Response of conductance and resistance coronary vessels to scalar concentrations of acetylcholine: Assessment with quantitative angiography and intracoronary Doppler echography in 29 patients with coronary artery disease

Abnormal vasoreactivity of the large conductance arteries has been observed in the presence of impaired endothelial function. More recently, experimental and clinical reports have shown that in early coronary atherosclerosis the impairment of the endothelium-mediated vasodilatation also involves the resistance arteries. The aim of this study is the correlation of endothelium-dependent vasodilatation of conductance and resistance vessels in coronary arteries without significant stenoses. In 29 patients (aged 57 ± 9 years, 24 men and 5 women) undergoing coronary angioplasty, a Doppler guide wire and a perfusion catheter were introduced into the proximal segment of an artery with less than 30% diameter stenosis. Selective infusions of papaverine (bolus of 7 mg), acetylcholine (continuous infusion of 0.036, 0.36, and 3.6 μg/ml at a flow rate of 2 ml/min), and isosorbide dinitrate (bolus of 3 mg) were sequentially performed. Heart rate, aortic blood pressure, and blood flow velocity were continuously measured. Mean cross-sectional areas of a proximal and a distal arterial segment were measured in baseline conditions, at the end of each infusion of acetylcholine, and at the peak effect of isosorbide dinitrate with quantitative angiography (CAAS System; Pie Medical Data, Maastricht, The Netherlands). Coronary blood flow was calculated from the time-averaged flow velocity and the cross-sectional area at the site of the Doppler sample volume. Coronary flow resistance was calculated as mean aortic pressure divided by coronary flow. All of the concentrations of acetylcholine induced a significant vasoconstriction of the studied artery. At the maximal concentration of acetylcholine all but three patients (90%) showed a reduction of cross-sectional area (-24% ± 20% and -22% ± 20% for the proximal and distal segments, respectively, $p < 0.00001$). Flow velocity showed a significant increase only with the two highest concentrations of acetylcholine. The maximal concentration induced a 105% ± 138% increase from the baseline flow velocity ($p < 0.001$). The coronary flow changes after acetylcholine showed a large interpatient variability, with a mean increase from baseline after the highest dose of $+43\%$ ± 85% (range, -60% ± 239%), with the presence of a flow reduction in 10 patients (35%). No clinical or angiographic variables showed a significant correlation with the cross-sectional area, flow velocity, and flow changes after infusion of acetylcholine. After acetylcholine infusion, angiographically normal or minimally diseased arteries of patients with symptoms of coronary artery disease show: (1) a significant coronary vasoconstriction of the epicardial arteries; (2) a variable change in coronary flow, with a trend toward a moderate increase; and (3) a dissociation between the impairment of endothelium-mediated vasodilatation of conductance and resistance vessels, suggesting the presence of different mechanisms underlying the endothelial dysfunction in these two arterial districts. (Am Heart J 1994;127:514-31.)

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The in vitro observations of Furchgott and Zawadzki[1] and the results of in vitro and in vivo studies by Palmer et al. and Vallance et al.[3] have shown that an endothelium-derived relaxing factor, identified as nitric oxide,[2] modulates vascular tone in response to physiologic and pathologic stimuli (increase in wall shear stress, serotonin, bradykinin, histamine, thrombin, sympathetic stimulation, acetylcholine, endotoxins, etc.). Endothelial damage, leading to a decreased formation or release of nitric oxide from its precursor L-arginine, or reduced penetration caused by the presence of subendothelial intimal thickening, are possible explanations of the impairment of endothelium-mediated vasodilatation observed in patients with systemic hypertension,[4] hypercholesterolemia, diabetes mellitus,[5] or atherosclerosis.[6]

The presence of paradoxical vasoconstriction induced by acetylcholine has been shown in patients with coronary artery disease at sites of severe stenosis or moderate wall irregularities[7] and in angiographically normal segments.[8-10] Coronary spasm after acetylcholine infusion has also been demonstrated in patients with variant angina, with and without angiographically visible changes.[11,12] The observed vasoconstriction or vasodilatation after acetylcholine infusion is the net effect of the conflicting action of this substance on the endothelial cells (stimulation to the release of endothelium-derived relaxing factor) and on the smooth muscle cells (vasoconstriction caused by the direct effect on the cholinergic receptors). With the use of intracoronary Doppler echography, an impairment of the endothelium-derived vasodilatation was also observed after more physiologic stimuli such as an increase in blood flow.[13-15] Flow-dependent vasodilatation is an essential mechanism of adjustment of coronary tone to prevent endothelial damage caused by a pathologic increase in wall shear stress.[12] Abnormal vasoconstriction in response to sympathetic stimulation[16] or release of platelet-derived vasoconstrictors[17,18] was observed if the direct effect of these substances on the muscular media was not antagonized by a preserved endothelium-mediated vasodilatation. Nitric oxide also has a powerful antiaggregatory activity. Yao et al.[19] showed a protective effect of endogenous nitric oxide in the prevention of cyclic flow variations caused by platelet aggregation at the site of endothelial injury. Endothelial dysfunction therefore is not only a potential mechanism of aggravation of ischemia in patients with coronary atherosclerosis; it also increases the risk of endothelial injury and impairs the antithrombotic reaction, thus facilitating the development of acute coronary syndromes and the release of platelet-derived growth factors, which may

predispose patients to progression of atherosclerosis. An impairment of endothelium-mediated vasodilatation has been shown in patients with risk factors for coronary atherosclerosis but without angiographically visible atherosclerotic changes.[9,20] A possible limitation of these studies is the poor sensitivity of angiography in the detection of early atherosclerotic changes. More recently, the presence of endothelial dysfunction in patients with structurally normal coronary arteries but with hypertension, hyperlipidemia, family history of coronary artery disease or smoking has been confirmed with two-dimensional intracoronary ultrasound.[21] A complete loss of endothelium-mediated vasodilatation was present in arteries with angiographically visible atherosclerotic changes. Angiographically normal arteries of patients with hypercholesterolemia showed a normal flow-mediated vasodilatation after infusion of papaverine but an abnormal vasoconstriction after infusion of acetylcholine.[21]

The possible presence of opposite effects of acetylcholine infusion on epicardial and resistance coronary arteries have been reported by Hodgson and Marshall.[22] The observed increase of coronary flow after acetylcholine infusion was prevented by pretreatment with methylene blue, an inhibitor of endothelium-derived relaxing factor. Zeiher et al.[23] reported a significantly lower flow increase after acetylcholine infusion in patients with coronary artery disease than in control subjects. These findings confirmed previous experimental results showing that the impairment of endothelial function in atherosclerotic arteries may extend into the coronary microcirculation.[24-26] The presence of an impaired endothelium-dependent vasodilatation of the resistance vessels may induce or facilitate the development of myocardial ischemia in response to neurohumoral stimulation or increased myocardial work.[27] The epicardial arteries and the arterioles have large structural differences and show a different involvement in the atherosclerotic process, mainly confined to the large epicardial coronary arteries. Both types of arteries, however, are likely to show a similar response to pathologic stimuli on the endothelial cells, impairing the intracellular production and release of nitric oxide. The presence of an impaired diffusion or of an increased extracellular degradation of nitric oxide in the thickened intima is a phenomenon limited to the epicardial arteries and may explain an earlier and more severe impairment of the endothelium-mediated vasodilatation of these vessels.

The aim of this study is the simultaneous assessment of the endothelium-mediated vasodilatation of conductance and resistance vessels in coronary ar-

teries without significant stenosis (<30% diameter stenosis). During selective infusion of scalar increasing concentrations of acetylcholine, the changes of coronary cross-sectional area over a proximal or mid segment and a distal segment of the studied artery were measured with quantitative coronary angiography and correlated with the changes in coronary flow, derived from the flow velocity measured with a Doppler guide wire and used as an index of vasodilatory response of the resistance vessels.

METHODS

Patient population. Twenty-nine patients (aged 57 ± 9 years, 24 men and 5 women) who were undergoing elective percutaneous transluminal angioplasty because of disabling stable angina pectoris were studied. Previous myocardial infarction was present in 8 of 29 patients (26%); in no case was the territory of distribution of the studied artery involved. Systemic hypertension was defined as a chronically elevated arterial blood pressure (≥150/90 mm Hg) and was present in 9 of 29 patients (31%). Three patients were current smokers (10%). A history of smoking was present in 18 patients (62%). No patient had anemia (mean hemoglobin, 8.8 ± 0.57 mmol/L) or anamnestic or biohumoral signs of diabetes mellitus or hyperthyroidism.

The angiographic selection criteria included less than 30% diameter stenosis in one of the three major coronary arteries without visible collaterals originating from this vessel and with a normal left ventricular contraction of the segments of distribution of the studied artery. Angiographically visible wall irregularities were present in 19 of 29 patients (66%). The studied artery was the left anterior descending coronary artery in seven patients (24%), the left circumflex coronary artery in 13 (45%), and the right coronary artery in nine (31%). Written informed consent was obtained in all cases. The protocol was approved by the Ethics Committee of Erasmus University-Rotterdam Dijkzigt Hospital. All vasoactive medication, with the exception of short-acting sublingual nitrates, was withheld for at least 48 hours before the catheterization. No sublingual, intravenous, or intracoronary nitrates were used in the 6 hours before or during the catheterization procedure.

Catheterization procedure. After systemic anticoagulation with 10,000 IU of heparin and 250 mg of acetylsalicylic acid administered intravenously and sedation with 5 to 10 mg diazepam administered intravenously, the artery to be studied was instrumented with a 9F giant lumen (inner lumen = 0.084 inches) Amplatz or Judkins guiding catheter (left coronary artery) or a 7F Judkins diagnostic catheter (right coronary artery). A 0.018 inch Doppler angioplasty guide wire was then advanced to a normal or near-normal straight proximal segment of the artery to be studied where a stable flow velocity signal could be obtained. A 3.6F flexible infusion catheter (Tracker 25, Target Therapeutics, San José, Calif.) was then inserted over the Doppler wire into the proximal segment of the coronary artery to obtain a selective injection into the left anterior descending or left circumflex coronary artery.[28] Care was taken to avoid a too selective cannulation of the large guiding catheter into the left main coronary artery to pre-

vent limitation of flow during maximal hyperemia. For the right coronary artery a selectively engaged 7F diagnostic catheter was used for the injection. Heart rate and mean aortic pressure were automatically measured with a previously described computer-assisted system[29] by averaging 16 consecutive seconds of recording. After baseline acquisition of flow velocity, heart rate, and blood pressure, the measurements were repeated 30 seconds after a bolus injection of 7 mg of papaverine diluted in 1.5 ml. After a recovery period of 8 minutes, new basal measurements were performed, followed by a cineangiogram suitable for quantitation. Scalar concentrations of acetylcholine (Dispersa, Wintertaur, Switzerland) at 37° C (0.036, 0.36, μg/ml) were infused at a flow rate of 2 ml/min with a precision pump-injector (Mark V, Medrad, Pittsburgh, Pa.). With these dilutions and flow rates and assuming a coronary blood flow of 80 ml/min in the studied artery, intracoronary blood concentrations of 10^{-8}, 10^{-7}, and 10^{-6} mol/L were estimated. Five minutes after the beginning of the infusion of each concentration, blood flow velocity and hemodynamic measurements were obtained, and a new cineangiogram was performed. Five minutes after the end of the series of acetylcholine infusions, a new baseline flow velocity was obtained, and 1 minute after a bolus injection of 3 mg of isosorbide dinitrate, a new cineangiogram was performed.

Doppler guide wire and flow velocity measurements. The Doppler angioplasty guide wire is an 0.018 inch (diameter = 0.46 mm), 175 cm long flexible and steerable guide wire with a floppy shapable distal end mounting a 12 MHz piezoelectric transducer at the tip (FloWire, Cardiometrics Inc., Mountain View, Calif.). The sample volume was positioned at a distance of 5.2 mm from the transducer to avoid the area of distortion of flow profile caused by the presence of the Doppler guide wire. At this distance the sample volume has a diameter of approximately 2.25 mm because of the divergent ultrasound beam, so that a large part of the flow velocity profile is included in the sample volume even in case of eccentric position of the Doppler guide wire. In order to increase the reliability of the analysis of the Doppler signal,[30] a real-time fast-Fourier transform algorithm was applied to the quadrature audio signal. The Doppler system used (FloMap, Cardiometrics Inc.) performs a real-time spectral analysis of the Doppler signal and calculates and displays on-line several spectral variables including the instantaneous peak velocity and the time-averaged (mean of 2 beats) peak velocity (Fig. 1). The flow velocity measurements obtained with this system have been validated in vitro and in an animal model in which simultaneous electromagnetic flow measurements were used for comparison.[31] Coronary flow reserve was defined as the ratio between maximal flow velocity at the peak effect of the papaverine injection and in baseline conditions. ECG, coronary pressure, and peak coronary blood flow velocity were continuously sampled at 125 kHz per channel with a 12-bits analog-to-digital converter. The ACodas software package (DataQ Instr., Akron, Ohio) was used for off-line analysis.

Quantitative angiographic measurements. The preformed coronary catheter, filmed not filled with contrast medium, was used as a scaling device.[32] Before the study,
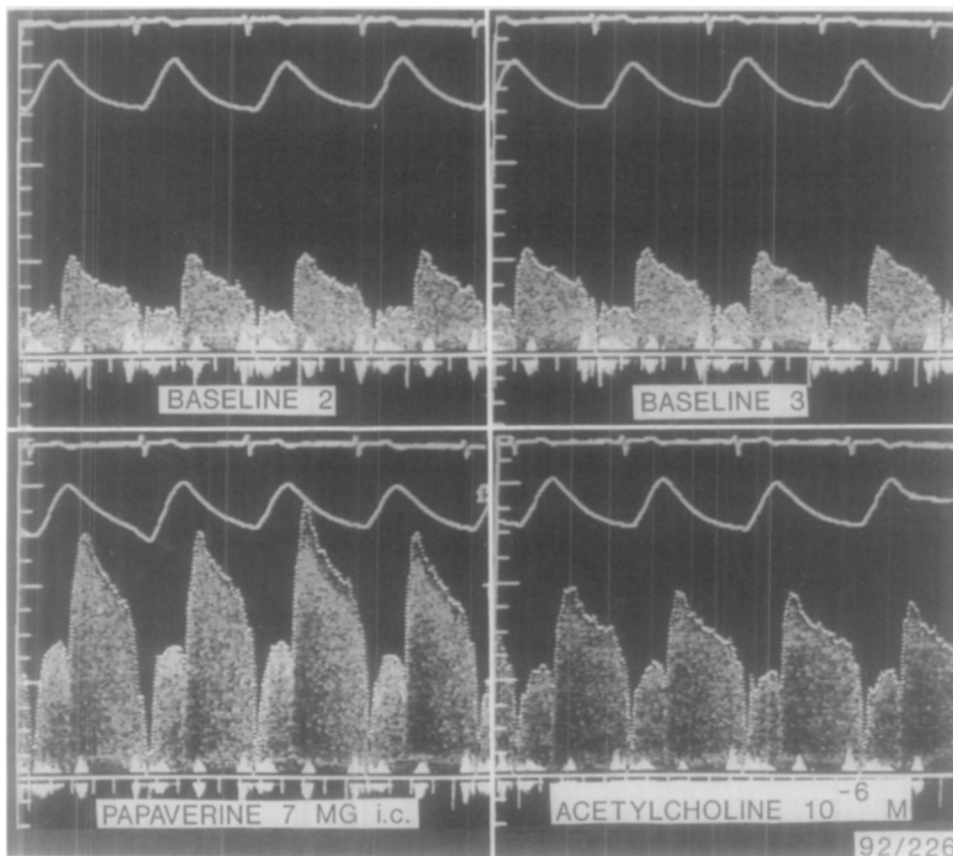
Fig. 1. Flow velocity measurement in proximal left circumflex artery before injection of increasing concentrations of acetylcholine *(baseline 2)*, 5 min after end of infusion of acetylcholine *(baseline 3)*, and at peak effect of papaverine *(lower left)* and acetylcholine 10^{-6} mol/L *(lower right)*. Note the stable flow velocity in baseline condition, large velocity increase after infusion of papaverine, and moderate increase after infusion of maximal concentration of acetylcholine.
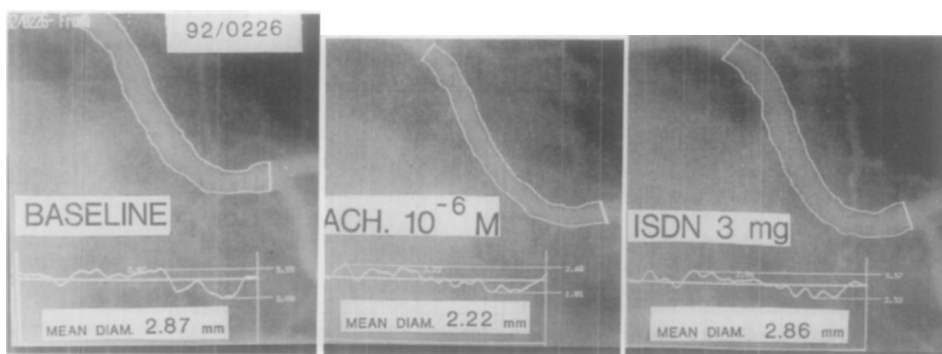
Fig. 2. Quantitative angiographic measurements of mean diameter of a proximal segment of left circumflex artery. Two side branches are used as landmarks to facilitate consistent repeated measurements of same segment throughout procedure. Note severe decrease in mean coronary diameter (-22%) after maximal concentration of acetylcholine *(ACH 10^{-6} M)*. *ISDN*, Isosorbide dinitrate.

when necessary, a previously validated on-line analysis system operating on digital images (ACA-DCI, Philips, Eindhoven, The Netherlands)[33] was used to exclude the presence of more than 30% diameter stenosis. Coronary angiography was performed with a manual injection of 6 to 10 ml of iopamidol (Iopamiro 370, Schering, Berlin, Germany). A 5- or 7-inch field-of-view of the image intensifier

was used. No changes of the position of the patient or of the x-ray gantry were performed throughout the procedure. The same angiographic view was maintained during the study, avoiding foreshortening or vessel superimposition of the arterial segments of interest. A previously validated[34] cinefilm-based off-line system (CAAS System, Pie Medical Data, Maastricht, The Netherlands) was used to measure

HEART RATE

BEATS/MIN

p< 0.002

A

BLOOD PRESSURE

mm/Hg

p< 0.001

p< 0.001

B
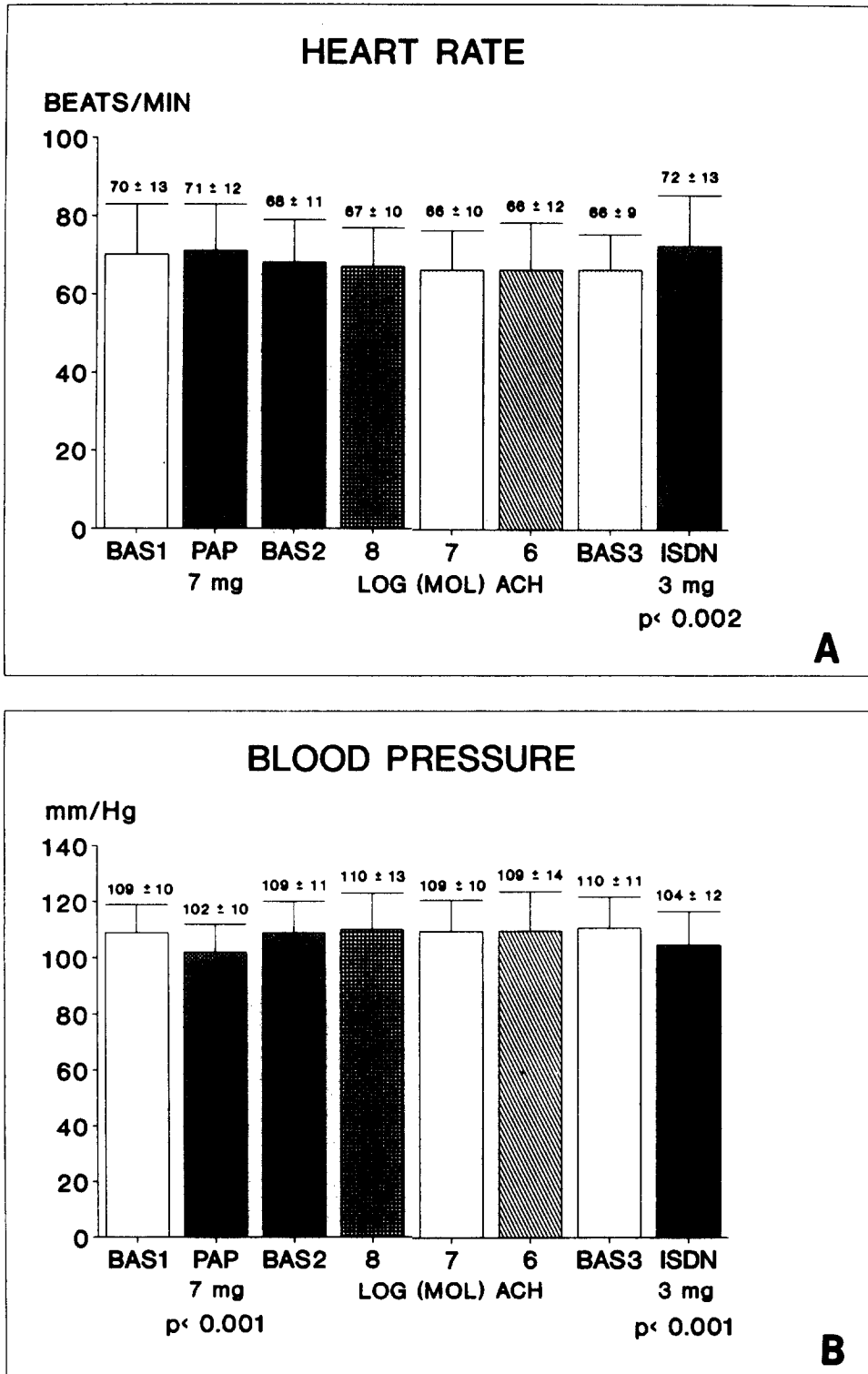
Fig. 3. A, Heart rate. **B,** Mean aortic blood pressure during various phases of the study. *ACH,* Acetylcholine; *BAS,* baseline; *ISDN,* isosorbide dinitrate; *PAP,* papaverine.

the mean diameter over a 2 to 3 cm long proximal or mid segment and distal coronary segment; easily visible side branches were used as anatomic landmarks to allow the analysis of the same segments in successive cineangiograms (Fig. 2). In 14 cases (48%) a second-order arterial branch

(diagonal, obtuse marginal, posterolateral, right ventricular branch) was analyzed as a distal segment. In the remaining 15 (52%) cases the distal segment of one of the three major coronary arteries was used. The quantitative angiographic system used automatically detects the vessel

BLOOD FLOW VELOCITY
PERCENT CHANGE VS BAS2
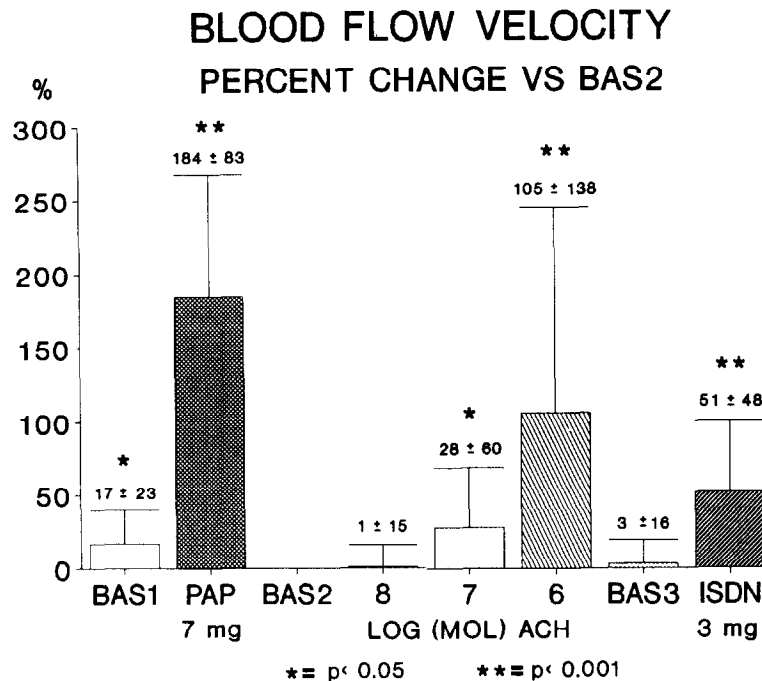
Fig. 4. Flow velocity changes expressed as a percent of baseline flow velocity. *ACH*, Acetylcholine; *BAS*, baseline; *ISDN*, isosorbide dinitrate; *PAP*, papaverine.

centerline and applies a weighted first- and second-derivative function with predetermined continuity constraints to the brightness profile of each scan line perpendicular to the vessel centerline.[35] A user-defined reference diameter was measured at the site of the Doppler sample volume.[36] Cross-sectional area was calculated from the corresponding diameter, assuming a circular arterial cross-section. Coronary flow was calculated as: Coronary flow (ml/min) = CSA (mm²) × Time-averaged peak velocity (cm/s) ÷ 2 × 0.6, where CSA is the arterial cross-sectional area at the site of the Doppler sample volume. Coronary flow resistance was calculated as: Coronary flow resistance (mm Hg min ml⁻¹) = Mean aortic pressure (mm Hg) ÷ Coronary flow (ml/min).

Statistical analysis. The significance of the differences between flow velocity and cross-sectional area measurements and derived indexes in baseline conditions and after papaverine, acetylcholine, and isosorbide dinitrate infusions was tested with a two-tailed Student's t test for paired data. A two-tailed Student's t test for unpaired data was used to compare the diameter and flow changes observed in patients with different clinical angiographic characteristics. Linear regression analysis was used to correlate the changes observed in cross-sectional area, in coronary flow, and in coronary flow resistance. Analysis of variance for repeated measurements was used to test the time-response and the variability of the flow velocity changes after infusion of acetylcholine. Statistical significance was defined as $p < 0.05$. All data were expressed as means ± SD.

RESULTS

Heart rate was stable throughout the study, with a significant increase only after the bolus injection of

isosorbide dinitrate (Fig. 3, *A*). In two cases during the maximal infusion of acetylcholine brief episodes of Mobitz I atrioventricular block, which did not require ventricular pacing, were observed. Aortic pressure was stable in baseline conditions and during the infusion of the different concentrations of acetylcholine. A slight but significant decrease was observed at the peak effect of the papaverine and isosorbide dinitrate infusions (−7% and −5%, respectively) (Fig. 3, *B*).

Flow velocity changes. Table I shows the individual changes in blood flow velocity in the studied patients. In Fig. 4 the changes of time-averaged peak blood flow velocity are expressed as a percent of the baseline velocity (baseline 2). A moderate decrease of blood flow velocity was observed between the first baseline measurement (beginning of the study) and the second and third baseline measurements (5 minutes after infusion of papaverine and after infusion of the maximal concentration of acetylcholine, respectively, $p < 0.05$). On the contrary, a large increase was observed after papaverine injection, with a peak velocity 2.8 ± 0.83 times higher than in basal conditions (baseline 2). The lowest concentration of acetylcholine did not induce significant changes in blood flow velocity. A 28% ± 60% increase was observed after 5 minutes of infusion of acetylcholine 10^{-7} mol/L ($p < 0.05$). At the end of the infusion of the highest acetylcholine concentration (10^{-6} mol/L) a more than twofold increase in flow velocity was observed (+105% ± 138%, $p < 0.001$).

Table I. Clinical characteristics and flow velocity changes after papaverine and acetylcholine infusions

Patient No.	Age (yr)	Sex	Hypertension	Cholesterol (mmol/L)	Vessel	Wall irreg.
1	70	M	Y	5.5	LAD	Y
2	63	M	N	5.7	RCA	Y
3	68	F	Y	7.3	RCA	Y
4	58	M	N	4.9	LCX	Y
5	49	M	Y	5.8	LCX	Y
6	59	M	N	6.5	LCX	Y
7	50	M	N	6.4	RCA	Y
8	53	M	Y	5.6	LCX	Y
9	48	M	Y	7.2	LAD	Y
10	53	M	N	5.5	LAD	N
11	66	F	Y	7.7	LCX	Y
12	52	F	N	7.4	LCX	Y
13	59	M	N	6.6	LCX	Y
14	42	M	N	5.2	LCX	N
15	54	F	N	5.9	LCX	Y
16	45	M	N	7.1	LCX	Y
17	45	M	Y	6.1	LAD	N
18	44	M	N	7.7	RCA	Y
19	67	M	N	5.8	LAD	N
20	67	M	N	6.9	RCA	Y
21	71	M	N	4.7	RCA	N
22	45	F	N	7.0	LAD	N
23	46	M	Y	6.8	LAD	N
24	58	M	N	5.4	RCA	N
25	66	M	N	4.9	LCX	Y
26	69	M	N	5.4	RCA	Y
27	65	M	N	5.9	LCX	N
28	54	M	Y	5.3	RCA	N
29	56	M	N	7.1	LCX	Y
Mean	57			6.2		
±SD	9			0.9		

Ach, Acetylcholine; *BAS*, baseline; *ISDN*, isosorbide dinitritate; *LAD*, left anterior descending coronary artery; *LCX*, left circumflex coronary artery; *PAV*, papaverine; *RCA*, right coronary artery.
*$p < 0.002$ versus baseline 2.
†$p < 0.02$ versus baseline 1.
‡$p < 0.05$ versus baseline 2.
§$p < 0.005$ versus baseline 1.

Time-response of the flow velocity change and flow velocity variability during acetylcholine infusion. In Fig. 5 the time-response of the flow velocity changes during the infusion of the maximal concentration of acetylcholine is reported for the entire group (Fig. 5, *A*) and for all individual cases. A significant increase of flow velocity was observed within 30 seconds from the beginning of the infusion. Afterwards, during the remaining infusion period, no significant flow velocity changes were observed in the study population overall. However, if the individual response is considered, (Fig. 5, *B*) a relatively stable flow velocity was observed only in 10 patients during infusion. In the remaining patients, despite the constant rate of infusion and the stable hemodynamic conditions, a large variability was observed, with cases showing a progressive increase or decrease during the final phase of infusion (Fig. 5, *C* and *D*, respectively) or a bell-shaped or biphasic response (Fig. 5, *E* and *F*, respectively; example in Fig. 6). Cyclic variations of flow velocity were observed in the following minutes (Fig. 5). The variability was more evident in the patients with a large velocity increase after acetylcholine.

Coronary artery cross-sectional area. Table II indicates the individual measurements of the mean cross-sectional area of the proximal and distal coronary segments. In Fig. 7 the changes in cross-sectional area after infusion of the three increasing concentrations of acetylcholine and the bolus of iso-

BAS1 cm/sec	PAV cm/sec	BAS2 cm/sec	Ach10^{-8} cm/sec	Ach10^{-7} cm/sec	Ach10^{-6} cm/sec	BAS3 cm/sec	ISDN cm/sec
22	44	22	21	18	20	21	30
20	48	19	16	22	33	22	35
19	40	20	20	21	32	24	22
33	60	26	29	24	50	26	24
44	79	26	25	25	23	23	28
32	89	22	29	30	49	25	33
11	41	14	19	19	26	15	24
23	70	21	23	35	53	23	65
17	39	17	22	41	66	20	32
38	56	32	29	30	32	26	42
27	65	20	19	20	37	16	26
25	80	19	19	35	44	17	25
22	52	24	26	28	46	24	38
33	53	24	24	23	24	22	36
28	71	28	28	27	43	28	43
42	120	39	39	41	65	39	66
27	77	25	25	32	48	26	29
32	68	23	21	22	67	23	43
27	70	17	19	25	31	19	22
25	66	27	27	36	72	30	39
25	57	21	19	34	69	23	23
36	90	55	40	35	120	n.r.	n.r.
23	44	21	16	24	17	17	18
26	66	24	20	20	18	21	30
15	52	11	14	42	89	17	26
28	65	28	28	27	29	25	40
21	34	15	15	22	39	21	24
12	43	9	10	9	15	9	21
32	65	23	17	19	18	23	19
26	62*	23†	23	27‡	44*	22§	32*
8	18	8	7	8	24	5	12

sorbide dinitrate are expressed as a percent of the basal cross-sectional area. The injection of the two lowest concentrations of acetylcholine induced a moderate but significant reduction of the mean cross-sectional area both in the proximal segment and in the distal segment. A larger decrease was observed after infusion of the highest concentration of acetylcholine (-24% ± 20% and -22% ± 20% of the mean cross-sectional area of the proximal and distal coronary segments, respectively, $p < 0.00001$). At this concentration almost all of the studied arteries showed a variable degree of vasoconstriction (26 of 29 arteries, 90%), (Fig. 8, A). In no case was a mean cross-sectional area reduction of more than 75% observed. At the end of the infusion of the highest concentration of acetylcholine, focal vasoconstriction of the more distal branches of the studied artery was observed in eight patients. In these cases quantita-

tive angiography also showed a more severe vasoconstriction (-32% ± 25% vs -18% ± 22% cross-sectional area reduction of the analyzed segments with and without focal arterial spasm, respectively, p = not significant). The presence of a preserved vasodilatory capacity of the studied artery was confirmed by the diffuse cross-sectional area increase after bolus injection of a direct smooth muscle vasodilator such as isosorbide dinitrate ($+16\%$ ± 26% and $+18\%$ ± 26% cross-sectional area increase vs baseline for the proximal and distal coronary segments, respectively, both $p < 0.002$).

Coronary flow changes. A significant increase of coronary flow was observed only after infusion of the maximal concentration of acetylcholine ($+43\%$ ± 83%, $p < 0.001$). The large variability in the individual measurements for the highest concentrations of acetylcholine is shown in Fig. 8, B). Note that at the
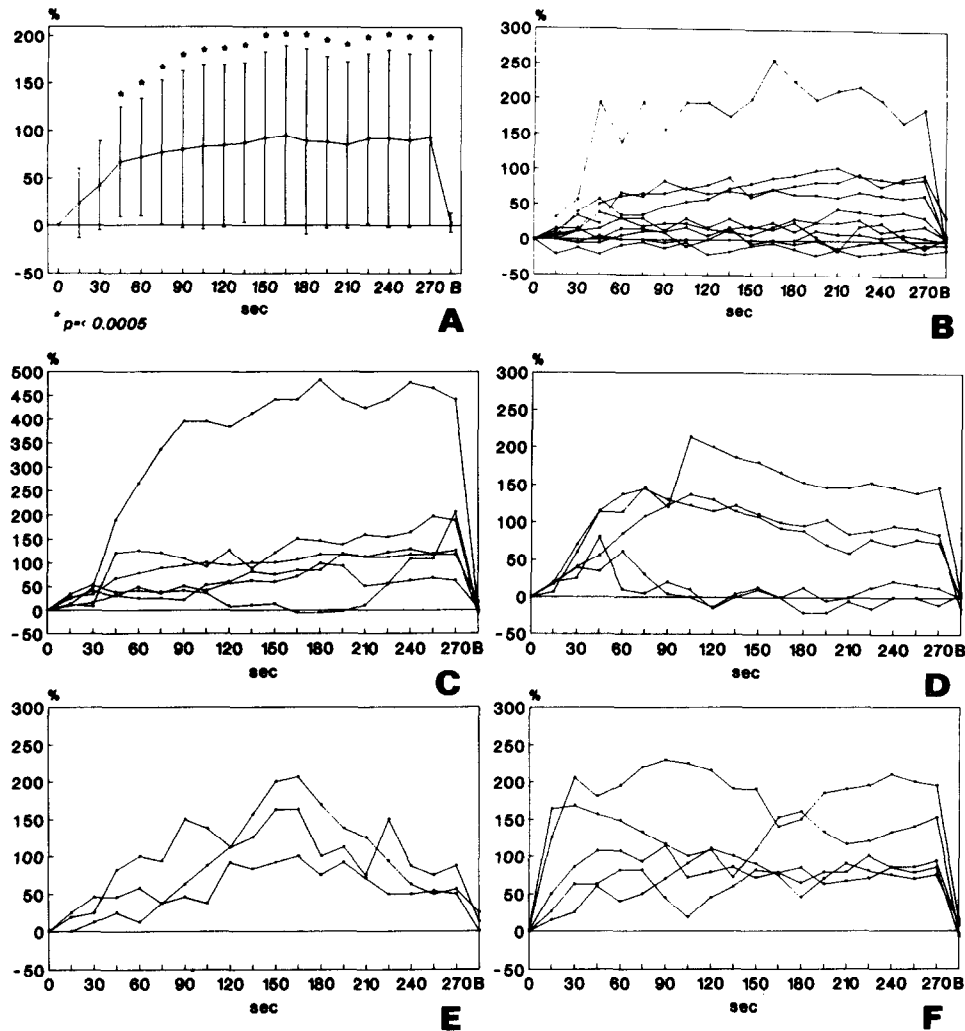
Fig. 5. Temporal changes in flow velocity during infusion of the highest concentration of acetylcholine expressed as a percent of the value before infusion *(time 0)*. **A,** mean ± SD. **B,** Ten individual curves showing a relatively stable flow velocity at baseline 3 (5 minutes after the end of the acetylcholine infusion). **C** and **D,** six and five patients showing, respectively, a progressive increase and a progressive decrease from the beginning to the end of the infusion. **E** and **F,** three and five cases with a bell-shaped or a biphasic response during infusion.

peak concentration of acetylcholine, 10 patients showed a decrease of absolute flow and an increase in coronary resistance.

Correlation of the observed results with the clinical-angiographic characteristics. The flow velocity, cross-sectional area, and flow changes after acetylcholine infusion showed no correlation with age, sex, presence of systemic hypertension, total cholesterol, high-density lipoprotein (HDL) cholesterol, HDL cholesterol/total cholesterol ratio, plasma triglycerides, type of studied artery, and basal coronary luminal diameter. The presence of wall irregularities was associated with a larger decrease in luminal cross-sectional area (−27% ± 20% change vs baseline in

the 19 arteries with angiographically visible wall irregularities and −16% ± 20% in the angiographically normal arteries). The difference, however, was not statistically significant. The arteries with wall irregularities also showed a smaller flow increase after the last concentration of acetylcholine (+47% ± 30% vs + 68% ± 56% in the group with smooth arterial contours, p = not significant).

A poor correlation was observed between flow velocity changes after acetylcholine and papaverine infusions (r^2 = 0.18 for the maximal concentration of acetylcholine). Similarly, the percent increase of lumen diameter after isosorbide dinitrate infusion was not correlated with the changes observed after ace-

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Usefulness of changes in exercise tolerance induced by nitroglycerin in identifying patients with syndrome X

Two exercise tests, one under basal conditions and one after sublingual nitroglycerin (NTG), were performed in 39 patients with stable angina pectoris—16 with critical coronary stenoses and 23 with normal coronary arteries (syndrome X). Under basal conditions, times at ischemic threshold, at peak exercise, and at complete ECG recovery were similar in the two groups. Peak ST depression was significantly higher in patients with coronary artery disease (CAD). In a similar proportion of patients, ST-segment depression developed earlier or at a low heart rate. Patterns of heart rate, blood pressure, and rate-pressure product during exercise and recovery were also similar. After NTG an increase in the ischemic threshold was observed in a significantly higher proportion of patients with CAD (93.8% vs 39.1%). Furthermore, a subgroup of patients with syndrome X showed a worsening of exercise performance. This suggests that NTG does not directly affect small coronary vessels. Our results confirm that no relevant differences exist in exercise responses between patients with CAD and those with syndrome X under basal conditions. NTG-induced changes in this response could be useful in identifying patients with normal coronary arteries. Moreover, this test could be used as a guide to therapeutic approaches. (AM HEART J 1994;127:531-5.)

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It has been suggested that the course of ST-segment depression during and after exercise may be useful in distinguishing patients with anginal pain with and without angiographically documented coronary lesions.[1-4] This contradicts the findings of other inves-

Table II. Quantitative angiographic changes after acetylcholine infusion

Patient No.	Mean diameter proximal segment (mm)					Mean diameter distal segment (mm)				
	BAS	$Ach10^{-8}$	$Ach10^{-7}$	$Ach10^{-6}$	ISDN	BAS	$Ach10^{-8}$	$Ach10^{-7}$	$Ach10^{-6}$	ISDN
1	1.89	1.74	1.65	1.58	1.84	1.24	1.30	1.20	1.14	1.46
2	2.82	2.82	2.50	2.36	2.87	2.87	2.40	2.59	2.21	2.69
3	2.36	2.33	2.67	2.35	3.05	2.74	2.71	2.57	2.35	3.02
4	2.41	2.39	2.49	2.30	2.79	1.78	1.82	1.69	1.60	1.94
5	2.48	2.09	2.15	1.99	2.60	1.74	1.50	1.34	1.36	1.8
6	2.87	2.19	2.12	2.22	2.86	2.27	1.86	1.66	1.83	2.42
7	4.00	4.08	4.13	4.28	4.21	2.23	1.95	1.89	1.92	2.06
8	2.75	2.57	2.73	2.60	3.16	1.58	1.55	1.40	1.57	1.95
9	1.48	1.28	1.41	1.15	1.54	1.17	0.97	1.12	1.10	1.44
10	1.67	1.42	1.52	1.52	1.92	1.32	1.30	1.37	1.26	1.67
11	3.00	2.98	2.85	2.62	3.06	1.40	1.42	1.39	1.21	1.47
12	1.66	1.62	1.62	1.59	1.82	1.48	1.39	1.33	1.31	1.43
13	2.30	n.a.	1.97	1.38	2.02	2.15	n.a.	1.66	1.05	1.8
14	2.91	2.90	3.00	2.85	2.90	1.66	1.66	1.82	1.77	1.67
15	3.20	3.18	3.15	2.96	3.12	1.50	1.37	1.28	0.98	1.36
16	3.11	2.77	2.59	2.43	3.41	1.42	1.21	1.29	1.32	1.71
17	2.17	1.91	1.90	1.74	1.94	1.99	1.78	1.82	n.a.	1.77
18	2.69	2.51	2.54	2.41	2.96	1.33	1.21	1.26	1.40	1.52
19	2.59	2.28	2.23	2.07	2.62	1.60	1.32	1.31	1.30	1.76
20	3.13	2.52	2.72	2.42	3.16	1.78	1.57	1.67	1.69	1.83
21	2.96	3.27	3.14	3.15	3.57	1.71	1.73	1.54	1.60	2.08
22	1.52	1.43	1.62	1.49	2.21	1.36	1.46	1.42	1.09	1.99
23	2.22	2.24	2.06	2.12	2.26	1.76	1.76	1.74	1.88	2.12
24	3.03	2.70	2.91	2.41	3.23	2.35	2.23	2.35	2.31	2.62
25	2.28	1.90	1.77	1.39	2.37	1.66	1.55	1.48	1.18	1.94
26	2.72	2.72	2.71	2.41	3.20	1.74	1.74	1.67	1.42	2.11
27	2.47	2.40	1.96	1.91	2.63	1.11	1.17	0.95	1.09	1.23
28	3.16	3.22	3.28	3.28	3.09	1.90	1.77	1.73	1.67	1.75
29	2.47	2.26	2.18	1.81	2.68	1.46	1.31	1.29	1.29	1.56
Mean	2.56	2.42*	2.39*	2.23†	2.73‡	1.73	1.61*	1.58‡	1.50†	1.85*
±SD	0.56	0.62	0.62	0.66	0.60	0.43	0.38	0.39	0.38	0.42

Ach, Acetylcholine; *BAS*, baseline; *ISDN*, isosorbide dinitrate.
*$p < 0.002$ versus baseline.
†$p < 0.00001$ versus baseline.
‡$p < 0.0005$ versus baseline.

tylcholine infusion. The flow changes and flow resistance changes after the maximal dose of acetylcholine infusion showed a poor correlation with the cross-sectional area changes observed in the proximal/distal coronary segments. Fig. 9 shows the linear regression analysis performed with the use of the cross-sectional area changes of the proximal segments. Also, in the distal coronary segment analyzed a very poor correlation was observed, with a squared correlation coefficient of 0.01 and 0.05 for coronary flow and flow resistance.

DISCUSSION

Acetylcholine is the prototype and the most frequently used pharmacologic stimulus with a primary endothelium-independent contractile action on the vascular smooth muscle cells and an opposite endo-thelium-mediated vasodilatory activity, which is predominant in normal conditions and at physiologic concentrations.[37, 38] Acetylcholine was used in the in vitro experiments in which the role of intact endothelium in the regulation of vascular tone was established[1] and in the first in vivo study showing that acetylcholine induces severe vasospasm in human coronary arteries with significant stenoses.[6] The induction of an endothelium-dependent vasodilatation in canine femoral[39] and coronary[40] arteries after the application of acetylcholine on the arterial adventitia suggests a role of acetylcholine, the mediator of the parasympathetic stimulation, in the modulation of vascular tone. The circadian rhythm of the parasympathetic activity has been suggested as an explanation for the higher incidence in the early morning of acute coronary syndromes, such as vasospastic an-
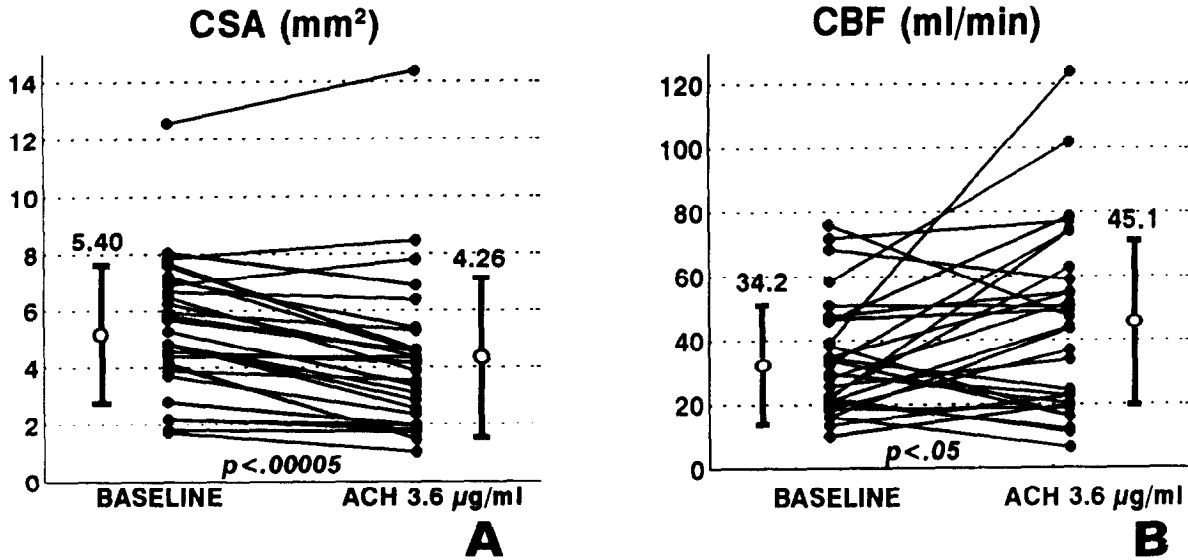
Fig. 8. Cross-sectional area *(CSA)* and coronary blood flow *(CBF)* in baseline conditions (baseline 2) and after infusion of maximal concentration of acetylcholine (ACH 10⁻⁶ M). Note almost uniform decrease in cross-sectional area and large variability of individual flow change.
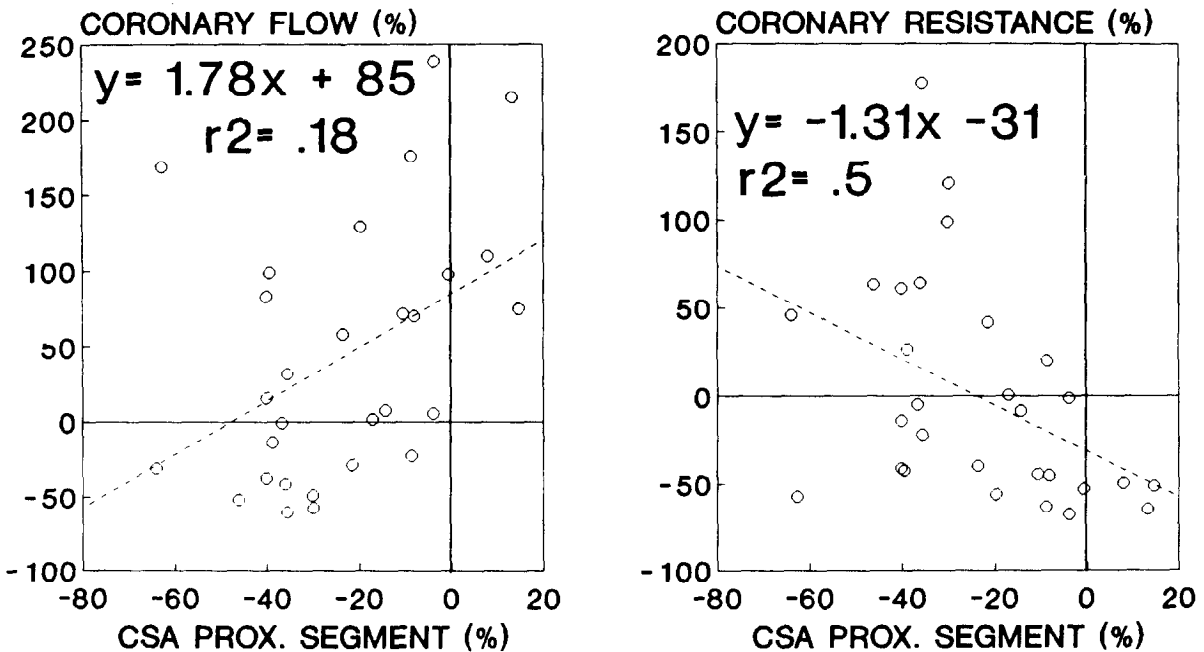
Fig. 9. Linear regression analysis of percent changes in mean cross-sectional area *(CSA)* of proximal segment studied and of percent changes of coronary flow and resistance. Note poor correlation over entire range of measurements.

gina and myocardial infarction. Selective intracoronary infusion of acetylcholine elicited vascular responses comparable to those observed after administration of serotonin,[41] a substance that is released after platelet activation and may contribute to the development of myocardial ischemia in acute coronary syndromes.[17, 42, 43]

In this study concentrations and flow rate of acetylcholine were the same as those used in recent reports[14, 20, 23] in order to facilitate the comparison of results. Papaverine and isosorbide dinitrate were infused selectively in doses sufficient to induce a maximal vasodilatation of the resistance and conductance coronary arteries with a limited systemic effect.[44] With these drugs, the presence of an aspecific impairment of vascular relaxation caused by structural changes of the epicardial and resistance coronary vessels could be excluded.

Cross-sectional area changes. The arteries studied included both angiographically normal and minimally diseased arteries. In this latter group the severity of coronary vasoconstriction was similar to that reported in two comparable series of patients by Zeiher et al.[16, 23] (-27% decrease in the present study vs -29% and -34% cross-sectional area decrease from baseline at the same concentration of acetylcholine). The angiographically normal arteries showed a 16% decrease in cross-sectional area from baseline, similar to the 23% decrease reported by Vrints et al.[12] for normal segments of the left anterior descending coronary artery at this concentration. In the present study the arteries without angiographically visible lesions showed a less pronounced vasoconstriction after infusion of the maximal concentration of acetylcholine. No significant differences were present, however, between arteries with and without angiographically visible wall irregularities. A possible explanation is that the atherogenic factors, which have already induced a severe symptomatic coronary stenosis in our study population, can be sufficient to induce the development of diffuse endothelial damage also in the absence of evident atherosclerotic changes. An alternative explanation is that angiography is not sufficiently sensitive to detect initial atherosclerotic changes. Epicardial and intracoronary ultrasound imaging have shown that diffuse atherosclerotic changes are present in patients with coronary artery disease also in segments that have an angiographically normal lumen and smooth vascular contours.[45-47] Pathologic reports have explained this phenomenon with the presence of an overall vascular enlargement able to preserve a normal vascular lumen despite large areas of wall encroachment.[48] In this study no patient showed a severe focal or diffuse

spasm that induced a critical flow reduction and the development of symptoms and signs of myocardial ischemia. The characteristics of the studied population, including only patients with stable angina and for the vast majority single-vessel coronary disease, may explain the different results observed in previous studies, which report frequent episodes of severe vasoconstriction after acetylcholine.[7, 11, 22] The absence of more than 75% cross-sectional area reduction from the basal measurement suggests that the impairment of flow after acetylcholine infusion is not due to a critical vasoconstriction of the epicardial arteries. A flow limitation caused by focal or diffuse vasoconstriction of small distal branches, not analyzable with quantitative angiography but visually detectable in eight cases, is more difficult to rule out. In these patients, however, the flow changes after infusion of the maximal concentration of acetylcholine were similar to those observed in the remaining cases.

Diffuse vasoconstriction was present after infusion of the maximal concentration of acetylcholine in 90% of the patients studied (26 of 29). A progressive dose response was observed with increasing concentrations of acetylcholine. The proximal and distal segments showed a similar decrease of lumen dimension. A moderate difference between proximal and distal segments was observed only after the intermediate concentration of acetylcholine (cross-sectional area decrease from baseline $-12\% \pm 17\%$ in the proximal segment and $-15\% \pm 15\%$ in the distal segment, $p < 0.05$). At the peak concentration of acetylcholine, however, no differences were observed between proximal and distal arterial segments. Vrints et al.[12] have confirmed the presence of similar changes of proximal and distal segments of the left anterior descending coronary artery. A more significant vasoconstriction after infusion of acetylcholine of the distal coronary segments was reported by Dubois-Randé et al.[49] in a very limited patient population (five cases). The variability of the response to acetylcholine of the proximal and distal segments observed in individual patients can explain this difference and probably reflects a different severity of atherosclerotic involvement of the two segments.

Coronary flow and flow velocity changes. Intracoronary Doppler echography was used to assess coronary flow velocity in this study. Technical improvements have recently increased the reliability of this technique for the assessment of coronary flow velocity. In particular, the large Doppler sample volume and the use of peak blood flow velocity, allowed by the spectral analysis of the signal, avoid changes of the measured velocity in response to minor variations of the

position of the Doppler probe inside the artery. It was thus possible to minimize manipulation and repositioning of the Doppler probe and to avoid excluding patients because of poor quality of the Doppler recordings. The accuracy in the calculation of absolute coronary flow from the corresponding mean flow velocity and cross-sectional area, however, is still limited by the difficulty of obtaining an exact measurement of the mean velocity and by the inability to acquire the two measurements simultaneously. In the presence of rapid changes of flow and cross-sectional area such as those after the injection of a bolus of nitrates, papaverine, or adenosine, the delay between flow velocity measurement and cineangiogram may result in a significant inaccuracy of the flow measurements. In this study no cineangiograms were performed at the maximal effect of the injection of papaverine. For this reason, absolute coronary flow after papaverine infusion could not be calculated. The presence of a larger cross-sectional area at the peak effect of papaverine than in baseline conditions may explain the relatively low coronary flow reserve (2.8 ± 0.8) observed in this study. On the basis of previously reported angiographic measurements after papaverine infusion, a 15% to 20% underestimation of the true flow reserve is expected.[50, 51] In this study, however, papaverine was used only to confirm a normal response of the coronary resistance vessels to a direct smooth muscle vasodilator to exclude structural alterations of the microvasculature as the factor limiting the flow increase.

The lack of simultaneous flow velocity/cross-sectional area measurements is also a serious limitation in explaining the changes observed during infusion of acetylcholine. Variations of flow velocity during infusion of acetylcholine have not been previously reported. With the previous generation of Doppler probes, the Doppler signal was highly dependent on minor changes in the position of the Doppler sample volume.[52] These changes therefore could have been misinterpreted as artifacts because of an unstable position of the catheter in the artery. The previously described characteristics of the Doppler guide wire and the modalities of signal analysis (fast Fourier transform, continuous automatic measurement of peak flow velocity) are ideal conditions for the assessment of these moderate flow velocity variations. Two causes can be suggested for these flow velocity variations: a true change in flow as the result of a variable vasodilatation of the resistance vessels over time or a change of the cross-sectional area at the site of the Doppler sample volume (flow velocity changes but coronary flow remains the same). In the absence of a simultaneous continuous assessment of

lumen cross-sectional area, the mechanism of these flow velocity changes remains speculative. In the near future the combination of intravascular imaging and Doppler ultrasound may allow a continuous assessment of coronary cross-sectional area and flow velocity and facilitate the assessment of the dynamics of the flow-area changes after acute pharmacologic interventions.[53]

In this study the flow velocity changes after acetylcholine infusion have been expressed as a percent change from baseline and not as a percent of the maximal flow increase (after papaverine infusion) because a moderate (−7%) but significant aortic pressure reduction was observed at the peak effect of papaverine. A constant pressure is a prerequisite for a reliable comparison of a flow measured in autoregulatory conditions and during maximal vasodilatation.[54] Recently, the slope of the instantaneous hyperemic diastolic pressure-flow relationship has been used in animal experiments as an index of coronary conductance and has been shown to be independent of changes in aortic pressure, heart rate, and cardiac contractility.[55, 56] The instantaneous hyperemic diastolic pressure–flow velocity relationship can be reproducibly assessed also in human beings.[57, 58] The possibility of measuring an index of coronary conductance independent of the hemodynamic conditions at the time of assessment during different pharmacologic interventions and during maximal vasodilatation is of great potential interest (Fig. 10). This approach, however, still requires more extensive clinical validation.

Experimental data have demonstrated that atherosclerotic animals show an abnormal endothelium-dependent vasodilatation of the coronary resistance arteries, despite the absence of structural atherosclerotic lesions.[24] The comparison of the flow response to acetylcholine in patients with coronary artery disease and in control subjects has confirmed an impaired flow increase in the patients with coronary artery disease, despite the absence of significant lesions of the epicardial coronary arteries.[23] In this study a large variability in the flow changes was observed after infusion of the highest doses of acetylcholine. A dose-dependent vasodilatation after acetylcholine infusion was present in most cases, with flow increase up to three times the baseline flow. In 10 patients, however, a flow decrease was observed after infusion of the maximal concentration of acetylcholine. The mean flow increase from baseline was +44% ± 24% at the maximal concentration of acetylcholine, an increase much lower than the flow increase observed in normal control subjects at the same acetylcholine concentration.[23, 59] No clinical or angiographic pre-

PAPAVERINE 7 MG i.c.

ACETYLCHOLINE 10-6 M
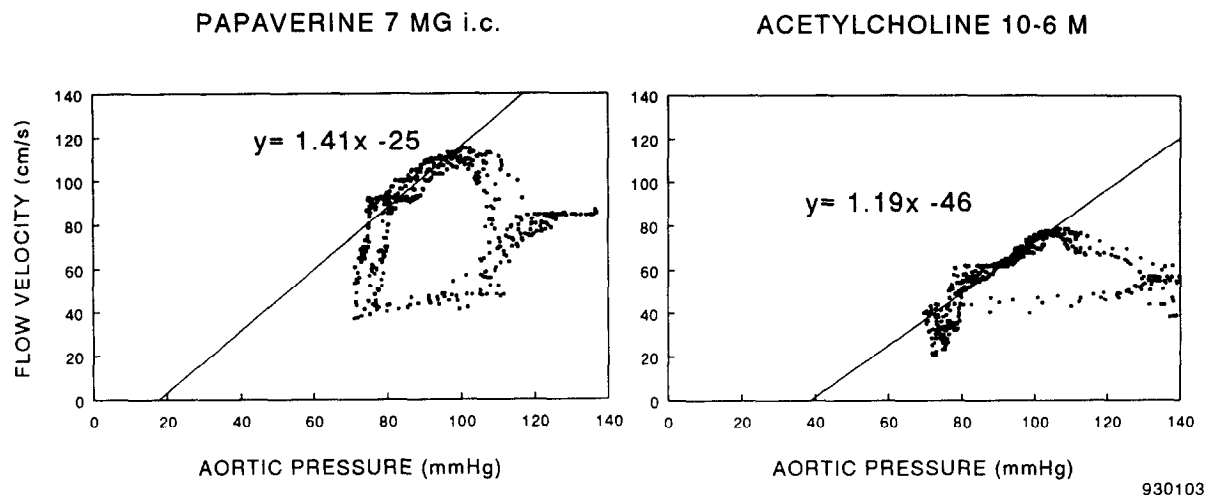
Fig. 10. Pressure-flow velocity loop of 4 consecutive beats at peak effect of injection of papaverine and after 5 minutes of infusion of highest concentration of acetylcholine. Regression line has been calculated from middiastolic data points. Note steeper slope of pressure-velocity relationship after papaverine infusion and lower pressure intercept.

dictors of these large individual differences could be observed.

A reduction of the endothelium-dependent relaxation is present in animals chronically maintained on an atherogenic diet with a high content of cholesterol.[60, 61] In patients with hypercholesterolemia without angiographic evidence of coronary artery disease, impaired endothelium-mediated vasodilatation of the epicardial coronary arteries and of the resistance coronary vessels has been demonstrated.[16] Thirteen of the study patients had a total cholesterol level of 6.4 mmol/L or (250 mg/dl) greater. This group, however, showed no significant differences in terms of flow increase and vascular diameter changes after acetylcholine infusion. The importance of the relative amounts of HDL and low-density lipoprotein cholesterol has recently been reported to correlate more closely than total cholesterol with the degree of impairment of the endothelium-mediated vasodilatation.[62] In the studied group, however, the use of the HDL/total cholesterol ratio did not identify a subset of patients with a different response to acetylcholine.

Correlation of coronary area-flow changes after acetylcholine infusion. In this study mean arterial cross-sectional area of the epicardial arteries and coronary flow have been considered as independent indices of response of conductance and resistance coronary vessels. This assumption has three potential limitations: (1) the possibility that a flow-limiting vasoconstriction occurs in an epicardial artery, (2) the development of a vasodilatation of the epicardial ar-

teries secondary to the increase of flow, and (3) the use of a cross-sectional area measured along the analyzed segment to calculate coronary flow. In spite of all these potential reasons for interdependence, the flow or flow resistance changes after infusion of the maximal concentration of acetylcholine showed only a poor correlation with the corresponding cross-sectional area changes. The discrepancy between flow and cross-sectional area reflects a different response of the conductance and resistance arteries to acetylcholine. The large arteries are the preferential target of the atherosclerotic process. At this level the presence of intimal thickening may constitute a barrier to the diffusion of nitric oxide from the endothelial cells to the muscular media.[63] A macrophagic infiltration or the presence of a lipidic component of the intimal plaque may also accelerate the degradation of nitric oxide and prevent its action on the underlying muscular layer.[37] The importance of these mechanisms in atherosclerotic human arteries is indirectly confirmed by the frequent development of focal vasoconstriction after acetylcholine infusion. Myocardial perfusion is regulated predominantly by resistance arteries with a diameter of less than 200 μm.[64] These arteries do not show signs of atherosclerotic involvement as determined by histologic examination, suggesting that biochemical or ultrastructural changes are the most likely mechanisms underlying the abnormal endothelium-dependent relaxation. These observations have potential clinical implications. Prolonged treatment aimed at the regression of the

atherosclerotic intimal changes may be required to restore an impaired endothelium-mediated response when the presence of an intimal barrier is the main operative mechanism.[65-67] On the contrary, acute pharmacologic interventions or brief treatment may be sufficient to normalize the endothelial function when metabolic abnormalities are involved. The possibility of normalizing the endothelial response in hypercholesterolemia with a short-term infusion of L-arginine has been shown in animal experiments,[68] as well as in human coronary arteries.[49, 69] Similarly, different classes of drugs have shown the ability to restore a normal endothelium-mediated vascular reactivity in experimental animals.[70-72]

Conclusions. In angiographically normal or minimally diseased arteries of patients with symptomatic coronary artery disease, very low doses of acetylcholine induced a significant coronary vasoconstriction of the epicardial coronary arteries. The resistance vessels showed a variable response, with a trend toward moderate vasodilatation (flow increase in two of three of the patients after infusion of the highest concentration of acetylcholine). The presence of hypercholesterolemia or wall irregularities was not correlated with the diameter/flow changes after acetylcholine infusion. The poor correlation observed between cross-sectional area and flow changes after acetylcholine infusion suggests that different mechanisms induce impairment of endothelium-mediated vasodilatation in conductance and resistance coronary vessels.

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Usefulness of changes in exercise tolerance induced by nitroglycerin in identifying patients with syndrome X

Two exercise tests, one under basal conditions and one after sublingual nitroglycerin (NTG), were performed in 39 patients with stable angina pectoris—16 with critical coronary stenoses and 23 with normal coronary arteries (syndrome X). Under basal conditions, times at ischemic threshold, at peak exercise, and at complete ECG recovery were similar in the two groups. Peak ST depression was significantly higher in patients with coronary artery disease (CAD). In a similar proportion of patients, ST-segment depression developed earlier or at a low heart rate. Patterns of heart rate, blood pressure, and rate-pressure product during exercise and recovery were also similar. After NTG an increase in the ischemic threshold was observed in a significantly higher proportion of patients with CAD (93.8% vs 39.1%). Furthermore, a subgroup of patients with syndrome X showed a worsening of exercise performance. This suggests that NTG does not directly affect small coronary vessels. Our results confirm that no relevant differences exist in exercise responses between patients with CAD and those with syndrome X under basal conditions. NTG-induced changes in this response could be useful in identifying patients with normal coronary arteries. Moreover, this test could be used as a guide to therapeutic approaches. (Am Heart J 1994;127:531-5.)

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It has been suggested that the course of ST-segment depression during and after exercise may be useful in distinguishing patients with anginal pain with and without angiographically documented coronary lesions.[1-4] This contradicts the findings of other inves-