

Coronary Vasomotor Response to Acetylcholine Relates to Risk Factors for Coronary Artery Disease

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In animals, acetylcholine dilates normal arteries and produces vasoconstriction in the presence of hypercholesterolemia, hypertension, or atherosclerosis, reflecting endothelial cell dysfunction. In patients with angiographically smooth coronary arteries, acetylcholine has been reported to produce both vasodilation and constriction. To test the hypothesis that the acetylcholine response relates to risk factors for coronary artery disease, acetylcholine 10^{-8} to 10^{-6} M was infused into the left anterior descending or circumflex coronary artery, and diameter changes were assessed with quantitative angiography in 34 patients with angiographically smooth coronary arteries. The acetylcholine response ranged from +37% (dilation) to -53% (constriction) at the peak acetylcholine dose. All coronary arteries dilated in response to nitroglycerin ($26 \pm 17\%$), suggesting an abnormality of endothelial function in the patients with a constrictor response to acetylcholine. By multiple stepwise regression analysis, serum cholesterol ($p < 0.01$), male gender ($p < 0.001$), family history ($p < 0.05$), age ($p < 0.05$), cholesterol level ($p < 0.01$), and total number of risk factors ($p < 0.0001$) were independently associated with the acetylcholine response. Thus, coronary risk factors are associated with loss of endothelium-dependent vasodilation. The development of vasoconstriction is likely to be an abnormality of endothelial function that precedes atherosclerosis or an early marker of atherosclerosis not detectable by angiography. (*Circulation* 1990;81:491-497)

In animals, acetylcholine produces endothelium-dependent dilation of normal arteries and vasoconstriction in pathologic states such as hyper-

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cholesterolemia, hypertension, or atherosclerosis.¹⁻⁹ Clinical studies have shown that intracoronary acetylcholine infusion constricts atherosclerotic coronary arteries.⁹⁻¹¹ Smooth segments may also constrict in patients with coronary artery disease evident in

other vessels, possibly reflecting early atherosclerosis.¹¹ In patients with angiographically "normal" coronary arteries, the vasomotor response to intracoronary acetylcholine is heterogeneous: both dilation and constriction have been reported.¹⁰⁻¹³

This study examines the hypothesis that coronary risk factors disturb endothelial function and may thereby influence the response to intracoronary acetylcholine in patients with angiographically smooth coronary arteries.

Methods

Patients

Prior studies have shown that coronary arteries with luminal irregularities or significant stenoses constrict in response to intracoronary acetylcholine infusion.^{10,11,13,14} To examine the relation between coronary risk factors and the acetylcholine response while avoiding the dominant effects of angiographic evidence of atherosclerosis, only patients with smooth coronary arteries were included in this study.

Between January 1985 and April 1989, the response to intracoronary acetylcholine was studied in 106 patients. Only 34 of these patients had angio-

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Supported in part by Grant RO1-HL-35295 and Training Grant 1T32-HL-07604 from the National Institutes of Health. R.D.F. is a recipient of a Physician-Scientist Award (HL-09157) from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland.

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Received May 31, 1989; revision accepted October 24, 1989.

graphically normal coronary arteries. These patients had been referred by their primary cardiologist for cardiac catheterization to exclude coronary artery disease. All had chest pain that was atypical for myocardial ischemia and a negative or nondiagnostic exercise tolerance test. No patient had a clinical history suggestive of variant angina. In each case, angiography revealed entirely smooth coronary arteries, without stenoses or even minimal luminal irregularities. In 32 patients, left ventricular function was normal by ventriculography (mean ejection fraction, $70 \pm 12\%$) or echocardiography. In two patients, there was global dysfunction (ejection fraction, 29% and 31%) and normal hemodynamics. The peak response to intracoronary acetylcholine was previously reported in 11 of these patients.^{10,14,15}

Definition of Coronary Risk Factors

The following risk factors for coronary artery disease were assessed by history and chart review.

Cholesterol. The total serum cholesterol level was measured in the hospital clinical chemistry laboratory with an enzymatic-colorimetric method (SMAC System, Technicon, Terrytown, New York) on a sample obtained the day before catheterization. No patient had a documented familial hyperlipidemia. No patients were taking cholesterol-lowering agents at the time of catheterization.

Hypertension. Patients with a history of elevated blood pressure that resulted in the initiation of antihypertensive therapy by the primary physician were considered to have a positive history of hypertension. The control blood pressure in a drug-free state in the catheterization laboratory was also recorded.

Cigarette smoking. Patients were classified as non-smokers if they had never smoked or had stopped smoking more than 3 months before study. All other patients were classified as smokers.

Family history. Family history was considered to be positive if a parent or sibling of the patient had documented clinical evidence of coronary artery disease before 60 years of age, such as a history of myocardial infarction, coronary artery bypass surgery, angina pectoris, exercise tolerance test diagnostic of ischemia, sudden cardiac death, or documented coronary artery disease by angiography.

Diabetes mellitus. None of the patients had a history of insulin-dependent diabetes mellitus or elevated blood glucose.

Protocol

The vasomotor response to intracoronary acetylcholine was studied using a previously described protocol.^{10,14,16} Informed consent was obtained in accordance with the requirements of the Committee for the Protection of Human Subjects from Research Risks at Brigham and Women's Hospital and University of Michigan Hospital. Vasoactive medications including calcium channel blockers, β -adrenergic-receptor blockers, angiotensin converting enzyme

inhibitors, and long-acting nitrates were withheld 18–24 hours before cardiac catheterization.

After completion of the diagnostic study, an additional dose of heparin 5,000 units was given intravenously (for a total of 10,000 units). An 8F standard angioplasty guiding catheter was positioned in the ostium of the left main coronary artery. Through the guide, a 2.5F infusion catheter was positioned in the proximal left anterior descending or circumflex coronary artery (one patient).

Serial infusions at 0.8 ml/min were made selectively into the coronary artery in the following sequence using an infusion pump (Harvard Apparatus, South Natick, Massachusetts): 1) 2-minute control infusion (5% dextrose in sterile water); 2) three 2-minute acetylcholine infusions with a final estimated intracoronary concentration of 10^{-8} to 10^{-6} M, assuming coronary blood flow to be 80 ml/min¹⁷; 3) 5-minute repeat control infusion; 4) nitroglycerin infusion of 40 μ g during 2.5 minutes. Throughout the protocol, heart rate, central aortic pressure, and the electrocardiogram (lead I) were monitored continuously. If marked coronary constriction was observed during acetylcholine infusion, the higher doses of acetylcholine were omitted.

Quantitative Angiography

At the end of each infusion, biplane quantitative coronary angiography was performed with a previously validated technique^{16,18,19}: Nonionic contrast (Omnipaque 350, Wintrop Breon Laboratories, New York, New York) was injected into the left coronary artery at 5–7 ml/sec for a total of 8–10 ml with a power injector (Medrad, Pittsburgh, Pennsylvania) to standardize vessel opacity. The biplane system (Polydiagnost-C, Phillips Medical Systems, Shelton, Connecticut) was set up to position the studied vessel in the center of each field of view and at a single position in space (isocenter).

Vessel morphology was judged by two experienced cardiologists unaware of patient identity, coronary risk factors, and vasomotor responses. Patients with angiographically smooth coronary arteries were included in the study. Technically suitable single-plane angiograms were selected from the biplane views for analysis. In each patient, two or three available segments in the middle to distal vessels, 10–25 mm in length, were analyzed. Each segment was centered and the single-frame cineimage was digitized (20–40 μ m/pixel) with the use of a video camera (Cohu, San Diego, California) connected to a video interface (Recognition Concepts, Incline Village, Nevada) and a Microvax II computer (Digital Equipment, Maynard, Massachusetts). Two line profile averaging was used to minimize anatomic noise, and 16 video images were summed to minimize video noise. Four cineframes in end diastole were scanned and averaged, with two anatomic features used to ensure accurate alignment. Calibrated grids in the field of view were used to scale the data from pixels to millimeters. A series of measurements of diameter

were recorded for the length of the arterial segment. Two anatomic features were used to reproduce the segment of interest after each drug infusion and to assess serial changes in vessel diameter. The 2-mm segment that showed the greatest mean diameter change at peak acetylcholine (either dilation or constriction) was chosen, and the response of this segment was used for calculation of the acetylcholine response of the patient. In each patient, the response of the 2-mm segment had the same directional change as the entire 10–25-mm segment. In five patients, a 6–8-mm segment distal to the catheter infusion site was analyzed. In these patients, the changes in vessel diameter were determined with the method described and validated by Mancini et al.¹⁹

Data Analysis

The vasomotor response to intracoronary acetylcholine infusion was analyzed in two ways and yielded similar results. First, the slope of the dose-response relation to acetylcholine was examined to correct for the fact that a few patients did not receive all three doses of acetylcholine. In the dose range of acetylcholine used in this study, a linear relation occurred between acetylcholine dose and the percent change in vessel diameter. Because of marked constriction at the lower acetylcholine doses, the 10^{-6} M dose was omitted in three patients. In five patients, the 10^{-8} M dose was omitted to limit the angiographic contrast load. Using linear regression, the slope of the acetylcholine dose-response relation (% change in coronary diameter/log [acetylcholine]) was calculated from the available doses for each patient, and this parameter was related to the presence of coronary risk factors. A positive slope (increasing diameter with increasing acetylcholine dose) reflects dilation, and a negative slope (decreasing diameter with increasing acetylcholine dose) reflects constriction of the coronary segment.

Second, the analysis was performed with the response to a single acetylcholine dose. Thirty-three patients received acetylcholine 10^{-7} M, and the relation between coronary risk factors and the percent diameter change at this dose was examined.

Statistical Analysis

Univariate analysis of the effects of coronary risk factors on the acetylcholine response was performed with linear regression for continuous variables (cholesterol level, age, blood pressure at time of study, diameter response to nitroglycerin, and baseline diameter), and one-way analysis of variance was performed for categorical variables (history of hypertension, family history, smoking, and sex). The interaction between risk factors and acetylcholine response was then examined using multiple step-wise regression analysis.

The number of coronary risk factors was considered a continuous variable and compared with the acetylcholine response by linear regression analysis. To calculate the number of risk factors, cholesterol level greater than 210 mg/dl, age greater than 40 years, male sex, positive family history of coronary

disease, hypertension, and cigarette smoking were considered to be risk factors.

The nitroglycerin response and response after repeat control infusion were compared with control responses using the paired *t* test.

The standard deviation of the slope of the acetylcholine dose-response relation was calculated for each patient, and the mean standard deviation for all patients was calculated to assess the variability of these slopes.

Statistical significance was assumed when the null hypothesis could be rejected at the 0.05 probability level. All values are expressed as mean \pm SD.

Results

Hemodynamic Response

Acetylcholine had no effect on heart rate, systemic arterial pressure, or the electrocardiogram in any patient. No patient developed chest pain or any other adverse effects. Resting left ventricular filling pressure (pulmonary capillary wedge pressure or left ventricular end-diastolic pressure) was measured in 23 patients and ranged from 4 to 19 mm Hg (mean, 10 ± 4 mm Hg). Linear regression analysis showed that the acetylcholine response was independent of left ventricular filling pressure ($r=0.18$, $p=NS$).

Diameter Responses

Acetylcholine infusion produced segmental changes in coronary diameter, with marked changes in some regions of the vessel and minimal change in others. However, no patient had both marked constriction and dilation within the same vessel. For the entire group of 34 patients, the maximum diameter response ranged from +37% (dilation) to -53% (constriction). The slope of the dose-response curve to acetylcholine in the maximally responding segment ranged from +13% to -25% change in diameter/log [acetylcholine] (mean, $-4.3 \pm 10.8\%$) (Table 1). The mean of the standard deviations of the slopes was $3.4 \pm 1.7\%$ change in diameter/log [acetylcholine] indicating good fit for the calculated regression lines used for the slope calculation.

The baseline diameter was 2.04 ± 0.89 mm. Nitroglycerin dilated all segments ($26 \pm 17\%$), suggesting preserved function of vascular smooth muscle and an abnormality of endothelial function in the patients with a constrictor response to acetylcholine. Univariate and multiple stepwise regression analysis revealed that the acetylcholine response was independent of the response to nitroglycerin ($r=0.01$, $p=NS$) and of the baseline diameter ($r=0.20$, $p=NS$) also suggesting that the response to acetylcholine was not primarily influenced by intrinsic disease of the vascular smooth muscle or by basal tone.

Risk Factors

The mean age was 43 years (range, 17–72 years). There were 24 men and 10 women. The mean cholesterol level was 213 ± 48 mg/dl (range, 124–322 mg/dl). The mean blood pressure at catheteriza-

TABLE 1. Coronary Risk Factors and Acetylcholine Response

Patient	Age (yr)	Gender	Serum cholesterol level (mg/dl)	Hypertension	Smoking	Family history	Coronary risk factors (n)	Acetylcholine response
1	45	M	167	—	+	+	3	13
2	32	F	179	—	+	—	1	11
3	41	M	169	—	—	—	2	10
4	30	M	169	—	—	—	1	9
5	34	M	180	—	—	—	1	8
6	17	M	126	—	—	—	1	6
7	37	M	219	—	+	—	3	6
8	30	F	211	—	—	+	2	6
9	46	F	170	—	—	—	1	5
10	57	F	160	—	—	—	2	4
11	43	F	179	—	—	—	2	4
12	35	M	207	—	+	+	2	4
13	56	F	252	—	—	—	2	4
14	39	M	124	—	+	—	2	3
15	19	M	209	—	+	—	2	3
16	47	M	200	+	—	—	3	1
17	72	F	298	+	—	+	4	−6
18	27	M	240	—	+	—	3	−9
19	47	M	227	—	+	—	5	−9
20	31	M	259	—	—	—	2	−10
21	42	M	206	+	—	—	3	−10
22	56	F	243	+	—	+	4	−10
23	45	M	167	+	—	+	4	−11
24	47	M	162	—	—	+	3	−11
25	44	M	322	—	+	—	4	−12
26	44	M	250	+	+	+	6	−12
27	56	M	310	+	+	+	6	−14
28	47	M	239	—	+	—	4	−14
29	48	M	218	—	+	+	5	−15
30	46	M	209	+	—	—	3	−17
31	40	M	220	—	—	—	4	−18
32	54	F	252	+	—	+	4	−19
33	56	M	280	—	—	—	3	−22
34	55	M	227	+	—	+	5	−25

Acetylcholine response, slope of the diameter response to intracoronary acetylcholine infusion (% change in coronary diameter/log[acetylcholine]): positive slope indicates dilation, negative slope indicates constriction (see text); +, present; —, absent.

tion was 94 ± 13 mm Hg. Ten patients had a history of hypertension requiring antihypertensive therapy. Twelve had smoked cigarettes within 3 months of the study. Thirteen had a positive family history of coronary artery disease (Table 1).

Univariate analysis revealed a negative correlation between the acetylcholine response and serum cholesterol level ($r = -0.58$, $p = 0.0003$) (Figure 1), and age ($r = -0.44$, $p = 0.01$) (Figure 2). Patients with a history of hypertension constricted more (slope, $-12.3 \pm 7.2\%$) than nonhypertensive patients (slope, $-1.0 \pm 10.4\%$) ($p = 0.004$). Patients with a positive family history of coronary disease constricted more (slope, $-9.2 \pm 10.7\%$) than patients without this coronary risk factor (slope, $-1.3 \pm 9.9\%$) ($p = 0.04$). The

acetylcholine response did not correlate with baseline blood pressure ($r = 0.16$, $p = 0.39$). There was no difference in the responses for cigarette smokers (slope, $-4.0 \pm 10.6\%$) and nonsmokers (slope, -4.5 ± 11.1) ($p = 0.90$). By univariate analysis, the difference in acetylcholine response for men (slope, $-6.6 \pm 10.4\%$) and women (slope, $1.2 \pm 9.9\%$) approached significance ($p = 0.053$).

However, stepwise multiple regression analysis revealed a negative correlation between the acetylcholine response and serum cholesterol level ($p < 0.01$), male gender ($p < 0.001$), positive family history ($p < 0.05$), and age ($p < 0.05$). For the final regression model, including these four coronary risk factors, $r^2 = 0.63$. The strongest single predictor of the

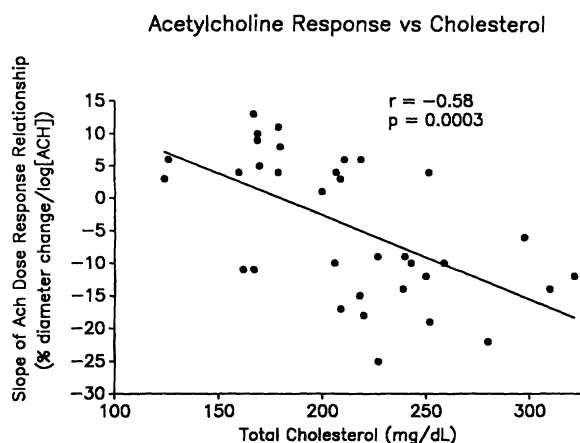


FIGURE 1. Plot of the relation between the vasomotor response to acetylcholine and serum cholesterol level. The acetylcholine response is expressed as the slope of the dose-response relation (% change in coronary diameter/log [acetylcholine]), where a positive slope reflects dilation and a negative slope reflects constriction. By univariate and multiple regression analysis, a significant negative correlation occurred between the acetylcholine response and serum cholesterol level.

acetylcholine response was the number of risk factors for coronary artery disease ($r = -0.73$, $p < 0.0001$), which ranged from 1 to 6 (mean, 2.9 ± 1.5) (Figure 3). Smoking and history of hypertension were not independent predictors of the acetylcholine response.

When the diameter response to the single acetylcholine dose of 10^{-7} M was examined (rather than the whole dose response), univariate analysis revealed a significant relation between the acetylcholine response and number of coronary risk factors ($r = -0.63$, $p = 0.0001$).

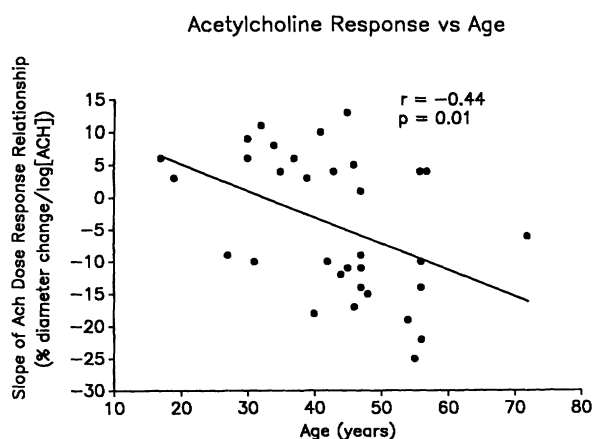


FIGURE 2. Plot of the relation between the vasomotor response to acetylcholine and age. The acetylcholine response is expressed as the slope of the dose-response relation (% change in coronary diameter/log [acetylcholine]), where a positive slope reflects dilation and a negative slope reflects constriction. By univariate and multiple regression analysis, a significant negative correlation occurred between the acetylcholine response and age.

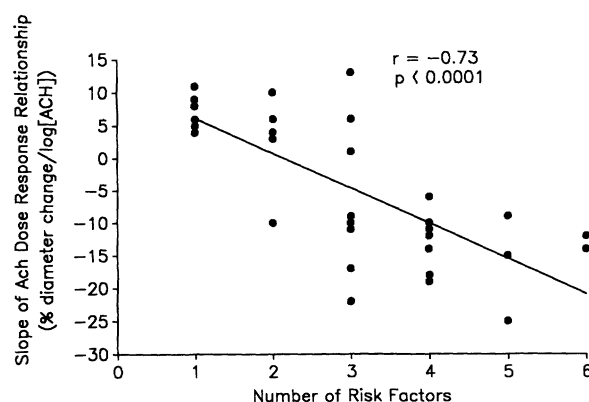


FIGURE 3. Plot of the relation between the vasomotor response to acetylcholine and the number of coronary risk factors. The acetylcholine response is expressed as the slope of the dose-response relation (% change in coronary diameter/log [acetylcholine]), where a positive slope reflects dilation and a negative slope reflects constriction. By univariate analysis, a significant negative correlation occurred between the acetylcholine response and the number of coronary risk factors.

The analysis was repeated after excluding the two patients with a decreased left ventricular ejection fraction, and the same significant relation between number of risk factors and acetylcholine response was observed ($r = -0.71$, $p < 0.0001$).

Discussion

This study showed that there is a significant relation between coronary risk factors and the vasomotor response to intracoronary acetylcholine infusion in patients with angiographically smooth coronary arteries. In this group of 34 patients, the response was heterogeneous and ranged from 37% dilation to 53% constriction. All vessels dilated in response to intracoronary nitroglycerin, a direct smooth muscle dilator, suggesting a disturbance of endothelial cell function in the constricting vessels. A constrictor response to acetylcholine was independently associated with increasing serum cholesterol, male gender, positive family history of coronary artery disease, and patient age. The number of risk factors for coronary artery disease was the best predictor of the response to intracoronary acetylcholine.

In animals, a number of pharmacologic agents, including acetylcholine, dilate normal arteries by stimulating the release of endothelium-derived relaxant factor(s) (EDRF).¹⁻⁹ Acetylcholine also constricts vascular smooth muscle, and the net acetylcholine response is believed to result from these two opposing actions.¹ Mechanical denudation and pathologic states affecting the endothelium impair the vasodilator response or convert it to a vasoconstrictor response.¹⁻⁹ Loss of the vasodilator response to acetylcholine has been shown in animal models of atherosclerosis and in atherosclerotic human coronary arteries studied in vitro.⁶⁻⁹ Sreeharan et al²⁰ suggested that the mechanism of impaired vasodilator function in atherosclerosis is failure of EDRF

release rather than impaired diffusion of EDRF or hypersensitivity of vascular smooth muscle to the direct effects of acetylcholine.

Coronary risk factors are known to impair endothelium-dependent vasodilation in experimental studies. Cholesterol-fed animals develop selective attenuation of endothelium-dependent vasodilation before and after developing histologic evidence of frank atherosclerosis.^{2,3,6-9} Even a brief incubation (30 minutes) of rabbit aorta with low-density lipoprotein (LDL) inhibits the vasodilator response to acetylcholine.² However, the serum level of cholesterol in these studies far exceeds those usually observed in patients. In animals, chronic and acute elevation of blood pressure disturbs endothelium-dependent vasodilation.^{4,5} Age may also be a factor because older rats have an impaired acetylcholine dilator response when compared with younger animals.²¹

Hodgson, using methylene blue (an inhibitor of the action of EDRF) suggested that acetylcholine is capable of releasing EDRF in humans.²² In clinical studies, intracoronary acetylcholine infusion constricts coronary arteries when there is angiographic evidence of atherosclerosis in the studied vessel.¹⁰⁻¹² However, controversy exists regarding the response of "normal" coronary arteries.^{10,11,13} Furchgott¹ described the endothelium-dependent vasomotor response of arteries to acetylcholine *in vitro* throughout a specific dose range. Variation in dose and rate of acetylcholine infusion may account in part for the reported heterogeneous response in patients.^{10,11,13} In addition, classification of patients with mild, nonocclusive coronary disease as normal may also explain the variation in observed response.¹³ Finally, angiography is not a sensitive method of detecting early atherosclerosis or diffuse involvement of an entire vessel with disease.^{23,24} Undetected atherosclerosis in angiographically smooth vessels may account for the constrictor response observed when there are stenoses in adjacent coronary arteries.^{11,23,24} For similar reasons, intracoronary acetylcholine has been suggested as a tool for early detection of the diffuse coronary atherosclerosis that develops in heart transplant recipients.¹⁶

Because coronary risk factors are associated with endothelial dysfunction and a constrictor response to acetylcholine in experimental studies, it seemed possible that known coronary risk factors would relate to the vasomotor response in patients. The Framingham Study identified total serum cholesterol level, hypertension, and cigarette smoking as primary risk factors.²⁵⁻²⁷ Family history of coronary artery disease is a significant risk factor, although clustering of other risk factors within families may in part explain the correlation.²⁸ Male sex, increasing age, and insulin-dependent diabetes are also well-established risk factors.^{25,27} These associations can be used to predict which individuals are at increased risk for the development of coronary atherosclerosis, and recent studies have concentrated on ways to modify the risk for the individual patient.^{25,27}

In this study, an abnormal response to the endothelium-dependent vasodilator acetylcholine was observed in patients with increasing coronary risk factors but with angiographically smooth coronary arteries. Experimental evidence suggests that several risk factors can directly alter endothelial cell vasodilator function, without histologic evidence of atherosclerosis.^{2,4,21} The presence of focal, early, or a diffuse form of atherosclerosis that is angiographically undetectable would also explain this finding. Thus, vasoconstriction in these patients possibly reflects an abnormality of endothelial function that precedes or is a predisposing factor for the development of atherosclerosis.

It is important to emphasize that despite the strong overall correlation between risk factors and a constrictor response to acetylcholine, the responses to acetylcholine were variable in adjacent segments within the same vessel exposed to the same risk factors. It is likely that as yet undefined local factors in the vessel wall such as variation in rates of healing after injury or variation in local shear stress may play important roles in modifying local endothelial function.^{27,29,30} This latter concept is supported by the recent observation that constrictor responses to acetylcholine are most marked at arterial branch points.²⁹

Further, there was important variability in the response to acetylcholine among patients with the same number of coronary risk factors, particularly in patients in the intermediate range (Figure 3). Thus, despite the overall correlation, there appears to be variation in the susceptibility of the individual patients to these coronary risk factors. In cardiac transplant patients, a constrictor response to acetylcholine predicted the subsequent development of diffuse coronary atherosclerosis.¹⁶ In patients with coronary artery disease, the acetylcholine response possibly relates to the susceptibility of the endothelium and vessel wall to the prevailing risk factors.

Limitations of the Study

A limitation of this study is the relatively small number of patients studied compared with epidemiologic trials such as the Framingham Study. It is possible that a significant independent correlation between the acetylcholine response and smoking and history of hypertension would be shown with a larger sample size. Despite this problem, highly significant relations were observed between the acetylcholine response and the other risk factors.

We cannot exclude the unlikely possibility that use of low osmolar contrast medium resulted in damage to the endothelium in patients with coronary risk factors but not in those without.

Conclusions

Patients with angiographically smooth coronary arteries and few risk factors for coronary disease tend to have coronary vasodilation in response to intracoronary acetylcholine infusion, whereas patients with the same apparently "normal" coronary arteries and

increased risk factors show segmental coronary vasoconstriction. The preserved response to the direct smooth muscle vasodilator nitroglycerin suggests a disturbance of endothelial function in the constricting segments. In patients undergoing cardiac catheterization, the response to intracoronary acetylcholine may provide incremental information about coronary function not provided by the standard arteriogram. A constrictor response to acetylcholine may reflect early atherosclerosis at a stage not detectable by angiography or a disturbance of endothelial function that precedes the development of atherosclerosis.

Acknowledgments

We acknowledge the statistical advice of John Orav, PhD, Harvard School of Public Health. We thank the nursing and technical staff of the Brigham and Women's Hospital Cardiac Catheterization Laboratory for their expert help in conducting these protocols. We also thank George Rebecca, MD, for his assistance in completing this study.

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KEY WORDS • endothelial cell function • acetylcholine