

Modulation of Coronary Vasomotor Tone in Humans

Progressive Endothelial Dysfunction With Different Early Stages of Coronary Atherosclerosis

Andreas M. Zeiher, MD; Helmut Drexler, MD;
Helmut Wollschläger, MD; and Hanjörg Just, MD

The endothelium plays a critical role in the control of vasomotor tone by the release of vasoactive substances. Because endothelial injury or dysfunction is considered important very early in atherogenesis, we hypothesized that abnormal endothelial function precedes the angiographic detection of coronary atherosclerosis in the human coronary circulation. The coronary vasomotor responses to three different endothelium-mediated stimuli (intracoronary infusion of acetylcholine 10^{-8} to 10^{-6} M, increase in blood flow to induce flow-dependent dilation, and sympathetic stimulation by cold pressor testing) were assessed by quantitative angiography and subselective intracoronary Doppler flow velocity measurements within the left anterior descending coronary artery in 38 patients. All three stimuli elicited epicardial artery dilation in all 11 patients with smooth coronary arteries and absence of risk factors for coronary artery disease (group 1). All nine patients with smooth coronary arteries but with hypercholesterolemia (group 2) demonstrated a selective impairment in endothelial function with vasoconstriction ($35 \pm 12.7\%$ decrease in mean luminal area) in response to acetylcholine but showed a preserved flow-dependent dilation ($15.5 \pm 4.4\%$ increase in mean luminal area) and vasodilation in response to cold pressor testing ($14.2 \pm 4.6\%$ increase in mean luminal area). In all nine patients with an angiographically defined smooth coronary artery segment but with evidence of atherosclerosis elsewhere in the coronary system (group 3), both acetylcholine and cold pressor testing induced vasoconstriction ($26.2 \pm 8.7\%$ and $18.7 \pm 7.9\%$ decrease in mean luminal area, respectively), whereas flow-dependent dilation was preserved ($20.4 \pm 8.7\%$ increase in mean luminal area). In the nine patients with angiographic evidence of wall irregularities (group 4), flow-dependent dilation was also abolished and vasoconstriction occurred in response to acetylcholine and cold pressor testing ($34.5 \pm 10.7\%$ and $19.9 \pm 6.3\%$ decrease in mean luminal area, respectively). All coronary artery segments dilated in response to nitroglycerin, suggesting preserved function of vascular smooth muscle. Despite similar reductions in coronary vascular resistance in response to the smooth muscle relaxant papaverin, patients with hypercholesterolemia demonstrated a selective impairment of vasodilation of the resistance vasculature in response to acetylcholine ($p < 0.05$ versus groups 1, 3, and 4). Thus, there is a progressive impairment of endothelial vasoactive functioning in coronary arteries of patients with different early stages of atherosclerosis, beginning with a selective endothelial dysfunction in angiographically defined normal arteries in patients with hypercholesterolemia and progressively worsening to a complete loss of endothelium-mediated vasodilation in angiographically defined atherosclerotic coronary arteries. The assessment of endothelial modulation of vasomotor tone in the intact human coronary circulation may add a new dimension to the angiographic evaluation of coronary artery disease by detecting early changes in endothelial vasoactive function important in the development of atherosclerosis rather than detecting atherosclerosis per se. (*Circulation* 1991;83:391-401)

The endothelium plays a critical role in the control of vasomotor tone.¹⁻³ The tonic release of endothelium-derived relaxing fac-

tors and the release induced by a variety of receptor-dependent agonists have been shown to modulate contraction of the underlying smooth muscle.⁴ Injury

From the Medical Clinic, Department of Cardiology, University of Freiburg, Freiburg, FRG.

Address for reprints: Andreas M. Zeiher, MD, Medical Clinic,

Department of Cardiology, University of Freiburg, Hugstetterstrasse 55, D-7800 Freiburg, FRG.

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or dysfunction of endothelial cells is considered an important factor in atherogenesis.^{5,6} Impaired endothelium-dependent relaxations in atherosclerotic arteries have been reported in experimental animals^{7,8} and humans.^{9,10} Moreover, recent experimental evidence indicates that endothelial dysfunction occurs very early in the development of atherosclerosis.^{11,12} In animals fed a high-cholesterol diet, impaired vasoactive function of the endothelium was demonstrated at a time when hypercholesterolemia had produced no abnormal structural alterations of the arterial wall as assessed by light and electron microscopy.¹³⁻¹⁵ Hypercholesterolemia is an important cause of coronary artery disease in humans,¹⁶ and elevated plasma concentrations of low density lipoproteins (LDL) are associated with accelerated atherogenesis.^{17,18}

Therefore, it would be very important to detect alterations in endothelial vasoactive function very early in the development of atherosclerosis in humans because an abnormal response to vasoactive stimuli is very likely to precede the angiographic recognition of coronary atherosclerotic lesions. Recent advances in interventional techniques with reasonably accurate methods of subselectively measuring coronary blood flow velocity by intracoronary Doppler catheters and quantitative approaches to assess arterial dimensions from coronary angiograms have provided a means to study vascular responses of the coronary circulation in humans during cardiac catheterization. We¹⁹ and others²⁰ recently demonstrated that substantial flow-mediated vasodilation occurs in normal coronary arteries of humans in vivo. However, in the presence of atherosclerosis this flow-mediated dilation is impaired.^{19,20} In addition, studies from our laboratory²¹ and those by Nabel et al²² have shown that coronary vasomotion in response to sympathetic stimulation in humans is dependent on the functional integrity of the endothelium.

Thus, the purpose of the present study was to assess the coronary vasomotor response to different stimuli of endothelium-dependent relaxation very early in the development of atherosclerosis in humans. Based on the results of experimental studies and preliminary data in patients from our laboratory,²³ we hypothesized that there is a hierarchical structure in the impairment of endothelium-dependent responses with progressive disease.

Methods

Patient Population

Thirty-eight patients undergoing routine diagnostic cardiac catheterization were studied. These patients were classified into four groups based on their history and the presence or absence of atherosclerosis on the diagnostic coronary angiogram. Patients with unstable angina, recent myocardial infarction, valvular heart disease, or clinical evidence of heart failure were excluded. Written, informed consent was obtained from all patients before the study. The

study protocol had been approved by the ethical committee of the University of Freiburg.

Group 1. Normal control patients. Eleven patients with angiographically normal coronary arteries served as control subjects. Patients with a history of arterial hypertension (systolic blood pressure >150 mm Hg and diastolic blood pressure >90 mm Hg), the presence of left ventricular hypertrophy, diabetes mellitus, and hypercholesterolemia (total cholesterol serum level >210 mg%) were excluded from the normal control group. The mean cholesterol serum level was 186 mg%, ranging from 143 to 208 mg%. The mean age of these patients was 49.2 years, three were women, and eight were men. All subjects had angiographically normal, smooth coronary arteries without luminal irregularities and without evidence of segmental wall motion abnormalities on left ventricular cineangiograms. The cause for referral for diagnostic coronary angiography was atypical chest pain in nine patients and intermittent left bundle branch block in two patients.

Group 2. Patients with hypercholesterolemia but with angiographically normal coronary arteries. Nine patients were studied who had angiographically normal, smooth coronary arteries without any luminal irregularities but with hypercholesterolemia (LDL serum levels >180 mg%). Mean total cholesterol value in these patients was 281.4 ± 22.7 mg%, ranging from 246 to 316 mg%. The mean LDL level was 198.2 ± 18.9 mg%, ranging from 182 to 243 mg% (198.2 ± 18.9 mg%). Two of these patients had a history of elevated blood pressure that resulted in the initiation of antihypertensive therapy by the primary physician. None of the patients had a history of diabetes mellitus or elevated blood glucose, and three patients were smokers. These patients were studied because of atypical chest pain. Their mean age was 48.2 years (range, 22 to 74 years), and five were men.

Group 3. Patients with coronary artery disease but with an angiographically normal proximal segment of the left anterior descending coronary artery. Nine patients were studied who had an angiographically normal, smooth proximal left anterior descending coronary artery segment (the vessel segment under study) but with angiographic evidence of atherosclerosis elsewhere in the coronary system. Three of these patients had more than 50% luminal narrowing of the right coronary artery, and six patients had luminal irregularities (<30% luminal narrowing) within the midportion of the left anterior descending coronary artery. Their mean age was 52.5 years (range, 38 to 63 years), and five were men.

Group 4. Patients with minimal disease of the left anterior descending coronary artery. Nine patients were studied who had angiographically visible luminal irregularities of the proximal left anterior descending coronary artery but no more than 30% luminal narrowing. Five of these patients had more than 50% luminal narrowing of the right coronary artery; four patients had only luminal irregularities of

the proximal left anterior descending coronary artery. Their mean age was 53.5 years (range, 43–66 years), and four were men.

Six of the patients with coronary artery disease (groups 3 and 4) had hypercholesterolemia with LDL serum levels exceeding 180 mg%.

Study Design

Vasoactive therapy including calcium channel blockers, angiotensin converting enzyme inhibitors, and long-acting nitrates was discontinued at least 24 hours before cardiac catheterization. No patient received β -adrenergic receptor blockers within 48 hours before the study. Diagnostic coronary angiography was performed by a standard percutaneous femoral approach using the Judkins technique. After the completion of the diagnostic catheterization, an additional 5,000 units heparin was given intravenously, and an 8F guiding catheter (Schneider, Zurich) 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 coronary artery by a 0.014-in. guide wire. The Doppler catheter was carefully positioned to obtain a stable flow velocity signal. Before the Doppler catheter was inserted in the guiding catheter, the flow velocity recordings were referenced to zero and were calibrated. A 5F bipolar pacing catheter was placed in the right ventricular apex and set in demand mode to prevent the heart rate from slowing to less than 40 beats/min. At least 40 minutes elapsed between the completion of diagnostic catheterization and the beginning of the study.

Five minutes after control angiography, cold pressor testing was performed by immersion of the patient's hand and forearm in ice water for 90 seconds. Ten minutes after cold pressor testing, the response of the proximal left anterior descending coronary artery segment to increased blood flow was assessed as previously published.¹⁹ In brief, flow-dependent vasodilation was evaluated by subselective infusion of 7 mg papaverin into the midportion of the left anterior descending coronary artery by the Doppler catheter. A previous study¹⁹ demonstrated that the dose of 7 mg papaverin, subselectively infused into the midportion of the left anterior descending coronary artery, elicits a maximal increase in coronary blood flow without affecting global hemodynamic parameters. In addition, this dose compares favorably with previous observations by Wilson and White,²⁴ who demonstrated that the administration of 12 mg papaverin, injected into the left main artery, produced maximal coronary hyperemia. Eighty seconds after papaverin-induced increase in blood flow in the left anterior descending coronary artery, a coronary angiogram was obtained for measuring the diameter of the proximal segment of the artery exposed to increased blood flow but not directly to papaverin itself. Reflux of papaverin into the proximal artery segment was excluded by power injection of contrast material through the Doppler catheter,

and potential effects of recirculating papaverin were assessed by measuring the diameter of the proximal circumflex coronary artery as the reference vessel not exposed to increased blood flow.

Ten minutes after papaverin infusion, acetylcholine was selectively infused into the left anterior descending coronary artery by the Doppler catheter. Increasing dosages were used to achieve the 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 2 ml/min, lasting 3 minutes for each concentration. Stepwise acetylcholine infusions were terminated when either vessel occlusion occurred or the largest dose (10^{-6} M) was reached. To compare the vasomotor response of identical coronary segments to acetylcholine and increased blood flow, the Doppler catheter was withdrawn into the proximal artery segment during the acetylcholine studies, where the response to increased blood flow was measured. The sequence of interventions with either papaverin or acetylcholine was randomly varied so that 21 patients received the papaverin infusion before the acetylcholine infusion and 17 patients received intracoronary infusion of increasing doses of acetylcholine before the assessment of flow-dependent dilation by infusion of papaverin. In any case, at least a 10-minute recovery period was allowed between all interventions.

Throughout the study, phasic and mean intracoronary blood flow velocities, heart rate, and aortic pressure (by the guiding catheter) were continuously measured. Serial hand injections of nonionic contrast material (Ultravist, Schering AG, Berlin), were administered during the control phase, at the end of cold pressor testing, at the recontrol phase after cold pressor testing, at the end of each acetylcholine infusion, at the recontrol phase after acetylcholine infusion, and 80 seconds after subselective infusion of papaverin. Before completion of the study, 0.25 mg nitroglycerin was injected into the left main stem by the guiding catheter, and a final angiogram was then obtained to assess the vasodilatory capability of the coronary arteries.

Quantitative Coronary Angiography

Coronary angiography was performed with a simultaneous biplane multidirectional isocentric radiological system (Siemens Bicor, Erlangen, FRG). The 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 frame rate of 25 frames/sec. In the patients with angiographic evidence of atherosclerosis, the angiograms were examined by two investigators, and the proximal segment of the left anterior descending coronary artery was classified as smooth or irregular by a consensus decision before analysis. For quantitative analysis, end-diastolic cineframes were videodigitized and stored in the image analysis system (Mipron I, Kontron electronics, Eching, FRG) in a 512×512 matrix with an

TABLE 1. Systemic Hemodynamic Response to Cold Pressor Testing

	Group 1 (n=11)	Group 2 (n=9)	Group 3 (n=9)	Group 4 (n=9)
Heart rate (beats/min)				
Before CPT	75.4±9.3	73.8±12.1	66.1±7.3	77.2±11.4
After CPT	84.8±11.5*	79.0±11.3*	75.6±9.5*	87.8±12.5*
Mean aortic pressure (mm Hg)				
Before CPT	101.3±13.8	96.8±8.1	95.8±12.9	98.2±9.2
After CPT	119.8±16.5*	111.1±11.9*	118.9±14.2*	115.9±7.9*
Double product				
% Change	34.1±12.5	29.1±17.9	41.9±10.5	34.4±14.5

Values are mean±SD.

Group 1, normal control patients; group 2, patients with hypercholesterolemia but with angiographically normal coronary arteries; group 3, patients with coronary artery disease but with an angiographically normal proximal segment of the left anterior descending coronary artery; group 4, patients with minimal disease of the left anterior descending coronary artery; CPT, cold pressor testing; Double product, heart rate×systolic aortic pressure; % change, [(double product before CPT/double product after CPT)×100]−100.

* $p<0.01$ vs. before CPT.

eight-bit gray scale. With the 12-cm field of view, the resulting pixel density was 7.3 pixels/mm. The geometrical resolution of the radiological imaging chain is greater than 4 line pairs/mm.

Quantitative coronary angiography by automatic contour detection was performed by a previously described and validated method that incorporates a geometric edge differentiation technique.^{19,21} Calculation of the exact radiological magnification factor of the measured segment was used to scale the data from pixels to millimeters as previously described.²⁵ The accuracy and precision of this technique, as well as the reproducibility of serial measurements under routine clinical conditions, have been established in previous studies.^{19,21}

Six- to 8-mm segments of the proximal left anterior descending coronary artery (the vessel under study) and of the circumflex coronary artery (the reference vessel during the selective infusion of papaverin and acetylcholine into the left anterior descending coronary artery) were measured. A series of diameter measurements were obtained for each scanline for the length of the arterial segment in which diameter versus segment length was plotted in graph form, and the mean diameter was calculated. Whenever possible, measurements were obtained for both views of the biplane images with the radiopaque tip of the Doppler catheter for identification of corresponding vessel segments, and the vessel's cross-sectional area was calculated from both views assuming an elliptical shape. Only single-plane analysis was performed for those coronary segments demonstrating overlapping with other parts of the coronary tree in one view; in those cases (eight of 38 patients or 21%), vessel cross-sectional area was calculated assuming a circular shape.

Derived Parameters

For estimating directional changes in coronary blood flow, a coronary flow index was calculated by multiplying the mean Doppler-derived blood flow

velocity with the computed cross-sectional area of the vessel segment. Because the injection of contrast material into the coronary circulation resulted in the typical biphasic response due to hyperemic effects, that is, an initial decrease followed by an increase in coronary blood flow velocity, the mean blood flow velocity immediately before the contrast injection was used for estimating coronary blood flow. To exclude limitations of coronary artery flow due to epicardial coronary artery constriction in response to acetylcholine, we calculated the coronary flow index only when the vessel's cross-sectional area reduction did not exceed 50% in the most constricting epicardial artery segment, as previously suggested by Treasure et al.²⁶ An index of coronary vascular resistance was calculated by the ratio of the mean aortic pressure divided by the coronary flow index.

Statistical Analysis

All data are expressed as mean±SD. Statistical comparisons were made by analysis of variance for repeated measures followed by the Student-Newman-Keuls test. Differences between groups were evaluated by analysis of variance followed by Bonferroni's *t* test. Statistical significance was assumed if the null hypothesis could be rejected at the 0.05 probability level.

Results

Systemic Hemodynamics

The systemic hemodynamic responses to sympathetic stimulation are summarized in Table 1. Cold pressor testing led to similar increases in heart rate and blood pressure in all four groups of patients. Subselective intracoronary infusion of 7 mg papaverin and infusion of acetylcholine to the final estimated blood concentration of 10^{-6} M had no effect on heart rate or aortic pressure in any of the four groups (Table 2). Injection of 0.25 mg nitroglycerin into the left main coronary artery slightly, but significantly ($p<0.01$), increased

TABLE 2. Systemic Hemodynamic Effects of Subselective Infusion of Acetylcholine and Papaverin

	Group 1 (n=11)	Group 2 (n=9)	Group 3 (n=9)	Group 4 (n=9)
Heart rate (beats/min)				
Before papaverin	71.1±11.0	67.9±10.9	68.4±10.9	76.0±9.9
After papaverin	73.4±10.5	72.6±12.9	72.1±12.4	78.9±9.9
Mean aortic pressure (mm Hg)				
Before papaverin	94.6±9.7	92.3±8.5	94.1±12.1	101.7±6.1
After papaverin	94.5±9.3	90.7±9.4	95.6±13.1	99.6±6.3
Heart rate (beats/min)				
Before max acetylcholine	74.4±14.1	71.3±15.1	69.7±9.6	73.4±9.2
After max acetylcholine	74.1±14.3	69.8±15.6	69.4±8.7	73.0±10.4
Mean aortic pressure (mm Hg)				
Before max acetylcholine	95.6±9.9	93.3±10.9	97.1±12.3	98.6±7.2
After max acetylcholine	96.2±10.0	92.9±10.8	95.5±10.0	100.0±7.3

Values are mean±SD.

Group 1, normal control patients; group 2, patients with hypercholesterolemia but with angiographically normal coronary arteries; group 3, patients with coronary artery disease but with an angiographically normal proximal segment of the left anterior descending coronary artery; group 4, patients with minimal disease of the left anterior descending coronary artery; before papaverine, before selective infusion of 7 mg papaverin; after papaverin, 80 seconds after selective infusion of 7 mg papaverin; before max acetylcholine, before selective infusion of acetylcholine; after max acetylcholine, at the end of selective infusion of the maximally attained acetylcholine concentration.

heart rate by $5.1\pm1.9\%$ and decreased mean aortic pressure by $3.9\pm1.8\%$ in all four groups.

Responses of Epicardial Coronary Arteries

There were no significant changes in the luminal area of the circumflex coronary artery of patients in any of the four groups during subselective infusion of acetylcholine or papaverin into the left anterior descending coronary artery. Thus, potential effects of the injected contrast material itself or those of recirculating papaverin were excluded.

Normal control arteries (group 1). In the normal control group, the proximal left anterior descending coronary artery segments of all patients dilated in response to all three stimuli (Figure 1). Cold pressor testing dilated the arteries by $18.5\pm7.6\%$ from a mean control luminal area of 9.4 ± 2.2 to 11.3 ± 2.5 mm² ($p<0.001$); the maximal dose of acetylcholine dilated the arteries by $24.7\pm15.9\%$ from a mean control luminal area of 9.2 ± 2.1 to 11.2 ± 3.1 mm² ($p<0.001$); and flow-dependent dilation increased the mean control luminal area by $22.2\pm7.9\%$ from 9.7 ± 1.9 to 11.6 ± 2.4 mm² ($p<0.001$). Intracoronary administration of nitroglycerin increased mean luminal area by $41.3\pm14.0\%$ (Figure 1).

Angiographically normal arteries in patients with hypercholesterolemia (group 2). In all nine patients with hypercholesterolemia but with angiographically normal coronary arteries, the proximal left anterior descending coronary artery segments dilated in response to cold pressor testing by $14.2\pm4.6\%$ from a mean control luminal area of 9.9 ± 2.1 to 11.5 ± 2.4 mm² ($p<0.001$) (Figure 1). However, all identical coronary artery segments demonstrated dose-dependent vasoconstriction to intracoronary infusion of acetylcholine; mean control luminal area was reduced by $35.9\pm12.7\%$ from 9.6 ± 2.3 to 6.4 ± 3.4 mm²

($p<0.001$) after the maximal dose (Figure 1). In one patient, 10^{-7} M acetylcholine produced profound vasoconstriction so that no further increase in dosage was attempted. In contrast, papaverin-induced increases in blood flow resulted in a $15.4\pm4.9\%$ increase (Figure 1) in mean control luminal area from 10.2 ± 2.6 to 11.8 ± 3.1 mm² ($p<0.001$). Intracoronary administration of nitroglycerin increased mean luminal area by $29.8\pm12.3\%$ (Figure 1).

Thus, the vasomotor response of angiographically normal coronary arteries in patients with hypercholesterolemia was characterized by a selective impairment of acetylcholine-induced vasodilation of the epicardial coronary arteries (Figure 2).

Angiographically normal proximal left anterior descending coronary artery segments in patients with coronary artery disease (group 3). All angiographically normal left anterior descending coronary artery segments in the nine patients with evidence of coronary artery disease elsewhere in the coronary system vasoconstricted in response to cold pressor testing by $18.7\pm7.9\%$ from a mean control luminal area of 8.9 ± 2.2 to 7.3 ± 2.5 mm² ($p<0.001$) after cold pressor testing (Figure 1). The identical coronary artery segments also demonstrated dose-dependent vasoconstriction to intracoronary administration of acetylcholine by $26.2\pm8.7\%$ from a mean control luminal area of 8.7 ± 2.3 to 6.7 ± 2.8 mm² after the maximal dose, at which the vessel was still patent. The infusion of acetylcholine caused temporary vessel occlusion in three of these patients at the maximal dose of 10^{-6} M. In contrast, increases in blood flow still elicited vasodilation of the proximal left anterior descending coronary artery segments by $20.4\pm8.7\%$ (Figure 1) from a mean control luminal area of 8.9 ± 2.3 to 10.6 ± 2.7 mm² ($p<0.001$) 80 seconds after subselective infusion of 7 mg papaverin into the

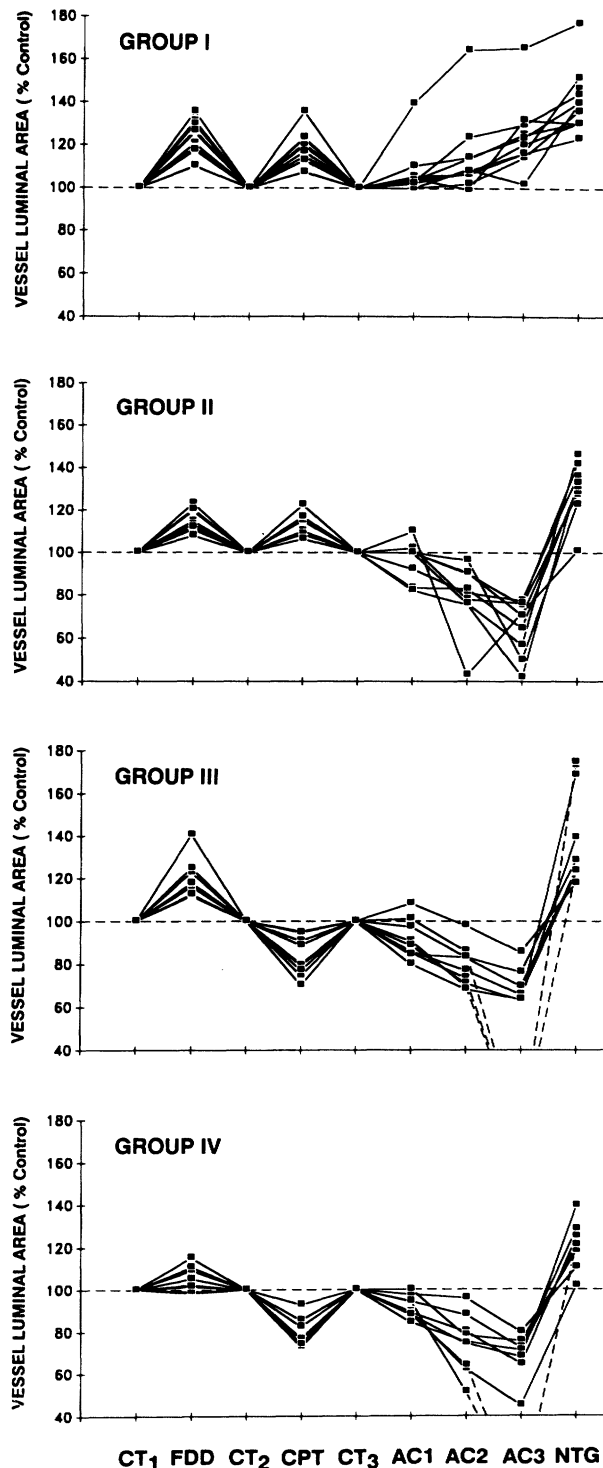


FIGURE 1. Plots of change in coronary artery luminal area (expressed as percent of control) in response to increased blood flow (flow-dependent dilation, FDD), cold pressor testing (CPT), increasing concentrations of acetylcholine (AC1, 10^{-8} M; AC2, 10^{-7} M; AC3, 10^{-6} M), and nitroglycerin (NTG) in the four groups of patients. CT1, CT2, and CT3, baseline values before FDD, CPT, and ACh infusions, respectively.

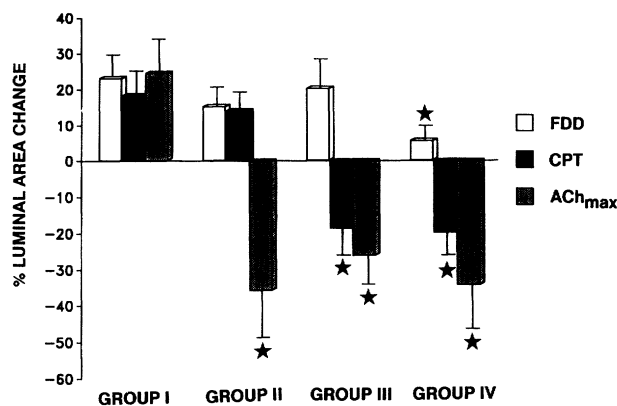


FIGURE 2. Bar graph of change in coronary artery luminal area (mean \pm SD, expressed as percent change from baseline) in response to increased blood flow (flow-dependent dilation, FDD), cold pressor testing (CPT), and the maximal concentration of acetylcholine (ACh_{max}) in the four groups of patients. * $p < 0.05$ vs. group I.

midportion of the artery. Intracoronary administration of nitroglycerin increased luminal area by $41.3 \pm 22.6\%$.

Thus, the vasomotor response of the group 3 patients was characterized by vasoconstriction to both cold pressor testing and acetylcholine but by preserved flow-mediated vasodilation of identical coronary artery segments (Figure 2).

Minimal disease of the left anterior descending coronary artery (group 4). All coronary artery segments with luminal irregularities vasoconstricted in response to cold pressor testing by $19.9 \pm 6.3\%$ (Figure 1) from a mean control luminal area of 8.4 ± 2.3 to 6.9 ± 2.6 mm² after testing ($p < 0.001$), and they vasoconstricted dose dependently to acetylcholine by $34.5 \pm 10.7\%$ (Figure 1) from a mean control luminal area of 8.2 ± 2.5 to 5.6 ± 3.1 mm² after the maximal dose. The infusion of acetylcholine at an estimated concentration of 10^{-6} M resulted in temporary occlusion of the left anterior descending coronary artery in two of these patients. Increases in blood flow caused only a slight, but statistically nonsignificant, increase in the luminal area of proximal artery segments by $5.8 \pm 5.4\%$ (Figure 1) from 8.2 ± 3.0 at control to 8.6 ± 3.1 mm². Intracoronary administration of nitroglycerin increased luminal area by $20.6 \pm 9.9\%$ ($p < 0.05$ versus groups 1 and 3).

Figure 2 illustrates the progressive impairment in endothelium-mediated vasoactive functions of epicardial coronary arteries with different early stages of atherosclerosis, beginning with a selective endothelial dysfunction in angiographically normal arteries of patients with hypercholesterolemia and progressively worsening to a complete loss of endothelium-mediated vasodilation in coronary arteries with angiographic evidence of atherosclerosis.

Responses of Coronary Resistance Vasculature

In the normal control group (group 1), coronary flow index increased by $442 \pm 123\%$ (range, 286–699%) in

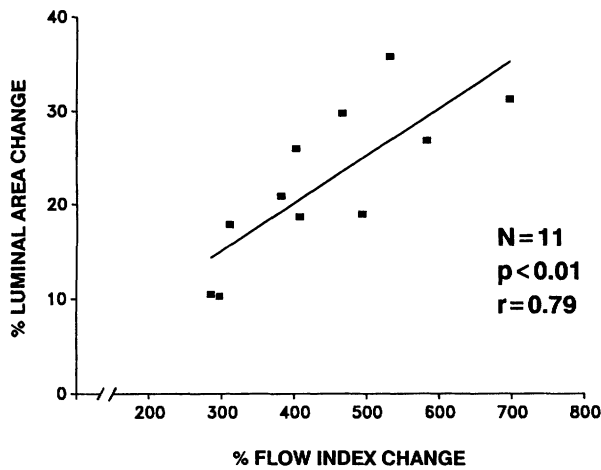


FIGURE 3. Plot of relation between flow-mediated change in coronary artery luminal area (expressed as percent change from baseline) and change in coronary blood flow index (expressed as percent change from baseline) in response to subselective infusion of 7 mg papaverin in the normal control patients (group 1).

response to subselective infusion of 7 mg papaverin, indicating a normal coronary flow reserve greater than 3.5 in all normal control patients. Figure 3 illustrates the linear relation between increases in flow indexes and flow-mediated vasodilation of the proximal artery segment in the normal control patients.

Comparable papaverin-induced increases in coronary flow indexes were observed in patients with hypercholesterolemia (group 2, $297 \pm 139\%$) and in patients with coronary artery disease but with normal proximal left anterior descending coronary artery segments (group 3, $356 \pm 146\%$) (both values are nonsignificant versus group 1). Papaverin-induced increases in flow indexes were slightly, but significantly, reduced in patients with minimal disease of the left anterior descending coronary artery (group 4, $258 \pm 77\%$, $p < 0.05$ versus group 1). There was a significant ($p < 0.05$), but weak ($r = 0.48$), correlation

between increases in flow indexes and flow-mediated vasodilation of the proximal artery segment in the group 2 patients. However, no significant relation between flow-mediated vasodilation and increases in flow indexes was observed in patients with coronary artery disease ($r = 0.16$ in group 3 and $r = 0.15$ in group 4).

The changes in coronary vascular resistance indexes in response to the three different stimuli are illustrated in Figure 4. Coronary flow indexes were calculated only when cross-sectional area reduction was less than 50% in the most constricting epicardial artery segment to exclude limitations of coronary artery flow due to epicardial artery constriction in response to acetylcholine. Therefore, in three patients of both group 3 and group 4, and in two patients of group 2, respectively, changes in maximal coronary vascular resistance induced by acetylcholine were calculated at a concentration of 10^{-7} M, whereas maximal decreases in coronary vascular resistance were assessed at the 10^{-6} M concentration of acetylcholine in all other patients. In patients with hypercholesterolemia but with angiographically normal coronary arteries (group 2), coronary vascular resistance was significantly ($p < 0.05$) less reduced in response to the maximal dose of acetylcholine compared with the normal control group (group 1) and with both groups with coronary artery disease (groups 3 and 4). In contrast, papaverin induced similar reductions in coronary vascular resistance in normal control patients (group 1), in patients with hypercholesterolemia (group 2), and in patients with coronary artery disease but with angiographically normal left anterior descending coronary arteries (group 3). The papaverin-induced decrease in coronary vascular resistance was slightly, but significantly, reduced in patients with minimal disease of the left anterior descending coronary artery (group 4) compared with the normal control group. If the patients with coronary artery disease (groups 3 and 4) were classified according to the presence or absence of

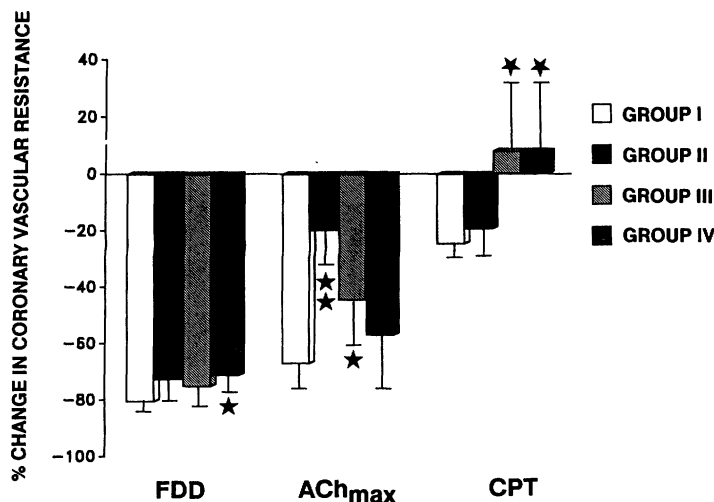


FIGURE 4. Bar graph of change in coronary vascular resistance (mean \pm SD, expressed as percent change from baseline) in response to papaverin-induced increases in blood flow (flow-dependent dilation, FDD), maximal concentration of acetylcholine (ACh_{max}), and cold pressor testing (CPT) in the four groups of patients. * $p < 0.05$ vs. group I; ** $p < 0.05$ vs. groups I, III, and IV.

hypercholesterolemia, those with elevated LDL serum levels ($n=6$) would have demonstrated a significantly ($p<0.05$) smaller reduction in coronary vascular resistance index in response to the maximal dose of acetylcholine ($37.6\pm 15.6\%$ versus $57.8\pm 10.7\%$ decrease) despite a normal response to papaverin ($397\pm 144\%$ increase in flow index).

Sympathetic stimulation by cold pressor testing decreased coronary vascular resistance similarly in the normal control group (group 1) and in patients with hypercholesterolemia (group 2), but it increased coronary vascular resistance in patients with coronary artery disease (groups 3 and 4, $p<0.05$ versus groups 1 and 2, respectively).

Thus, the coronary resistance vasculature demonstrated a selective impairment of vasodilation of the resistance vessels in response to the endothelium-dependent mediator acetylcholine in patients with hypercholesterolemia despite a normal response to the smooth muscle relaxant papaverin.

Discussion

The results of this study demonstrate that there is a progressive impairment in endothelium-mediated modulation of coronary vasomotor tone with different stages of early atherosclerosis in humans. In normal patients with angiographically normal coronary arteries and without risk factors for coronary artery disease, the vasomotor response to intracoronary administration of acetylcholine, increased blood flow, and sympathetic stimulation is characterized by an increase in epicardial artery luminal area. Patients with angiographically normal coronary arteries but with hypercholesterolemia and elevated plasma concentrations of LDL demonstrate a selective endothelial dysfunction with vasoconstriction in response to acetylcholine but preserved vasodilation in response to sympathetic stimulation and increased coronary blood flow. This selective endothelial dysfunction is not confined to the large epicardial conductance vessels but also includes the human coronary resistance vasculature. Angiographically normal coronary artery segments in patients with evidence of atherosclerosis elsewhere in the coronary system have lost their ability to dilate in response to acetylcholine and sympathetic stimulation, whereas their ability to dilate flow dependently is still intact. Atherosclerotic coronary arteries with angiographically visible luminal irregularities demonstrate a complete loss of endothelium-mediated vasoactive functions.

To interpret the progressive alterations in endothelium-dependent responses observed in the intact coronary circulation of patients with different early stages of atherosclerosis, the mechanisms of vasodilation induced by the different endothelium-dependent stimuli must be considered. Experimental studies strongly suggest that the vasodilation in response to increased flow is mediated by release of endothelium-derived relaxing factor (EDRF),²⁶⁻²⁸ possibly mediated by the signal of shear stress on the endothelium by stretch-activated ion channels.²⁹⁻³¹ Our

results demonstrated a strong linear correlation between relative increases in blood flow and luminal area changes of epicardial conductance vessels in the normal coronary vasculature, thereby indicating that changes in shear stress may at least, in part, contribute to the flow-mediated dilation in the intact human coronary circulation. Similar results were recently reported for the brachial artery in humans.³²

The mechanisms of vasodilation of normal epicardial coronary arteries in response to sympathetic stimulation is very likely to be mediated by endothelial cell α_2 -adrenergic receptors³³ and increased shear stress due to augmentation of coronary blood flow and driving pressure,^{34,35} thereby counteracting the direct α -adrenergic receptor-mediated vasoconstrictor effects.³⁶ Acetylcholine causes endothelium-dependent dilation by acting on endothelial muscarinic receptors to initiate the release of EDRF.^{2,4} The EDRF-mediated vasorelaxation counteracts acetylcholine's direct vasoconstrictor effect on the muscarinic receptors of the vascular smooth muscle.

The most important finding of this study is the hierarchical structure in the impairment of endothelium-dependent responses to the three different stimuli in patients with progressive stages of atherosclerosis. Previous studies demonstrate that the normal vasodilator response of epicardial arteries to acetylcholine^{10,21,37} is reversed to a constrictor response in patients with risk factors for coronary artery disease despite angiographically smooth coronary arteries.^{38,39} These results suggest an abnormality of endothelial function, which either precedes atherosclerosis or represents an early marker of atherosclerosis not detectable by angiography.³⁸ In the present investigation, the angiographically normal epicardial arteries of patients with hypercholesterolemia also exhibited a vasoconstrictor response to acetylcholine; however, the vasodilator response to increased coronary blood flow and to sympathetic stimulation was well preserved. Thus, our results demonstrate the loss of selective endothelium-mediated vasoactive functions very early in the development of atherosclerosis in patients with angiographically normal coronary arteries but with elevated plasma levels of LDL. These findings closely correspond to a number of experimental studies. Hypercholesterolemic animals without any evidence of structural alterations of the arterial wall demonstrate an impairment of receptor-mediated endothelium-dependent relaxations,^{7,40,41} but those mediated by norepinephrine or by the calcium ionophore A23187 are still preserved.^{9,11,13,15}

By combining these previous experimental findings with our present findings in patients with intact coronary circulation, we can hypothesize that very early in the process of atherosclerosis during hypercholesterolemia in humans endothelium-dependent responses are impaired first by a depressed receptor-mediated initiation of the production or release of EDRF. This hypothesis is further substantiated by our finding that hypercholesterolemia selectively im-

pairs endothelial function also in the coronary resistance vessels, even though small vessels do not develop atherosclerotic lesions with intimal or medial thickening. In patients with coronary artery disease, contrasting effects of acetylcholine with constriction of epicardial conductance arteries but with vasodilation of coronary resistance vessels have been reported^{42–44} and have been attributed to the lack of atherosclerotic lesions in the resistance vasculature, even when high-grade atherosclerotic lesions are present in the epicardial arteries. The present study is the first to demonstrate an impaired vasodilator response of coronary resistance vessels to acetylcholine in patients with hypercholesterolemia and a preserved vasodilator response of the resistance circulation to acetylcholine in patients with coronary artery disease but with normal LDL levels. Therefore, rather than reflecting atherosclerosis per se, the alterations in endothelial vasoactive function induced by hypercholesterolemia may implicate early changes in endothelial cell function important in the development of atherosclerosis in humans. However, although a number of experimental studies suggest that impairment of endothelium-dependent relaxation in diet-induced hypercholesterolemia may be specific for relaxations mediated by receptors, we cannot exclude that atherosclerosis not detectable by coronary angiography was already present in the patients with hypercholesterolemia and angiographically smooth coronary arteries. To ascertain that hypercholesterolemia itself induces alterations of muscarinic receptors with preservation of other features of endothelium-mediated relaxation would require histological data of the vessel wall, which obviously cannot be obtained from patients undergoing diagnostic coronary angiography.

The loss of a vasodilator response to sympathetic stimulation by cold pressor testing, in addition to the vasoconstrictor effects of acetylcholine, in angiographically normal epicardial coronary artery segments but with evidence of atherosclerosis elsewhere in the coronary system suggests a further impairment in endothelium-mediated vasoactive function in these patients. Intraoperative echocardiographic studies have demonstrated that in vivo coronary arterial atherosclerosis is far more extensive than that predicted by coronary angiography.⁴⁵ 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, the angiographically “normal” appearing coronary arteries demonstrating a vasoconstrictor response to sympathetic stimulation are probably in an early stage of atherosclerosis with mainly diffuse intimal thickening. Previous studies using intracoronary infusion of acetylcholine also demonstrated endothelial dysfunction in angiographically normal arteries of patients with coronary artery disease^{21,37} and of cardiac trans-

plant patients, which is a population prone to accelerated graft atherosclerosis.⁴⁶

Whether the vasoconstrictor response of both epicardial and resistance vessels in patients with coronary artery disease represents an altered sensitivity of vascular adrenergic receptor stimulation or is mediated by vasoactive substances released from activated platelets during sympathetic stimulation⁴⁷ remains to be determined. A number of experimental studies demonstrated the pivotal role of an intact endothelium for preventing intracoronary platelet aggregation with subsequent reductions in coronary blood flow during sympathetic stimulation.^{48,49}

Despite impaired endothelial function in response to acetylcholine and sympathetic stimulation, flow-mediated vasodilation was still preserved in angiographically normal arteries in patients with coronary artery disease. Thus, in the absence of a concomitant vasoconstrictor stimulus, increases in blood flow still elicited vasodilation, indicating preservation of the effects of EDRF released through the signal of shear stress in these vessel segments. However, when atherosclerosis was more advanced as evidenced by angiographically visible luminal irregularities of the analyzed vessel segments, then flow-mediated vasodilation was also abolished. In these atherosclerotic vessel segments, the vasodilator response to the smooth muscle relaxant, endothelium-independent, nitroglycerin was significantly reduced, too, indicating a general reduction in vasodilatory capability. Although the precise mechanisms of endothelium-mediated vasodilation for the three different stimuli used in the present study cannot be directly assessed in the intact human coronary circulation, our results indicate that vasodilation in response to increased coronary blood flow is preserved until there is angiographic evidence of myointimal thickening of the arterial wall as indicated by luminal irregularities.

These observations in the intact human coronary circulation favorably correspond with recently published experimental results in pigs with different early stages of atherosclerosis.¹⁵ Endothelium-dependent responses progressively deteriorated during the process of atherosclerosis from an impairment of a variety of receptor-mediated endothelial functions during the early stages of atherosclerosis to a complete loss of endothelium-mediated relaxation when myointimal thickening with concomitant medial thickening of the arteries was present.¹⁵ However, we cannot exclude the possibility that, in coronary atherosclerosis, vasoconstrictor substances may be released with a smaller amount of EDRF.^{50,51}

In summary, this study demonstrates a progressive impairment of endothelial vasoactive functions in coronary arteries with advancing early stages of atherosclerosis in the intact human coronary circulation, beginning with a selective endothelial dysfunction in angiographically normal arteries in patients with elevated serum LDL concentrations and progressively worsening to a complete loss of endothelium-mediated vasodilation in coronary arteries with angiographic ev-

idence of atherosclerosis. The assessment of endothelial modulation of vasomotor tone in the intact human coronary circulation may add a new dimension to the angiographic evaluation of coronary artery disease by detecting early changes in endothelial cell vasoactive function important in the development of atherosclerosis rather than reflecting atherosclerosis per se. Clinically and most importantly, this may provide unique and critical information for evaluating therapeutic interventions very early in the process of atherosclerosis in patients with a high risk of developing coronary artery disease, for example, in hypercholesterolemia.¹⁶ In fact, preliminary results indicate that endothelial dysfunction in response to acetylcholine in patients with hypercholesterolemia may be reversed by a dietary supplementation of fish oil.⁵²

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KEY WORDS • coronary artery disease • hypercholesterolemia • endothelium • sympathetic stimulation • coronary vascular resistance • acetylcholine • papaverin • angiography