo.

We thank the nurses and technicians of the Cardiac Catheterization Laboratory at the University of Freiburg for invaluable assistance in the performance of this study.

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