Responses of Angiographically Normal Human Coronary Arteries to Intracoronary Injection of Acetylcholine by Age and Segment Possible Role of Early Coronary Atherosclerosis

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We examined the response of left coronary arteries to intracoronary injection of acetylcholine (ACh) 50 \( \mu \)g in 74 patients by measuring the diameter changes with a videodensitometric analysis system. Patients with angiographically normal coronary arteries were subdivided into a younger group of 26 patients (age, 9–29 years) and an older group of 23 patients (age, 31–68 years). In the younger group, the diameter at the distal segment of the left anterior descending artery (LAD) and at the proximal, middle, and distal segments of the left circumflex artery (LCx) increased significantly (16.7±19.3%, \( p<0.01 \), for LAD and 8.0±18.8%, \( p<0.05 \); 11.0±16.1%, \( p<0.01 \); and 19.8±17.5%, \( p<0.01 \), for LCx segments, respectively) in response to ACh. In the older group, on the other hand, the diameter at the proximal and middle segments of LAD and LCx decreased significantly (−20.8±16.9%, \( p<0.01 \); and −17.9±28.4%, \( p<0.01 \), for LAD segments and −14.6±17.4%, \( p<0.01 \); and −11.3±21.4%, \( p<0.05 \), for LCx segments, respectively). The dilator response to ACh in the younger group was significantly greater in the distal segment than in the proximal segment in both LAD and LCx (\( p<0.01 \) for LAD and \( p<0.05 \) for LCx). The constrictor response to ACh in the older group was significantly greater in the proximal than the distal segment in both LAD and LCx (\( p<0.05 \) for LAD and LCx, respectively). Nearly all coronary arteries with angiographically evident atherosclerosis constricted to ACh in 25 patients. We conclude that most of the angiographically normal coronary arteries in subjects more than 30 years old have endothelial dysfunction or atherosclerosis and that the proximal segment is more prone to atherosclerosis than the distal segment. (Circulation 1990;81:482–490)

Coronary spasm plays an important role in the pathogenesis of not only variant angina but also other forms of angina, acute myocardial infarction, and sudden death.\(^1\)\(^-\)\(^4\) However, precise mechanism(s) by which coronary spasm occurs remains unknown. We and others have shown that coronary spasm can be induced in most patients with variant angina by intracoronary injection of acetylcholine (ACh).\(^5\)\(^-\)\(^7\) We also have shown that intracoronary injection of ACh causes constriction of coronary arteries in the majority of adult humans.\(^8\)

However, ACh has been shown to cause endothelium-dependent relaxation in normal vessels of most species.\(^9\)\(^,10\) Previous investigators reported that isolated human coronary arteries obtained from hearts of transplant recipients\(^11\) or cadavers\(^12\) constrict in response to ACh and emphasized that the human coronary artery is unlike that of other mammals and lacks the phenomenon of muscarinic endothelium-dependent relaxation. More recent study, however, demonstrated that the isolated human coronary artery, like that of other large mammals, relaxes in response to ACh via an endothelium-dependent mechanism and that the muscarinic endothelium-dependent relaxation is impaired in atherosclerotic arteries.\(^13\)

Recently, Ludmer and coworkers\(^14\) reported that angiographically normal coronary arteries dilate, whereas evidently atherosclerotic coronary arteries
with luminal irregularities or stenoses constrict in response to the intracoronary infusion of ACh; they concluded that atherosclerotic coronary arteries paradoxically constrict to ACh. Atherosclerosis, however, may be present in the coronary arteries that appear normal angiographically. Recently, Werns and coworkers reported that angiographically normal coronary arteries of patients with coronary artery disease (CAD) constrict in response to intracoronary injection of ACh. Coronary atherosclerosis is known to begin in childhood and progress with increasing age. Atherosclerosis also is known to occur more frequently in the proximal than the distal segments of coronary arteries.

We designed the present study to examine whether there is any difference of response of the angiographically normal coronary arteries to intracoronary injection of ACh between younger and older subjects and between the proximal and distal segments. We also compared the response of the evidently atherosclerotic coronary arteries to ACh with that of angiographically normal coronary arteries in patients who had one or two major coronary arteries evidently involved in atherosclerosis.

**Methods**

**Study Patients**

We studied 74 patients who underwent diagnostic catheterization. These patients were divided into a normal coronary artery group and a CAD group based on the coronary angiographic findings after administration of nitroglycerin. All three major coronary arteries were smooth and normal in the former group, whereas either one or two of the three major coronary arteries had either minimal irregularities (less than 25% narrowing of the luminal diameter) or evidently stenotic lesions (more than 25% narrowing of the luminal diameter) in the latter group. The administration of nitroglycerin did not alter the classification of any arteries. The classification decisions were determined by a consensus of three investigators.

**Normal coronary artery group.** Forty-nine patients had angiographically normal smooth coronary arteries. These patients were subdivided further into a younger group and an older group according to whether they were less than or more than 30 years old. The younger group consisted of 26 patients (15 men and 11 women; age range, 9–29 years; mean age, 18.7 years). Nine had congenital heart disease, six had valvular heart disease, four had cardiomyopathy, three had ventricular arrhythmias, three had atypical chest pain, and one had myocarditis. In three patients with atypical chest pain, the possibility of Kawasaki disease could not be excluded. The older group consisted of 23 patients (nine men and 14 women; age range, 31–68 years; mean age, 51.6 years). Fifteen of them had atypical chest pain, three had ventricular arrhythmias, two had cardiomyopathy, two had valvular heart disease, and one had atrial fibrillation. Patients with a clinical history suggestive of coronary spastic angina were excluded from the study.

**CAD group.** This group consisted of 25 patients (20 men and five women; age range, 35–67 years; mean age, 55.7 years) who had atherosclerotic lesions in the left coronary artery. Eleven of them (seven men and four women) had luminal irregularities in the left coronary arteries. Eight of them had lesions in the left anterior descending artery (LAD), two in the left circumflex artery (LCx), and one in both arteries. Eight of them had atypical chest pain, two had cardiomyopathy, and one had an arrhythmia. Patients with a history suggestive of coronary spastic angina were excluded from the study.

Fourteen patients (13 men and one woman) in this group had 25–90% luminal diameter narrowings in the left coronary arteries. Six of them had lesions in the LAD, four in the LCx, and four in both arteries. Eleven of the 14 patients had stable exertional angina, two had old myocardial infarction, and one had hypertrophic cardiomyopathy. Patients with unstable angina or recent myocardial infarction were excluded from the study.

We also examined risk factors for coronary atherosclerosis in the study subjects. The risk factors included hypercholesterolemia (>240 mg/dl plasma cholesterol), hypertension (>160/90 mm Hg), and tobacco smoking.

This study protocol was approved by the ethics committee at our institution, and a written informed consent was obtained from each patient and his or her family.

**Study Protocol**

All drugs were withdrawn at least 72 hours before cardiac catheterization, except sublingual nitroglycerin, which was withdrawn at least 2 hours before catheterization. After the diagnostic right and left heart catheterization and coronary arteriography were done, a cardiac pacing catheter (USCI tripolar electrode catheter) was positioned in the right ventricle, and pacing rate was set at 5 beats/min above the baseline rate to avoid the possible reflex sympathetic discharge derived from bradycardia and hypotension during injection of ACh in the normal coronary group. In the CAD group, the pacing rate was set in the demand mode at 50 beats/min because ischemic changes on the electrocardiogram that might be induced by ACh injection would not be detected under a constant right ventricular pacing. Control coronary arteriograms of the left coronary artery in the right anterior oblique projection were obtained by injection of 8 ml Urografin 76 (Schering AG, Berlin, Germany) using a Sones catheter. Relations between focal spot, patient, and height of image tube were kept constant. Five milliliters of warmed 0.9% saline or 50 µg ACh chloride (Daichichi Seiyaku, Tokyo, Japan) dissolved in warmed 0.9% saline was injected into the left coronary artery with injection rate of 0.25 ml/sec. After each injection, coronary arteriography was done. Arterial blood pressure and
three electrocardiographic leads (I, aVF, and V₃) were monitored continuously during the procedure. Then, nitroglycerin (0.3 mg) was given sublingually, and coronary arteriograms were obtained.

**Quantitative Coronary Angiography**

We measured the luminal diameters of the coronary artery quantitatively using the cinevideodensitometric analysis system (model XR-70 coronary analyzer, Vanguard Instruments Corp), which has been described and validated in previous studies.²⁷,²⁸ Briefly, the 35-mm cine film was projected with a Vanguard projector into a Panasonic WV-1500 video camera. The RS-170 video signal produced then was digitized and analyzed by the preprogrammed microprocessor system that consisted of a processor, graphics display, output, and video-input printed circuit boards. The images were projected on a 12-in. video screen with twofold magnification and analyzed by positioning regions of interest on either side of the catheter shaft and a segment of the coronary artery. Lines connecting the pairs of regions of interest were microprocessor generated, and videodensity values for the pixels delineated by these lines were measured five times and averaged. The portion of the catheter just proximal to the primary curve was used as a scaling device to yield the absolute diameter in millimeters. The cine frame was selected for analysis on the basis of clear visualization with optimal opacification of the coronary artery at end diastole. Coronary arteries that were inadequately opacified and segments that were overlapped by other structures were not analyzed. After the analysis, a photograph (Polaroid 331) of the video image was taken.

In the normal coronary artery group, the diameters of the proximal, middle, and distal LAD and LCx segments were measured. The proximal, middle, and distal LAD segments and the proximal and middle LCx segments were defined as LAD segments 6–8 and LCx segments 11 and 13 distal to the branching of LCx segment 12 according to the classification of American Heart Association Committee Report.²⁹ The distal LCx segment was defined as segment 14 or 13 distal to the branching of segment 14, depending on the individual variation of the LCx.

In the CAD group, coronary artery diameters were measured at the segments with luminal irregularities or stenoses and at the angiographically normal segments. The diameters of angiographically normal left coronary arteries also were measured at the proximal, middle, and distal segments in this group.

The coronary arteriograms were analyzed by two investigators who were unaware of the identity of the patient or the study protocol and used anatomic references to reproducibly measure the same segment after each injection. Analysis of intraobserver and interobserver variability for the measurement of the coronary diameter showed high reproducibility ($r=0.99$, SEE = 3.4%, $p<0.001$; and $r=0.98$, SEE = 3.8%, $p<0.001$, respectively).

**Statistical Analysis**

Responses of coronary arteries to ACh or nitroglycerin were expressed as percent change of the diameter from control calculated with the following formula:

\[
\text{% change} = 100\% \times \frac{D_b - D_s}{D_b}
\]

where $D_s$ and $D_b$ are the diameters before and after ACh or nitroglycerin, respectively. Paired and unpaired Student’s $t$ tests were used in analysis of the percent changes in the coronary arterial diameter and the changes in blood pressure and heart rate. A linear regression analysis was performed to evaluate the relation between coronary arterial diameter change and age. An analysis of variance with the Bonferroni procedure was used to compare the response to ACh and nitroglycerin between each of the coronary arterial segments.³⁰ Incidence of coronary risk factors was compared using $\chi^2$ test. Intraobserver and interobserver correlations were determined by the linear regression equation. All values were expressed as the mean±SD, and a $p$ value of less than 0.05 was considered statistically significant.

**Results**

**Response to Saline**

Fifteen LAD and 15 LCx were analyzed in 15 patients from the normal coronary artery group (five from the younger group and 10 from the older group). Neither LAD nor LCx diameters changed significantly in any of the coronary segments in response to the intracoronary injection of saline ($-1.4±3.8\%$, $-1.8±4.0\%$, and $1.9±4.8\%$ for the proximal, middle, and distal LAD segments; $-2.2±3.3\%$, $-0.1±4.9\%$, and $-0.9±4.5\%$ for the proximal, middle, and distal LCx segments, respectively).

**Responses to Acetylcholine**

**Normal coronary artery group.** Twenty-six LAD and 26 LCx were analyzed in 26 patients in the younger group, and 22 LAD and 22 LCx were analyzed in 23 patients in the older group. One LAD and one LCx from the older group were excluded because of inadequate opacification of the cine film (see Figure 1). In the younger group, the LAD diameter did not change significantly in the proximal and middle segments ($-2.2±14.9\%$ and $5.9±20.6\%$, respectively) but increased significantly in the distal segment ($16.7±19.3\%$, $p<0.01$) in response to ACh. In the older group, on the other hand, the LAD diameter decreased significantly in the proximal and middle segments ($-20.8±16.9\%$, $p<0.01$; and $-17.9±28.4\%$, $p<0.01$, respectively) but did not change significantly in the distal segment ($-9.7±23.1\%$) in response to ACh. Thus, there were significant differences in the response to ACh in the proximal, middle, and distal LAD segments between the two groups ($p<0.01$ for the proximal, middle, and distal segments, respectively).

In the younger group, the LCx diameter increased significantly to ACh in the proximal, middle,
distal segments (8.0±18.8%, p<0.05; 11.0±16.1%, p<0.01; and 19.8±17.5%, p<0.01, respectively). In contrast, in the older group, the LCx diameter decreased significantly in the proximal and middle segments (−14.6±17.4%, p<0.01; and −11.3±21.4%, p<0.05, respectively) but did not change significantly in the distal segment (−4.8±20.8%) in response to ACh. Thus, there were significant differences in the response to ACh in the proximal, middle, and distal LCx segments between the two groups (p<0.01 for the proximal, middle, and distal segments, respectively).

There were significant negative linear correlations between the percent diameter change of coronary artery in response to ACh and patient’s age at the proximal, middle, and distal LAD and LCx segments (r = −0.4062, p<0.01; r = −0.3912, p<0.01; and r = −0.4728, p<0.01, for proximal, middle, and distal LAD segments; r = −0.4353, p<0.01; r = −0.4490, p<0.01; and r = −0.4437, p<0.01, for the proximal, middle, and distal LCx segments, respectively), as shown in Figure 2. However, the scatter was wide, and the absolute value of correlation coefficient (r) was less than 0.5 at all segments; thus, only a minor portion of the variation in the diameter change could be attributed to its linear regression on the age.

The dilator response to ACh in the younger group was significantly greater in the distal segment than in the proximal LAD and LCx segment (p<0.01 for

![Figure 1](http://circ.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.116.024420)

**Figure 1.** Bar graphs of effects of intracoronary injection of acetylcholine on the coronary artery diameter change from control in patients with angiographically normal coronary arteries. LAD, left anterior descending artery; LCX, left circumflex artery; prox, proximal segment; mid, middle segment; dis, distal segment. *p<0.05 vs. control; **p<0.01 vs. control; ††p<0.01, the younger group vs. the older group. (See text for details.)

![Figure 2](http://circ.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.116.024420)

**Figure 2.** Scatterplots of relations between diameter change in response to acetylcholine and age at the proximal, middle, and distal segments of the left anterior descending coronary artery (LAD) (upper panel) and the left circumflex artery (LCX) (lower panel). Prox-LAD, mid-LAD, and dis-LAD indicate the proximal, middle, and distal segments of the LAD, respectively. Prox-LCX, mid-LCX, and dis-LCX indicate the proximal, middle, and distal segments of the LCX, respectively. The linear lines denote the regression line.
LAD; p<0.05 for LCx) as shown in Figure 3. The proximal LAD segment tended to constrict in response to ACh. The constrictor response to ACh in the older group was significantly greater in the proximal segment than in the distal LAD and LCx segment (p<0.05 for both LAD and LCx) as shown in Figure 3.

CAD group. Seven angiographically normal LAD and 15 angiographically normal LCx were analyzed in 25 patients with CAD (see Figure 4).

The LAD diameter decreased significantly in the proximal and middle segments (−22.1±9.2%, p<0.01; and −17.4±11.3%, p<0.01, respectively) but did not change significantly in the distal segment (−8.9±11.1%) in response to ACh. The LCx diameter decreased significantly in the proximal and middle segments (−14.2±12.1%, p<0.01; and −9.7±13.5%, p<0.05, respectively) but did not change significantly in the distal segment (−2.8±20.6%) in response to ACh.

The constrictor response to ACh was greater in the proximal segment than in both LAD and LCx distal segments (p<0.05 for both LAD and LCx).

There were significant differences in the response to ACh between the younger group and the CAD group in each angiographically normal LAD and LCx segment (p<0.05 for each LAD segment; p<0.01 for each LCx segment). There were, however, no significant differences in the response to ACh between the older group and the CAD group in each angiographically normal LAD and LCx segment.

Twenty-seven arteries with irregularities or stenoses (18 LAD and nine LCx) were analyzed. The segments with irregularities or stenoses in these arteries constricted to ACh (−22.2±20.8%, p<0.01). In one patient, ACh caused a total occlusion of the stenotic lesion of the LCx in association with ST-segment depression on the electrocardiogram. Thirteen angiographically normal proximal or middle segments of these arteries (nine LAD and four LCx segments) were analyzed. Nearly all of them constricted to ACh (−21.2±15.0%, p<0.01). There was no significant difference in the response to ACh between the angiographically normal segments and the segments with irregularities or stenoses of these arteries.

Responses to Nitroglycerin

Responses of coronary arteries to nitroglycerin in the normal coronary artery group are shown in Figure 5.

The proximal, middle, and distal LAD segments dilated significantly to nitroglycerin in the younger group (17.1±9.1%, p<0.01, for the proximal; 25.7±22.7%, p<0.01, for the middle; and 25.5±24.1%, p<0.01, for the distal segment, respectively) and in the older group (17.4±17.1%, p<0.01, for the proximal; 18.5±14.6%, p<0.01, for the middle; and 18.6±24.1%, p<0.01, for the distal segment, respectively). The proximal, middle, and distal LCx segments dilated significantly to nitroglycerin in the younger group (32.9±17.2%, p<0.01, for the proximal; 25.7±25.3%, p<0.01, for the middle; and 28.6±14.6%, p<0.01, for the distal segment, respectively) and in the older group

**Figure 3.** Bar graphs of effects of intracoronary injection of acetylcholine on the coronary artery diameter change from control in patients with angiographically normal coronary arteries. LAD, left anterior descending artery; LCX, left circumflex artery; prox, proximal segment; mid, middle segment; dis, distal segment. *p<0.05 vs. each segment; **p<0.01 vs. each segment.

**Figure 4.** Bar graphs of effects of intracoronary injection of acetylcholine and sublingual administration of nitroglycerin on the coronary artery diameter change from control in patients with coronary artery disease. LAD, left anterior descending artery; LCX, left circumflex artery; prox, proximal segment; mid, middle segment; dis, distal segment. NTG, nitroglycerin; ACh, acetylcholine.
(24.1±15.2%, p<0.01, for the proximal; 30.9±21.7%, p<0.01, for the middle; and 25.7±20.9%, p<0.01, for the distal segment, respectively).

Thus, all LAD and LCx segments dilated significantly to nitroglycerin in both the younger and the older groups. There were no significant differences in the degree of response to nitroglycerin between the two groups and among the segments of coronary arteries.

The responses of coronary arteries to nitroglycerin in the CAD group are shown in Figure 4. The proximal, middle, and distal segments dilated significantly to nitroglycerin in angiographically normal LAD (19.8±16.7%, p<0.01, for the proximal; 21.3±14.0%, p<0.01, for the middle; and 35.5±22.8%, p<0.01, for the distal segment, respectively) and in angiographically normal LCx (29.4±23.2%, p<0.01, for the proximal; 31.6±22.1%, p<0.01, for the middle; and 35.7±27.3%, p<0.01, for the distal segment, respectively). Thus, all segments dilated significantly to nitroglycerin, and there were no significant differences in the degree of response among the coronary segments.

The coronary arteries with irregularities or stenoses dilated significantly to nitroglycerin in both the segments with irregularities or stenoses (28.4±22.9%, p<0.01) and the angiographically normal segments (31.3±20.6%, p<0.01).

Thus, there were no significant differences in the response of coronary arteries to nitroglycerin between the normal coronary artery group (including the younger group and the older group) and the CAD group. There were also no significant differences in the response to nitroglycerin among the coronary segments.

Systemic Hemodynamic Responses to ACh

Heart rate was kept constant during the ACh study by right ventricular pacing in the normal coronary artery group. Consequently, there were no significant changes in the systemic hemodynamic parameters during the injection of ACh. In the younger group, the heart rate was kept constant at 79±11 beats/min and the systolic arterial pressure was 113±11 mm Hg before ACh and 111±10 mm Hg after ACh. In the older group, the heart rate was kept constant at 74±13 beats/min and the systolic arterial pressure was 123±17 mm Hg before ACh and 120±15 mm Hg after ACh. In the CAD group, the heart rate and systolic arterial pressure was 70±14 beats/min and 134±20 mm Hg before ACh and 69±13 beats/min and 132±21 mm Hg after ACh. Neither heart rate nor systolic arterial pressure changed significantly after ACh.

Risk Factors for Coronary Atherosclerosis

Four of the 26 subjects (15.4%) in the younger group, 10 of the 23 subjects (43.5%) in the older group, and 22 of the 25 patients (88.0%) in the CAD group had one or more of the coronary risk factors. Thus, coronary risk factors were significantly more prevalent in the CAD group than in either the older group (p<0.01) or the younger group (p<0.01) and were significantly more prevalent in the older group than in the younger group (p<0.01) as shown in Figure 6.

Discussion

ACh has been shown to dilate normal vessels by releasing an endothelium-derived relaxing factor (EDRF).9,10 When the endothelium is removed or dysfunctional, the vasodilator response is replaced by vasoconstriction resulting from the direct effects of this agent on vascular smooth muscle.

Injury of endothelium is known to be essential to the development of atherosclerosis.22,23 Bossaller and
his coworkers\textsuperscript{13} showed in a recent in vitro study that atherosclerosis impairs the endothelium-dependent relaxation response of human coronary arteries to ACh.

In a previous study, we reported that angiographically normal or almost normal coronary arteries constrict in a significant number and dilate in a small number of adult humans in response to ACh.\textsuperscript{9} In the previous study, however, heart rate was not kept constant in all the study subjects, and bradycardias and hypotension that occurred in response to ACh injection into the right coronary artery might have confounded the interpretation of the effect of ACh on the right coronary artery because sympathetic discharge may occur under such conditions. Moreover, normal smooth coronary arteries and coronary arteries with minimal irregularities were put together, and the diameters of coronary arteries were measured with calipers.

In the present study, heart rate was kept constant by the right ventricular pacing in all the patients in the normal coronary artery group, and the injection of ACh was done only into the left coronary artery in both the normal coronary artery and the CAD groups. Consequently, no significant systemic hemodynamic changes occurred in response to the intracoronary injection of ACh, and direct effects of ACh on coronary arteries could be observed. Moreover, coronary arteries with minimal irregularities were considered as diseased coronary arteries, and the diameters of coronary arteries were measured more accurately with the cinevideodensitometric analysis system that has been validated.\textsuperscript{27,28}

The present study showed that ACh dilated most segments of the angiographically normal coronary arteries except the proximal segment of the LAD in the younger group, whereas the agent constricted most segments of the angiographically normal coronary arteries in the older and the CAD groups. ACh constricted nearly all segments of coronary arteries with irregularities or stenoses. There was no difference in the response of angiographically normal coronary arteries to nitroglycerin, an endothelium-independent vasodilator,\textsuperscript{10} between the younger subjects and the older subjects. There also was no difference in the response of coronary arteries to nitroglycerin between the normal coronary artery and CAD groups. These results strongly suggest that most of the angiographically normal coronary arteries in the older subjects and in patients with CAD have injured endothelium and are involved in atherosclerosis even though they appear normal angiographically. The fact that the coronary risk factors were more prevalent in the older than in the younger group and were most prevalent in the CAD group also supports this interpretation.

It must be pointed out, however, that defective cholinergic vasorelaxation is a relatively nonspecific sign of endothelial injury that may occur in patients with coronary risk factors such as hypertension, diabetes, and hypercholesterolemia without true atheromas. Recently, Hodgson and coworkers\textsuperscript{31} reported that methylene blue, an inhibitor of EDRF, potentiated epicardial coronary artery constriction with ACh but had no effect on the response to nitroglycerin in heart transplant recipients. Their results support the hypothesis that the net response to ACh depends on the interplay between direct vasoconstriction and EDRF-mediated vasodilation.

Autopsy studies have shown that coronary atherosclerosis begins in childhood and progresses with increasing age.\textsuperscript{20–24} The earliest lesion of atherosclerosis, the fatty streak, is present at 10–14 years of age. By age 20, fatty streaks have increased threefold, and the advanced lesion, the fibrous plaque, or atheroma begins to appear. After age 30, fatty streaks are replaced by fibrous plaques and other advanced lesions. The roughly inverse linear relations between the coronary artery diameter change in response to ACh and the age shown in this study were, thus, in agreement with the previous pathologic findings.

The present study also shows that the proximal segment constricted more to ACh than the distal segment in both LAD and LCx in the older group. The proximal segment dilated less to ACh than the distal segment in both LAD and LCx in the younger group. The proximal segment of the LAD actually tended to constrict rather than dilate in the younger group. However, there was no difference in the response to nitroglycerin among the coronary segments in both the younger and the older groups. These results strongly suggest that the proximal segments are more susceptible to endothelial injury or atherosclerosis than the distal segments in the coronary arteries and that atherosclerosis may be present in the angiographically normal proximal segment of the LAD in some of even the younger subjects.

Indeed, pathologic studies have shown that atherosclerosis is more prevalent and severe in the proximal segment than in the distal segment of coronary arteries and that the proximal segment of the LAD is particularly vulnerable to fatty streaks as well as to other, more-advanced lesions.\textsuperscript{21–25}

Ludmer and coworkers\textsuperscript{14} reported that angiographically normal coronary arteries dilate, whereas evidently atherosclerotic coronary arteries constrict in response to the intracoronary infusion of ACh (\textsuperscript{10}\textsuperscript{-9} to \textsuperscript{10}\textsuperscript{-6} M solution in estimated blood concentration). Werns and coworkers\textsuperscript{19} reported that ACh caused coronary dilatation in patients without coronary artery disease, whereas it constricted angiographically normal coronary arteries as well as coronary arteries with evident atherosclerosis in patients with CAD. In the present study, however, intracoronary injection of 50 $\mu$g ACh caused coronary constriction in most of the subjects who are more than 30 years old and had angiographically normal coronary arteries. The discrepancy between our results and those of Ludmer et al and Werns et al may be explained by the difference of concentration of infused ACh. The dose of ACh in our study corre-
sponds to about $10^{-5}$ M in estimated blood concentration, which is higher than that of Ludmer et al and Werns et al. Newman and coworkers\textsuperscript{32} reported that lower doses ($10^{-7}$ to $10^{-4}$ M solution) of ACh induced vasodilatation and that higher doses ($10^{-3}$ to $5 \times 10^{-3}$ M solution) induced vasoconstriction. Thus, direct effect of ACh to contract smooth muscle may over-ride the effect of EDRF in relatively higher doses of ACh.

The discrepancy may also be due to the difference of the number and gender of the patients studied. Ludmer et al studied four women, whereas we studied nine men and 14 women. Also, the possibility that the Japanese population are more susceptible to coronary vasoconstriction than the Western population cannot be excluded.

Besides impaired release of EDRF, an increased sensitivity of the vascular smooth muscle in the regions of atherosclerosis\textsuperscript{33-35} to ACh's direct contractile effect, a defect in the coupling mechanisms between EDRF and smooth muscle cells, or an increased release of endothelium-derived vasoconstricting factors\textsuperscript{36} including the newly discovered endothelin\textsuperscript{37} may also be involved in the constrictor response of atherosclerotic coronary arteries to ACh.

**Clinical implications.** Coronary atherosclerosis remains the leading cause of death in industrialized nations. Many patients with this disease, however, are asymptomatic and may die suddenly or within the first hours of myocardial infarction. At present, coronary arteriography is the only method of accurately diagnosing coronary atherosclerosis in living patients. Pathologic and intraoperative echocardiographic studies, however, demonstrated that coronary atherosclerosis is more prevalent than angiography predicts.\textsuperscript{15-18} The present study shows that most of the angiographically normal coronary arteries in the older subjects as well as in the patients with CAD constrict to the intracoronary injection of ACh as do the coronary arteries with evident atherosclerosis, whereas those in the younger subjects dilate in response to this agent. Thus, the intracoronary injection of ACh may provide a useful tool to detect early coronary atherosclerosis. Coronary atherosclerosis can be delayed or reversed,\textsuperscript{22,23} and this may be achieved most effectively in its early stages. Recent studies showed that the coronary arteries of heart transplant patients\textsuperscript{1,38,39} or of patients with CAD\textsuperscript{19} constrict to ACh despite the absence of angiographically evident atherosclerosis.

The intracoronary injection of ACh causes coronary vasoconstriction in nearly all patients with variant angina, including those with angiographically normal coronary arteries.\textsuperscript{5-7} Moreover, the intracoronary injection of ACh induces coronary spasm (total or almost total occlusion of a major coronary artery resulting in myocardial ischemia) in most of these patients.\textsuperscript{5-7} This strongly suggests that coronary arteries in patients with variant angina are all involved in endothelial injury or atherosclerosis, even though some of them may appear normal angiographically. The fact that nearly all of the patients with variant angina are over the age of 30\textsuperscript{40} also supports this concept. Thus, the present study implies that coronary atherosclerosis is a prerequisite to the pathogenesis of coronary spasm.\textsuperscript{5}

**References**


**KEY WORDS** • age • atherosclerosis • acetylcholine
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