JACC Vol. 26, No. 1
July 1995:41–9

Epicardial Vasomotor Responses to Acetylcholine Are Not Predicted by Coronary Atherosclerosis As Assessed by Intracoronary Ultrasound

RICK A. NISHIMURA, MD, FACC, AMIR LERMAN, MD, JAMES H. CHESEBRO, MD, FACC, DUANE M. ILSTRUP, MS, DAVID O. HODGE, MS, STUART T. HIGANO, MD, DAVID R. HOLMES, JR., MD, FACC, A. JAMIL TAJIK, MD, FACC

Rochester, Minnesota

Objectives. **The purpose of this study was to use intravascular ultrasound to determine the morphologic appearance of the coronary arteries, relating the absence, presence and extent of atherosclerosis to the response of the coronary arteries to acetylcholine infusion.**

Background. **Endothelial function plays a major role in the pathophysiology of myocardial ischemia and angina pectoris. The response of the coronary arteries to selective infusion of acetylcholine has been used to examine endothelial function, with vasoconstriction occurring in the absence of intact endothelial function. Vasoconstriction to acetylcholine infusion in humans without overt coronary artery disease has been attributed to early atherosclerosis not detected by coronary angiography.**

Methods. **Twenty-nine patients without overt coronary artery disease underwent selective coronary angiography and selective intracoronary infusion of increasing concentrations of acetylcholine (10^{-6}, 10^{-5} and 10^{-4} mol/liter), followed by intravascular ultrasound imaging.**

Results. **The response of the coronary arteries to acetylcholine infusion was not dependent on the absence or presence of atherosclerotic plaque, as detected by intravascular ultrasound. The percent change in epicardial coronary artery diameter during acetylcholine infusion versus baseline was −14 ± 28% (mean ± SD) in the seven patients with no visible atherosclerosis on intravascular ultrasound versus −9 ± 20% in the 22 patients with visible atherosclerosis on intravascular ultrasound (p = NS, confidence interval −14% to 25%). There was a greater vasoconstrictive response to acetylcholine infusion in patients with risk factors for coronary artery disease than in those without risk factors (p = 0.003).**

Conclusions. **The vasoreactive response to acetylcholine is not necessarily dependent on ultrasound detection of the presence or absence of atherosclerosis.**

(J Am Coll Cardiol 1995;26:41–9)

Changes in vasomotor tone of the epicardial coronary arteries play a major role in the pathophysiology of myocardial ischemia and angina pectoris (1,2). The role of the vascular endothelium in the control of arterial tone has been recognized (3–5). Vasomotor responses to various stimuli are thought to be mediated by the release of vasodilators, such as endothelial-relaxing factor, from intact endothelial cells. The lack of a vasodilator response implies endothelial dysfunction.

Acetylcholine has been used to examine this response by the vascular endothelium. Normal arterial segments in vitro will dilate when exposed to acetylcholine in a specific dose range but will constrict in response to acetylcholine after mechanical removal of the endothelium (3). When acetylcholine is infused into coronary arteries of patients with angiographic evidence of diffuse coronary disease, vasoconstriction occurs, which is presumed to be a direct effect of acetylcholine on the medial smooth muscle in the absence of release of vasodilators, such as endothelial-relaxing factor (6–8). When acetylcholine is infused into angiographically normal coronary arteries in young patients with no risk factors for coronary artery disease, vasodilatation occurs, thought to be due to release of endothelial-relaxing factor by normal endothelium. Angiographically smooth vessels constrict in response to infusion of acetylcholine in older patients (9), in patients with visible disease in other vessels (10) and in patients with risk factors for coronary artery disease (11–15). It has been postulated (6,7,9,11,16) that these angiographically smooth coronary vessels that constrict in response to acetylcholine have early atherosclerosis not detectable by routine coronary angiography.

There are many well known limitations of coronary angiography in diagnosing coronary artery disease, especially when there is mild or diffuse disease (10,17). Intravascular ultrasound is a new technology that allows high resolution images of the vascular structure and overcomes these limitations of coronary angiography (18,19). The purpose of the present study was to use intravascular ultrasound to determine the presence and extent of atherosclerosis in patients with mild irregularities or smooth epicardial coronary arteries, as as-

From the Division of Cardiovascular Diseases and Internal Medicine and Section of Biostatistics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota. There was no financial support for this study.

Manuscript received June 20, 1994; revised manuscript received January 19, 1995, accepted March 1, 1995.

Address for correspondence: Dr. Rick A. Nishimura, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905.

0735-1097/95/$9.50
0735-1097(95)00142-Q

JACC Vol. 26, No. 1
July 1995:41–9

sessed by coronary angiography, relating the ultrasound findings to the response of these vessels to infusion of acetylcholine.

Methods

Patient selection. Patients referred to the Cardiac Catheterization Laboratory for evaluation of chest pain syndromes or for determination of coronary anatomy in association with other cardiac diseases were considered for this study. Patients with known, documented coronary artery disease were excluded from the study (recent or remote myocardial infarction, unstable angina, previous coronary artery bypass grafting). Patients with clinically documented coronary artery spasm (transient ST segment elevation at rest during chest pain) were also excluded. Patients with severe chronic obstructive lung disease, renal failure (creatinine concentration >2 mg/dl [180 μmol/liter]) or known cerebrovascular disease were excluded from the study. Any patients taking long-acting nitrates or calcium channel blocking agents had these medications withheld for 24 to 48 h before the study. The study was approved by the Mayo Clinic Institutional Review Board, and written informed consent was obtained from all patients.

Diagnostic coronary angiography was performed in all patients before the study by using 6F catheters with a standard femoral percutaneous approach. Twenty-five hundred units of heparin was given intravenously after the arterial puncture. No nitroglycerin was given during the diagnostic procedure. Nonionic contrast material was used for all patients. Patients were included in this study if they had the following: 1) angiographically smooth coronary arteries, or 2) mild irregularities with no coronary artery lesion >30% lumen diameter stenosis by visual assessment in any major epicardial vessel. Patients were excluded from the study if the proximal coronary arteries were <2.0 mm in diameter. Left ventriculography or echocardiography was performed in all patients for measurement of left ventricular function.

Study design. After the diagnostic angiogram, an 8F guide catheter was positioned in the ostium of the left main coronary artery, and an infusion catheter (length 135 cm, outer diameter 3F, inner lumen >0.038 in. [0.10 cm]) was placed through the guide catheter selectively into the proximal left anterior descending or circumflex coronary artery. An additional 5,000 U of heparin was given intravenously. Blood pressure, electrocardiography (standard MCL_1 lead) and heart rate were monitored throughout the procedure. A single monoplane angiographic view that best displayed the left anterior descending or circumflex coronary artery without foreshortening was selected from the diagnostic angiograms, and the image intensifier was fixed at this angle and height throughout the procedure.

A baseline angiogram was obtained after the catheters had been properly positioned. Nonionic contrast material (8 to 12 ml) was injected by hand into the guide catheter to achieve optimal opacity of the coronary arteries, and the images were recorded on 35-mm film. After this, a control infusion of isotonic saline solution was performed through the small infusion catheter positioned in the left anterior descending coronary artery by using a Harvard infusion pump at a rate of 0.8 ml/min for a total duration of 3 min. At the end of 3 min, blood pressure, heart rate and electrocardiographic recordings were measured, and another angiogram was obtained. The infusion was continued until after the angiogram was completed. Three different infusions of acetylcholine were performed in a similar manner. The concentrations of acetylcholine were 10^{-6}, 10^{-5} and 10^{-4} mol/liter. If a blood flow of 80 ml/min in the coronary arteries is assumed (20), then these infusions would achieve estimated coronary blood concentrations of acetylcholine of 10^{-8}, 10^{-7} and 10^{-6} mol/liter (11). If there was severe constriction of the coronary arteries with any dose of acetylcholine, then the infusions were stopped. After each dose, angiography was repeated. After infusions of acetylcholine, 300 μg of nitroglycerin were given by an intracoronary route, and angiography was repeated within 2 min after the nitroglycerin was given.

After the selective coronary infusions, intravascular ultrasound imaging was performed. One of three intracoronary ultrasound systems (Cardiovascular Imaging Systems, Intertherapy and Hewlett-Packard) was used in this study. Details of these systems have been described elsewhere (18,19). The intracoronary ultrasound catheters were inserted through the 8F guide catheter and placed into the proximal and middle portions of the selected artery over a 0.014-in. (0.036 cm), high torque, floppy guide wire. After optimization of the ultrasound image, continuous real-time images were recorded on 0.5-in. videotape. Four to five segments of the coronary artery distal to the infusion catheter 0.5 to 1.0 cm apart were selected independently of the response to acetylcholine. These segments were identified on the videotape recording of the ultrasound imaging, and the exact position of the ultrasound catheter in relation to the artery was recorded on cine film at each position. The location of the catheter as seen on the cine film at each segment position was used to correlate the identified ultrasound image with the angiographic segment.

Study analysis. Analysis of artery diameter from the cine films was done with a modification of the technique previously described from this institution (21,22). A diastolic still frame at each infusion (baseline, saline solution, acetylcholine times three and nitroglycerin) was selected from the cine film and an 8 × 10-in. spot film was made. The different segments of the artery selected at the time of intracoronary ultrasound imaging were identified on the radiograph. By using a computer-interactive digitizing system, the outline of the contrast material within the lumen was digitized at each specific region of interest identified (21,22). The absolute diameter of the vessel lumen perpendicular to the long axis of the artery at the selected specific points of the artery was measured by using the guide catheter as the calibration standard. These measurements were made by an experienced observer with no knowledge of the ultrasound findings.

There was a progressive directional change in the vessel diameters during acetylcholine infusions of increasing concentrations. The response of each segment to the highest infusion

dosage of acetylcholine was calculated in two ways: 1) The percent change in vessel diameter during acetylcholine infusion compared with vessel diameter at baseline (%Δacetylcholine) was calculated; 2) to control for variations in underlying vasomotor activity, the response to acetylcholine was normalized to the maximally vasodilated diameter after nitroglycerin administration. Thus, the ratio of the vessel diameter during acetylcholine infusion to the diameter after administration of intracoronary nitroglycerin (acetylcholine/nitroglycerin) was calculated. For each patient, the average response of all measured segments was calculated for both the %Δacetylcholine and the acetylcholine/nitroglycerin ratio measurements. The average response of all measured segments was used in this study to account for the segmental heterogeneity seen in response to acetylcholine infusion (23). However, the average response of all measured segments for each patient had the same directional response as segments showing the greatest mean diameter change at peak acetylcholine infusion, as has been shown previously (11).

Intraobserver variability in coronary artery diameter measurement was assessed by independent, blinded measurement of random segments in 20 patients. The percent intraobserver variability was 6 ± 5% (mean ± SD). In 10 patients, two angiograms in the baseline state were obtained within 5 min of each other. The percent variability in the measurements of random segments from the two separate angiograms was 4 ± 5%.

For each coronary segment, a visual subjective analysis was performed from the cineangiogram to categorize the segments into 1) angiographically normal segments, and 2) segments with mild irregularities. This was arrived at by the consensus of two experienced angiographers who had no knowledge of the other results of the study. For each patient, angiographically normal segments were defined as *no irregularities in any segment*.

An off-line computer-interactive analysis system was used to digitize the intracoronary ultrasound video images onto a 256 × 256-bit matrix (24). Standard calibration markers directly from the ultrasound image were used for calibration of absolute measurements. Histopathologic correlations have been established for interpretation of intravascular ultrasound images (19,24–26). In the presence of atherosclerotic plaque or diffuse intimal thickening, there is a three-layered appearance. A thick homogeneous inner layer represents the atherosclerotic plaque; a darker middle layer represents the thinned media; and a bright surrounding area represents the adventitia (19,24,25). The following measurements were made at each specific segment of the artery that had been previously identified: measured lumen area and potential lumen area (measured at the leading edge of the dark medial band [18]). Percent area stenosis was defined as the plaque area divided by the potential lumen area.

Coronary artery segments were considered free of measurable atherosclerotic plaque if the ultrasound appearance of the vessel wall was homogeneous without layering or consisted of a thin homogeneous layer ≤0.1 mm in diameter (26) (group 1).

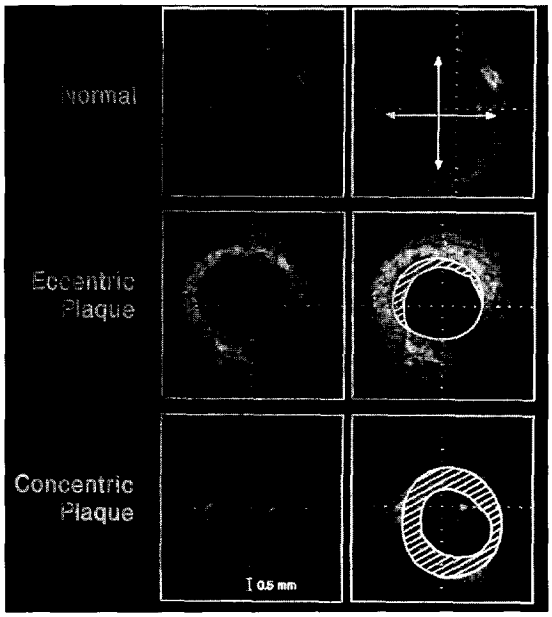


Figure 1. Intravascular ultrasound images demonstrate a visually normal coronary artery **(top)**, an artery with an eccentric plaque **(middle)** and an artery with a concentric plaque **(bottom). Arrows** indicate the lumen borders; **hatched areas** outline the measured plaque area. **Calibration markers** of 0.5 mm are shown.

Eccentric plaque was defined as measurable plaque covering <100% of the circumference of the vessel (group 2). *Concentric plaque* was defined as measurable plaque covering the entire circumference of the vessel (group 3) (Fig. 1).

For each patient, normal intravascular ultrasound segments were defined as all segments visualized being free of measurable atherosclerotic plaque.

Statistics. The two-sample *t* test was used to test for differences in normally distributed data, and the Wilcoxon rank-sum test was used in the nonnormally distributed cases.

The relation between continuous factors was assessed with the Pearson or the Spearman correlation coefficient. The Spearman coefficient was used for nonnormally distributed data, and the Pearson coefficient was used for normally distributed data.

For this sample size, adequate power was available to detect a 30% point difference in percent change in %Δacetylcholine, if such a difference truly existed (beta 0.1, alpha 0.05). Adequate power was available to detect a 30% point difference in percent change in acetylcholine/nitroglycerin, if such a difference truly existed (beta 0.01, alpha 0.05).

Results

Patients. There were 32 patients who were initially entered into the study. Of these, three patients were excluded, one because the infusion catheter moved into a diagonal artery, one because of inadequate ultrasound images and one because of angiographic images inadequate for measurement. Therefore, data from 29 patients were available for analysis (left

anterior descending coronary artery in 28, left circumflex coronary artery in 1; 15 men, 14 women; mean [±SD] age 55 ± 11 years, range 31 to 75). Twenty-seven patients were referred to the Cardiac Catheterization Laboratory for chest pain, and two had cardiac catheterization for symptoms of dyspnea. The mean ejection fraction was 66 ± 15%, and the end-diastolic volume index was 85 ± 22 ml/m^2.

The mean cholesterol level was 215 ± 51 mg/dl. Seventeen of the 29 patients had a cholesterol level >200 mg/dl. Eleven patients had a history of tobacco use (four active and seven in the past); eight had a positive family history of coronary disease (first-degree relative <50 years old with documented coronary artery disease); two had hypertension on therapy; and one had insulin-dependent diabetes. Medications at the time of the study included beta-adrenergic receptor blocking agents in four patients, salicylates in three and gemfibrozil in one. Three patients were receiving calcium-channel blocking agents, but these were stopped at least 24 to 48 h before the procedure. None were receiving long-acting nitrates.

Intravascular ultrasound findings. There were no complications from insertion of the intravascular ultrasound catheter. The average time of the intravascular ultrasound examination was 3 min.

There were 144 coronary artery intravascular ultrasound segments analyzed. Of these, 76 had no visible plaque, and 68 had definite atherosclerotic disease by intravascular ultrasound. Forty-two segments had eccentric plaque, and the remaining 26 had concentric plaque.

Seven of the 29 patients had no visible atherosclerotic plaque as assessed by intravascular ultrasound (group 1). The remaining 22 patients had visible atherosclerotic plaque in at least one segment on ultrasound images (group 2).

Angiographic measurements. Fourteen of the 29 patients had angiographically normal arteries. The remaining 15 patients had mild irregularities on angiography in at least one segment.

There were no complications as a result of acetylcholine infusions. There was no significant change in blood pressure or heart rate response during the various infusions. Chest pain developed in two patients during the final infusion of 10^{-4} mol/liter of acetylcholine, which was reversed by intracoronary nitroglycerin infusion.

There was no significant difference in coronary artery diameter measurement at baseline and during saline infusion (2.31 ± 0.5 mm vs. 2.27 ± 0.5 mm, p NS). The overall percent change during acetylcholine infusion in all patients was −9 ± 24%. With nitroglycerin infusion, there was an overall vasodilation compared with that at baseline, with a mean increase in coronary artery segment diameter of 13 ± 15%.

Relation of response to acetylcholine and intravascular ultrasound findings. The response of the coronary arteries to acetylcholine infusion was not related to the presence or absence of atherosclerosis as assessed by intravascular ultrasound (Fig. 2). Percent change in baseline segment diameter to that during acetylcholine infusion was −14 ± 28% in the seven patients with no visible atherosclerosis on intravascular ultrasound (group 1). This was not different from the −9 ± 20% decrease in the 22 patients with visible atherosclerosis on intravascular ultrasound (group 2) (Fig. 3, top). The estimate of the difference in percent change was only 5% (95% confidence interval [CI] −14% to 25%). The ratio of the vessel diameter during acetylcholine infusion to that diameter after nitroglycerin was 0.78 ± 0.26 in the seven patients with no visible atherosclerosis on intravascular ultrasound (group 1) and 0.82 ± 0.19 in the 22 patients with visible atherosclerosis on intravascular ultrasound (group 2) (p = NS) (Fig. 3, bottom). The estimate of the difference in acetylcholine/nitroglycerin ratio was 0.04 (95% CI −0.14 to 0.23).

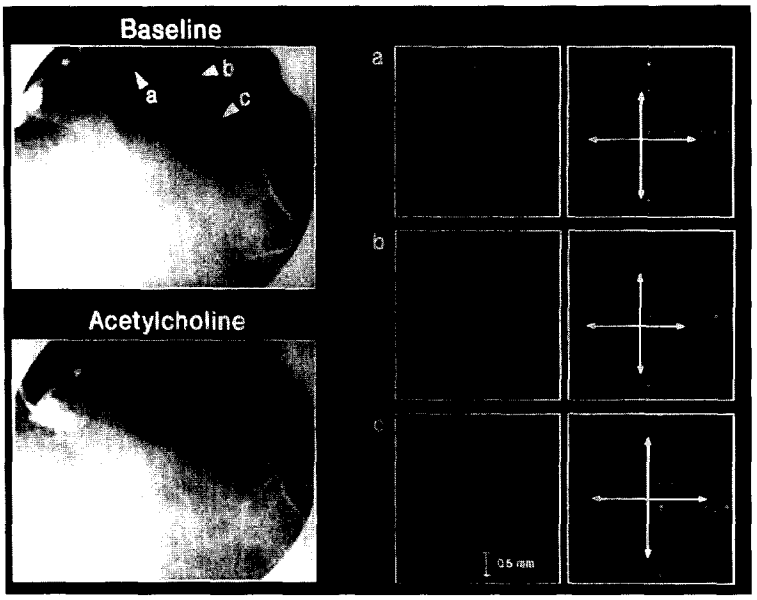
Table 1 shows the relation between the intravascular ultrasound and angiographic findings in the 29 patients. Eight of the 14 patients with angiographically normal coronary arteries had atherosclerotic disease on intravascular ultrasound imaging. Fourteen of 15 patients with mild irregularities noted on angiography had documented atherosclerotic disease on intravascular ultrasound imaging. There was no significant difference in the changes with acetylcholine when patients were compared with regard to the following findings: 1) normal angiographic segments/normal intravascular ultrasound segments versus 2) normal angiographic segments/abnormal intravascular ultrasound segments versus 3) abnormal angiographic segments/abnormal intravascular ultrasound segments. There was no significant difference in either percent change with acetylcholine or the ratio of acetylcholine/nitroglycerin within these three groups.

There were seven patients with no clinical risk factors and 22 with at least one or more clinical risk factors, including serum cholesterol level >200 mg/dl, positive family history of coronary artery disease, history of hypertension with current medical therapy, diabetes mellitus or history of or current tobacco use. There was a greater percent change in vessel diameter during acetylcholine infusion than at the baseline state in patients with than without risk factors (−14 ± 23% vs. 4 ± 8%, p = 0.003) (Fig. 4).

Discussion

The present study examined the in vivo vasomotor response in human coronary arteries of known atherosclerotic morphology documented by intravascular ultrasound. The results of this study in patients with chest pain syndromes are similar to those of a previous study in patients after cardiac transplantation (27,28): The vasoactive response to acetylcholine in these patients is not related to the absence, presence or degree of underlying atherosclerosis. Vasoconstriction may occur in normal segments and vasodilation in diffusely atherosclerotic segments.

The present study is not in disagreement with previous angiographic studies because a more sensitive and specific method to determine the presence or absence of atherosclerotic lesions was used with intravascular ultrasound. Our patients were limited to those with angiographically normal or near-normal coronary arteries. Thus, patients with definite



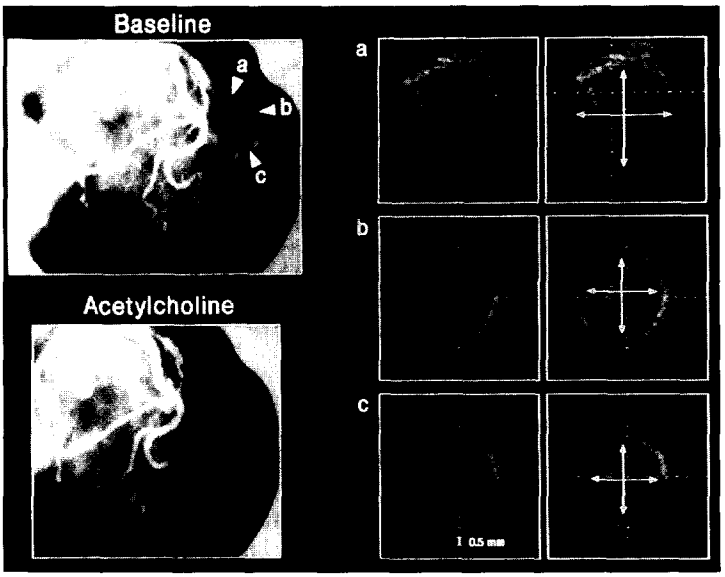
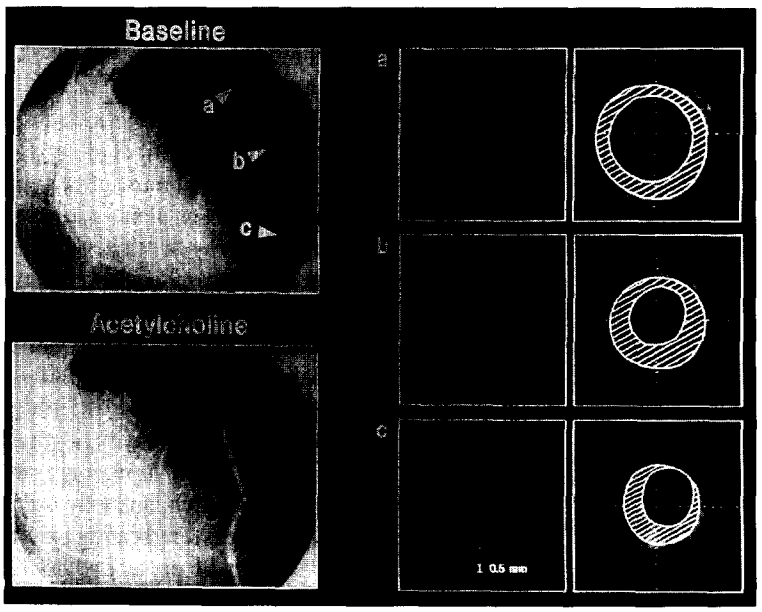


Figure 2. Coronary angiograms (**left**) obtained at baseline and after infusion of 10^{-4} mol/liter of acetylcholine and intravascular ultrasound images (**right**) of three representative segments (**a, b, c**) in three patients after intracoronary administration of nitroglycerin. **Top,** There is diffuse angiographic vasoconstriction of the entire left anterior descending coronary artery after infusion of acetylcholine, with no measurable atherosclerotic plaque on the intravascular ultrasound images. **Middle,** There is vasoconstriction in the mid- and distal left anterior descending coronary artery on coronary angiography after infusion of acetylcholine, with no measurable atherosclerotic plaque on the intravascular ultrasound images. **Bottom,** There is vasodilation of the entire left anterior descending coronary artery on coronary angiography after infusion of acetylcholine. The intravascular ultrasound images demonstrate a thick-layered appearance of the vessel wall, indicating diffuse atherosclerosis. Symbols as in Figure 1.



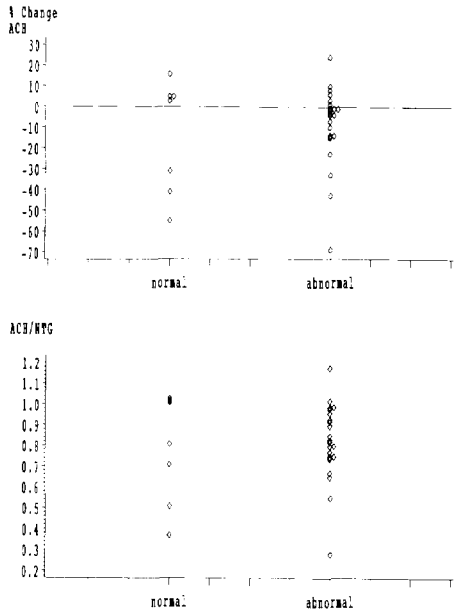


Figure 3. Top, Percent change in coronary artery diameter during acetylcholine infusion versus the baseline state (% change ACH). There is no significant difference in overall percent change during acetylcholine infusion in patients with no evidence of atherosclerotic plaque on intravascular ultrasound (normal) versus those with visible atherosclerotic plaque in any segment on intravascular ultrasound (abnormal). **Bottom,** Plot of the ratio of the coronary artery diameter during acetylcholine infusion to the coronary artery diameter during maximal vasodilation with intravenous nitroglycerin (ACH/NTG). There was no significant difference in this ratio in the patients with no visible atherosclerotic disease on intravascular ultrasound versus those with disease on intravascular ultrasound.

advanced atherosclerosis by angiography, who would be expected to have a vasoconstrictive response (6), were not included in the study. Previous studies (6–8) have shown a vasoconstrictive response in patients with mild irregularities of the left anterior descending coronary artery but with advanced stenosis in at least one other vessel; such patients with more advanced atherosclerosis were not included in our study. As previously shown (7–9,11–15), there was a relation between the

Table 1. Relation of Intravascular Ultrasound and Angiographic Findings in Individual Patients

	Angiographic Findings	
IVUS	Normal	Irregularities
Normal	n = 6	n = 1
%ΔACH	17 ± 29	3
ACH/NTG	0.74 ± 0.26	1.03
Plaque present	n = 8	n = 14
%ΔACH	−4 ± 9	−12 ± 24
ACH/NTG	0.87 ± 0.09	0.80 ± 0.22

Data presented are mean value ± SD or number of patients. ACH/NTG = ratio of vessel diameter during acetylcholine infusion to that after intracoronary nitroglycerin; IVUS = intravascular ultrasound; %ΔACH = percent change in diameter of coronary vessel during acetylcholine infusion versus diameter at baseline.

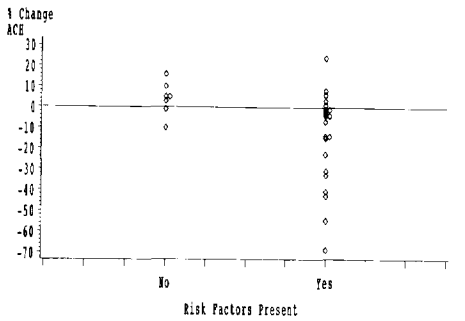


Figure 4. Percent change in coronary artery diameter during acetylcholine infusion versus baseline (% change ACH) in patients without versus with risk factors. There was a significantly larger degree of vasoconstriction in patients with than without risk factors ($p = 0.003$).

vasoconstrictive response to acetylcholine and the risk factors for coronary artery disease. All segments dilated in response to nitroglycerin, indicating that the changes in response to acetylcholine were the result of a disturbance in endothelial function and were not related to underlying changes in vasomotor tone.

Previous clinical studies. A vasoconstrictor response usually occurs in patients with definite angiographic coronary artery disease (6,8,9,29). A variable response to infusion of acetylcholine occurs in patients with angiographically normal coronary arteries or in those with mild irregularities (6–9,11–15,29,30). The variable response in earlier studies (6,30) was initially explained on the basis of different dosages of acetylcholine, different methods of infusion (bolus administration vs. continuous infusion) and changes in heart rate and blood pressure during infusion. However, subsequent studies found that vasodilation occurred when acetylcholine was infused into angiographically normal arteries in young patients with no risk factors and that vasoconstriction occurred in angiographically normal arteries in older (9), hypertensive (12,14), or hyperlipidemic patients (8,13,15) or in those with angiographic evidence of disease in other arteries (7). The vasoconstrictive response was directly related to the number of risk factors present (11).

Recent studies (31) showed that vasodilation in response to acetylcholine is attributed to release of endothelium-derived relaxing factor by muscarinic receptors (32) and is not due to prostaglandins or alpha-adrenoceptor mechanisms because it is inhibited by methylene blue, which blocks the cyclic guanosine monophosphate pathway. Thus, vasoconstriction in response to acetylcholine in human coronary arteries is due to endothelial dysfunction and was proposed (6,7,16,33) to occur in patients with angiographically normal coronary arteries because of early atherosclerosis that was not detected by conventional coronary angiography.

Others (27,28) have suggested that a vasoconstrictor response to acetylcholine may occur in patients without atherosclerotic disease, indicating that an abnormality of endothelial function may be present without morphologic atherosclerosis. The highly sensitive and specific information on the presence

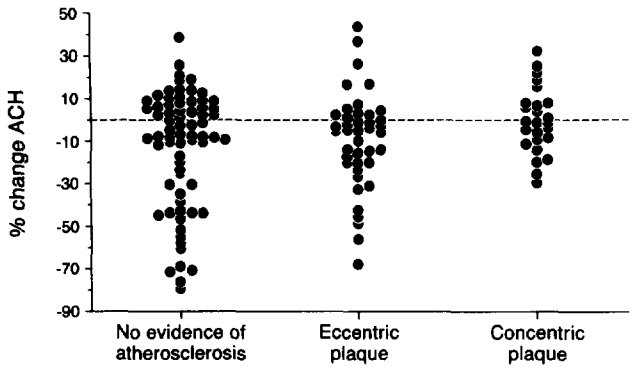


Figure 5. Percent change in coronary artery diameter during acetylcholine infusion versus baseline (% change ACH) in the 144 individual segments.

or absence of atherosclerotic plaque provided by intravascular ultrasound in the present study supports that hypothesis. In the animal model, abnormal responses to acetylcholine in the presence of hyperlipidemia were shown (34–36) to occur in the absence of atherosclerosis with the endothelium generally morphologically intact. Thus, early endothelial dysfunction without atherosclerosis in coronary arteries may represent an important early event predisposing to vasospasm. Mechanisms that have been proposed include attenuation of receptor-mediated stimulation, decreased synthesis of endothelium-derived relaxing factor (34), intimal trapping of endothelium-derived relaxing factor (37), a direct effect of low density lipoprotein (36) or decreased responsiveness of smooth muscle to endothelium-derived relaxing factor. Others (38) have suggested that a decreased endothelium-dependent relaxation may be due to the simultaneous release of a contracting substance. In patients with variant angina, substance P will cause coronary dilation in segments that constrict in response to acetylcholine, indicating that the mechanism of the production of endothelium-derived relaxing factor and the response of smooth muscle remains preserved (39).

The presence of diffuse atherosclerosis identified by intravascular ultrasound in the present study did not necessarily predict a vasoconstrictive response to acetylcholine. A recent study (23) has also shown that vasodilation may occur in patients with known coronary artery disease, indicating that endothelial function is not irreversible and diffusely lost in the presence of atherosclerotic disease. Patchy segmental responses in the same artery have been documented, suggesting that local factors may be important in determining endothelial function (23). There was less vasoconstriction in response to acetylcholine in segments consisting of concentric plaques (Fig. 5). This suggests that concentric atherosclerotic involvement may produce a cicatrization effect that prevents vasoconstriction.

Role of endothelium-derived relaxing factor in humans. The role of endothelium-derived relaxing factor in clinical coronary disease syndromes remains uncertain, but there have been recent studies implicating its importance. Impaired endothelium-dependent cholinergic coronary vasodilation in patients with visually normal arteries on coronary angiography may contribute to the pathogenesis of myocardial ischemia and angina in patients with syndrome X (40,41). There is an increased sensitivity of coronary arteries that respond with a vasoconstrictive response mediated by acetylcholine to constriction with mental stress (29) or catecholamines (16), suggesting that altered vasomotor tone may play a role in the production of coronary ischemia in stable disease. Infusion of acetylcholine immediately after myocardial infarction produced a much greater degree of vasoconstriction in the infarct-related than the noninfarct-related artery, suggesting that this response may be related to the pathogenesis of myocardial infarction (42). An impaired response in arteries that are prone to develop atherosclerosis, such as in branch points (43) and transplanted hearts (33), emphasizes the potential role of endothelial dysfunction in the development of future atherosclerosis. Thus, the response of coronary arteries to acetylcholine as a measure of endothelial function may be an important tool for future investigations into clinical coronary artery syndromes.

Limitations of the study. Multiple studies (19,24,25) documented the ability of intravascular ultrasound to detect and quantitate the amount of atherosclerosis when images are correlated with histopathologic specimens. Thus, the presence of a thick-layered appearance of the vessel wall on intravascular ultrasound is a reliable marker for atherosclerosis. However, mild degrees of intimal thickening may not be observed on intravascular ultrasound imaging until the intimal layer is sufficiently thick, so that the overall thickness of the vessel becomes resolvable at 30 MHz. Intimal thickening will normally occur with age and may even exceed the dimension of the underlying medial layer by age 30 years (44). The threshold on the instrument used in the study for detection of intimal thickening is ~178 μm (26), and intravascular ultrasound may not be sensitive to intimal thickening below this threshold.

The number of patients in the present study was relatively small, and the confidence intervals of the difference in response to acetylcholine between groups 1 and 2 were relatively large. However, the demonstration that several group 1 patients had vasoconstriction and several from group 2 had vasodilation was supportive of the hypothesis that endothelial function is not solely dependent on coronary artery morphology (Fig. 2). The quantitative analysis of the diameter of the coronary artery segments did not use the biplane videodensitometrically-automated analysis averaging multiple end-diastolic frames, as has been described in previous studies. Nonetheless, the intraobserver variability of <6% and the low variability between measurements on two separate angiograms at baseline demonstrate the reliability and reproducibility of this technique. There was no measurement of blood flow and, thus, the present study did not control for the potential confounding effects of patient-to-patient variability on flow-dependent vasodilation, which averages 7% (28). The concentration of acetylcholine used in our study was similar to that used in several previous studies (6,8,11,28); however, these

concentrations assume a blood flow of 80 ml/min to achieve final concentrations of 10^{-8} to 10^{-6} mol/liter. Thus, in the presence of higher coronary flow rates, the final intracoronary concentration would be less.

A bias may be present in the patient cohort because the majority of patients selected for our study had coronary angiography for evaluation of chest pain syndromes. It is possible that these patients, therefore, may have some intrinsic abnormality and coronary reactivity to vasoactive stimuli and may not be representative of all patients with angiographically normal coronary arteries. Nonetheless, even in this patient subgroup, these findings indicate that the relation between morphologic atherosclerotic disease and endothelial dysfunction is more complex than previously suggested.

We thank David T. Smyrk, BA for work on the angiographic and ultrasound images, Denise A. Skoda for secretarial assistance and Kristine Menke, BA for technical support.

References

1. Maseri A. The changing face of angina pectoris: practical implications. Lancet 1983;1:746–9.
2. Maseri A. Role of coronary artery spasm in symptomatic and silent ischemia. J Am Coll Cardiol 1987;9:249–62.
3. Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Circ Res 1983;53:557–73.
4. Bassenge E, Busse R. Endothelial modulation of coronary tone. Prog Cardiovasc Dis 1988;30:349–80.
5. Vanhoutte PM. The endothelium—modulator of vascular smooth-muscle tone. N Engl J Med 1988;319:512–3.
6. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
7. Werns SW, Walton JA, Hsia HH, Nabel EG, Sanz ML, Pitt B. Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease. Circulation 1989;79:287–91.
8. Zeiher AM, Drexler H, Wollschläger H, Just H. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 1991;83:391–401.
9. Yasue H, Matsuyama K, Matsuyama K, Okumura K, Morikami Y, Ogawa H. Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment: possible role of early coronary atherosclerosis. Circulation 1990;81:482–90.
10. Arnett EN, Isner JM, Redwood DR, et al. Coronary artery narrowing in coronary heart disease: comparison of cineangiographic and necropsy findings. Ann Intern Med 1979;91:350–6.
11. Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491–7.
12. Treasure CB, Manoukian SV, Klein JL, et al. Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. Circ Res 1992;71:776–81.
13. Drexler H, Zeiher AM. Endothelial function in human coronary arteries in vivo: focus on hypercholesterolemia. Hypertension 1991;18 Suppl II:II-90–9.
14. Brush JE Jr, Faxon DP, Salmon S, Jacobs AK, Ryan TJ. Abnormal endothelium-dependent coronary vasomotion in hypertensive patients. J Am Coll Cardiol 1991;19:809–15.
15. Kuhn FE, Mohler ER, Satler LF, Reagan K, Lu DY, Rackley CE. Effects of high-density lipoprotein on acetylcholine-induced coronary vasoreactivity. Am J Cardiol 1991;68:1425–30.
16. Vita JA, Treasure CB, Yeung AC, et al. Patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. Circulation 1992;85:1390–7.
17. McPherson DD, Hiratzka LF, Lamberth WC, et al. Delineation of the extent of coronary atherosclerosis by high-frequency epicardial echocardiography. N Engl J Med 1987;316:304–9.
18. Pinto FJ, St. Goar FG, Fischell TA, et al. Nitroglycerin-induced coronary vasodilation in cardiac transplant recipients: evaluation with in vivo intracoronary ultrasound. Circulation 1992;85:69–77.
19. Tobis JM, Mallery J, Mahon D, et al. Intravascular ultrasound imaging of human coronary arteries in vivo: analysis of tissue characterizations with comparison to in vitro histological specimens. Circulation 1991;83:913–26.
20. Ganz W, Tamura K, Marcus HS, Donoso R, Yoshida S, Swan HJC. Measurement of coronary sinus blood flow by continuous thermodilution in man. Circulation 1971;44:181–95.
21. Lam JYT, Chesebro JH, Steele PM, Badimon L, Fuster V. Is vasospasm related to platelet deposition? Relationship in a porcine preparation of arterial injury in vivo. Circulation 1987;75:243–8.
22. Steele PM, Chesebro JH, Stanson AW, et al. Balloon angioplasty: natural history of the pathophysiologic response to injury in a pig model. Circ Res 1985;57:105–12.
23. El-Tamimi H, Mansour M, Wargovich TJ, et al. Constrictor and dilator responses to intracoronary acetylcholine in adjacent segments of the same coronary artery in patients with coronary artery disease: endothelial function revisited. Circulation 1994;89:45–51.
24. Nishimura RA, Edwards WD, Warnes CA, et al. Intravascular ultrasonic imaging: in vitro validation and pathologic correlation. J Am Coll Cardiol 1990;16:145–54.
25. Gussenhoven EJ, Essed CE, Lancée CT, et al. Arterial wall characteristics determined by intravascular ultrasound imaging: an in vitro study. J Am Coll Cardiol 1989;14:947–52.
26. Fitzgerald PJ, St. Goar FG, Connolly AJ, et al. Intravascular ultrasound imaging of the coronary arteries: is three layers the norm? Circulation 1992;86:154–8.
27. Anderson TJ, Meredith IT, Uehata A, et al. Functional significance of intimal thickening as detected by intravascular ultrasound early and late after cardiac transplantation. Circulation 1993;88:1093–100.
28. Drexler H, Fischell TA, Pinto FJ, et al. Effect of L-arginine on coronary endothelial function in cardiac transplant recipients: relation to vessel wall morphology. Circulation 1994;89:1615–23.
29. Yeung AC, Vekshtein VI, Krantz DS, et al. The effect of atherosclerosis on the vasomotor response of coronary arteries to mental stress. N Engl J Med 1991;325:1551–6.
30. Horio Y, Yasue H, Rokutanda M, et al. Effects of intracoronary injection of acetylcholine on coronary arterial diameter. Am J Cardiol 1986;57:984–9.
31. Linder L, Kiowski W, Bühler FR, Lüscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo: blunted response in essential hypertension. Circulation 1990;81:1762–7.
32. Hodgson J McB, Marshall JJ. Direct vasoconstriction and endothelium-dependent vasodilation: mechanisms of acetylcholine effects on coronary flow and arterial diameter in patients with nonstenotic coronary arteries. Circulation 1989;79:1043–51.
33. Fish RD, Nabel EG, Selwyn AP, et al. Responses of coronary arteries of cardiac transplant patients to acetylcholine. J Clin Invest 1988;81:21–31.
34. Verbeuron TJ, Jordaens FH, Zonnekeyn LL, Van Hove CE, Coene M-C, Herman AG. Effect of hypercholesterolemia on vascular reactivity in rabbit: I. Endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits. Circ Res 1986;58:552–64.
35. Jayakody L, Senaratne M, Thomson A, Kappagoda T. Endothelium-dependent relaxation in experimental atherosclerosis in the rabbit. Circ Res 1987;60:251–64.
36. Andrews HE, Bruckdorfer KR, Dunn RC, Jacobs M. Low-density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. Nature 1987;327:237–9.
37. Kanamuru K, Waga S, Tochio H, Nagatani K. The effect of atherosclerosis on endothelium-dependent relaxation in the aorta and intracranial arteries of rabbits. J Neurosurg 1989;70:793–8.
38. Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 1986;8:344–8.
39. Yamamoto H. Preserved endothelial function in the spastic segment of the human epicardial coronary artery in patients with variant angina—role of substance P in evaluating endothelial function. Eur Heart J 1993;14 Suppl I:118–22.

40. Vrints CJM, Bult H, Hitter E, Herman AG, Snoeck JP. Impaired endothelium-dependent cholinergic coronary vasodilation in patients with angina and normal coronary arteriograms. J Am Coll Cardiol 1992;19:21–31.
41. Kessler KM. Syndrome X: the epicardial view [editorial comment]. J Am Coll Cardiol 1992;19:32–3.
42. Okumura K, Yasue H, Matsuyama K, et al. Effect of acetylcholine on the highly stenotic coronary artery: difference between the constrictor response of the infarct-related coronary artery and that of the noninfarct-related artery. J Am Coll Cardiol 1992;19:752–8.
43. McLenachan JM, Vita J, Fish RD, et al. Early evidence of endothelial vasodilator dysfunction at coronary branch points. Circulation 1990;82: 1169–73.
44. Velican C, Velican D. Study of coronary intimal thickening. Atherosclerosis 1985;56:331–44.