JACC Vol. 27, No. 2
February 1996:304–10

Flow-Mediated Vasodilation of Human Epicardial Coronary Arteries: Effect of Inhibition of Nitric Oxide Synthesis

NOBUO SHIODE, MD, NOBUYUKI MORISHIMA, MD, KENSYO NAKAYAMA, MD, TOGO YAMAGATA, MD, HIDEO MATSUURA, MD, GORO KAJIYAMA, MD

Hiroshima, Japan

Objectives. **This study sought to investigate the role of nitric oxide, an endothelium-derived relaxing factor, in flow-mediated vasodilation in human epicardial coronary arteries.**

Background. **Endothelium-derived relaxing factors may be released from the coronary artery endothelium in response to increases in blood flow.**

Methods. **We studied the effect of the nitric oxide synthesis inhibitor N^G-monomethyl-L-arginine (L-NMMA) on the flow-mediated vasodilation of epicardial coronary arteries in 12 patients, using quantitative angiographic and Doppler flow velocity measurements. Adenosine at 100 μg/min was infused into the left anterior descending coronary artery to test the dilator response of the proximal artery to increases in blood flow. Acetylcholine at 3 and 30 μg/min was infused into the left coronary ostium to determine endothelium-dependent vasodilation of the proximal left anterior descending artery. Adenosine and acetylcholine were infused before and after the intracoronary infusion of L-NMMA (25 μmol/min for 5 min).**

Results. **Infusion of L-NMMA caused a significant decrease in the baseline diameter of the proximal left anterior descending artery (from 2.90 ± 0.14 to 2.74 ± 0.13 mm [mean ± SEM], $p < 0.01$). Adenosine increased coronary blood flow before and after L-NMMA (+399.5 ± 27.5% and +511.9 ± 33.3%, respectively). Flow-mediated vasodilation was observed in the proximal left anterior descending artery before and after L-NMMA (+9.2 ± 1.5%, $p < 0.01$ and +8.6 ± 2.1%, $p < 0.01$, respectively). A dose of 3 μg/min of acetylcholine significantly dilated the proximal left anterior descending artery before L-NMMA (+7.6 ± 1.0%, $p < 0.01$), but acetylcholine-induced vasodilation was attenuated after L-NMMA (−1.8 ± 1.0%).**

Conclusions. **Our data suggest that nitric oxide modulates basal coronary artery tone but that mediators other than nitric oxide may be responsible for the flow-mediated vasodilation of human epicardial coronary arteries.**

(J Am Coll Cardiol 1996;27:304–10)

Dilation of large conduit arteries has been shown to occur in response to increases in blood flow (1). This phenomenon has been observed in isolated, perfused canine coronary artery segments in situ (2) and during reactive hyperemia following brief coronary artery occlusion (3,4). Inoue et al. (5) have demonstrated that the flow-mediated dilation of epicardial coronary arteries in conscious dogs is attenuated by removal of the endothelium. They have suggested that the endothelium modifies smooth muscle tone, depending on the level of coronary blood flow. Rubanyi et al. (6) have found that, in perfused canine femoral arterial segments, increases in flow will trigger the release of a vasodilating substance from endothelial cells that has characteristics similar to the endothelium-derived relaxing factor released by acetylcholine.

Flow-mediated vasodilation also has been demonstrated in human femoral, brachial and coronary arteries (7–9). Flow-mediated vasodilation is considered an important regulatory mechanism in the coronary circulation in humans. Indeed, physiologic stimuli that lead to an increase in coronary blood flow, including the use of papaverine or adenosine (10–12), bicycle exercise testing (13), cold pressor testing (14,15), and mental arithmetic (16), all have been shown to increase coronary artery diameter in patients without atherosclerosis or risk factors for coronary artery disease. The vascular tone of intact coronary arteries in humans appears to be markedly modulated by changes in blood flow. However, an impaired response is seen in patients with atherosclerosis. The parallel response of coronary arteries to physiologic stimuli and to acetylcholine led investigators to the conclusion that endothelium-derived relaxing factor may be released from the endothelium in response to these stimuli.

Endothelium-derived relaxing factor was first demonstrated more than a decade ago by Furchgott and Zawadzki (17). The first proof that nitric oxide was at least one of the relaxing factors resulted from studies by Palmer et al. (18). However, the role of nitric oxide in flow-mediated vasodilation is still unknown. The goal of the present study was to evaluate the role of nitric oxide in flow-mediated vasodilation in human epicardial coronary arteries.

From the First Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan.

Manuscript received April 26, 1995; revised manuscript received August 21, 1995, accepted September 8, 1995.

Address for correspondence: Dr. Nobuo Shiode, The First Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi-cho, Minami-ku, Hiroshima 734, Japan.

0735-1097/96/$15.00
0735-1097(95)00465-3

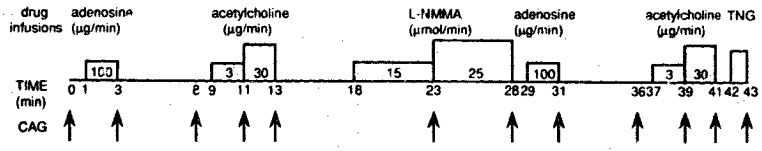


Figure 1. Sequence of drug infusions and timing of coronary arteriography (CAG), which was performed just after the end of each infusion. L-NMMA = N^G-monomethyl-L-arginine; TNG = nitroglycerin.

Methods

Study patients. Twelve patients (three women, nine men; mean [±SEM] age 57 ± 3 years, range 40 to 69) undergoing coronary angiography for the diagnosis of a chest pain syndrome were studied. All patients had angiographically normal smooth epicardial coronary arteries, normal left ventricular function (contrast ventriculographic ejection fraction ≥50%) and normal coronary flow reserve. Written informed consent was obtained from all patients before the diagnostic angiogram, and all studies were approved by the Human Investigation Ethical Committee of the University of Hiroshima.

Study design. Antianginal therapy was discontinued 48 h before catheterization except for the unrestricted use of sublingual nitroglycerin, which was withheld 1 h before catheterization. Patients were brought to the catheterization laboratory in the fasting state after premedication with hydroxyzine (25 mg intramuscularly) and promethazine hydrochloride (25 mg intramuscularly).

Diagnostic right and left heart catheterization and coronary angiography were performed through a standard percutaneous femoral approach. After vascular access had been obtained, 10,000 U of heparin was given intravenously. A 7F guide catheter was introduced into the left main coronary artery. A 3F infusion catheter (Tracker 325, Target Therapeutics) was advanced through the guide catheter into the middle segment of the left anterior descending coronary artery. A 0.014-in. (0.36-mm) Doppler flow guide wire (FloWire, Cardiometrics) was then advanced through the infusion catheter into the middle segment of the artery. The tip of the Doppler flow guide wire was positioned distal to the end of the infusion catheter. The diameter of the central lumen of this infusion catheter is 0.024 in. (0.61 mm), so that we could infuse vasoactive agents through the infusion catheter in the presence of the 0.014-in. Doppler flow wire.

Protocol. After completion of the diagnostic catheterization, the following interventions were performed (Fig. 1): After control conditions had been established, adenosine was infused at a dose of 100 μg/min into the distal left anterior descending coronary artery through the infusion catheter (1 ml/min for 2 min). After reestablishment of control conditions, serial infusions of intracoronary acetylcholine (3 and 30 μg/min) were delivered into the left coronary ostium through the guide catheter (1 ml/min, each for 2 min). Then L-NMMA, at doses of 15 and 25 μmol/min, was infused into the left coronary ostium through the guide catheter (1 ml/min, each for 5 min). Infusions of adenosine and acetylcholine were repeated after the infusion of L-NMMA (25 μmol/min). Finally, 200 μg/min of nitroglycerin was infused into the left coronary ostium.

We waited 5 min after each infusion of adenosine and acetylcholine before beginning the next drug infusion. Coronary arteriography was performed under control conditions and just after the end of each infusion. Heart rate, arterial pressure, coronary blood flow velocity and electrocardiogram (ECG) were monitored continuously through each infusion. Each measurement also was recorded under steady-state conditions.

Quantitative coronary arteriography. Coronary cineangiograms were recorded on 35-mm cinefilm (30 frames/s) using a Siemens cineangiographic system after selection of the view that allowed the best visualization of the left anterior descending coronary artery. Nonionic contrast medium was injected into the left coronary artery at the rate of 5 to 10 ml/s to a total of 7 to 10 ml. A power injector (Medrad) was used to optimize the quality and reproducibility of the opacification (19). Angiograms were obtained while the patients held their breath to avoid possible effects of respiration (20). A segment of left anterior descending coronary artery that was free of side branches and was at least 5 mm proximal to the infusion catheter tip (the site of infusion of the adenosine) was selected for quantitative analysis (Fig. 2). This segment was exposed to changes in blood flow but was not directly exposed to adenosine. Serial infusions of acetylcholine were delivered through the guide catheter to this segment so that the same segment could be analyzed for the response to increasing blood flow and to acetylcholine. A segment of the artery at least 10 mm distal to the infusion catheter tip was selected for analysis of

Figure 2. Coronary artery segments analyzed by quantitative angiography. A proximal segment of the left anterior descending (LAD) coronary artery (a segment proximal to the end of the infusion catheter) was analyzed for flow-mediated effects. A segment at the tip of the Doppler wire was analyzed to estimate the volume from flow velocity measurements. A segment distal to the end of the infusion catheter (Distal LAD) was analyzed for the direct effect of adenosine. A proximal segment of the left circumflex coronary artery (LCx) was analyzed to exclude a second-pass effect of adenosine.

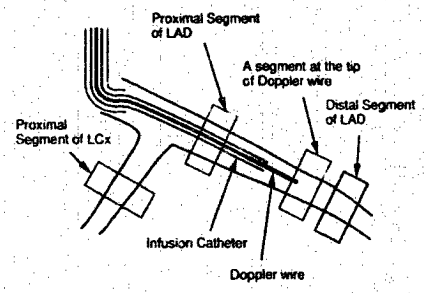


Table 1. Hemodynamic Measurements

					ACh (μg/min)		
	L-NMMA	Baseline 1	Adenosine	Baseline 2	3	30	TNG
HR (beats/min)	Before	64 ± 3	65 ± 3	64 ± 3	65 ± 3	66 ± 3	
	After	64 ± 3	63 ± 3	64 ± 3	63 ± 3	62 ± 3	68 ± 3*
BP (mm Hg)	Before	100 ± 3	100 ± 4	100 ± 3	100 ± 3	97 ± 3	
	After	103 ± 4	102 ± 4	103 ± 4	103 ± 3	100 ± 4	94 ± 3*
RPP (beats/min · mm Hg)	Before	9,046 ± 409	9,242 ± 572	9,114 ± 453	9,082 ± 446	9,079 ± 512	
	After	9,440 ± 478	9,363 ± 521	9,181 ± 531	9,406 ± 559	9,220 ± 609	8,855 ± 400

*$p < 0.01$ versus baseline control diameter (Baseline 1) before N^2-monomethyl-L-arginine (L-NMMA). Data presented are mean value ± SEM. ACh = acetylcholine; Baseline 2 = repeat control diameter; BP = mean blood pressure; HR = heart rate; RPP = rate–pressure product; TNG = nitroglycerin.

the direct effects of adenosine. The proximal segment of the left circumflex coronary artery, which was not exposed to adenosine or to increased blood flow, was analyzed to exclude a second-pass effect of the adenosine.

Measurement of the lumen diameter of each arterial segment was performed quantitatively with the aid of a computer-assisted coronary angiographic analysis system. The arterial segments under study were videodigitized at end-diastole and then stored in the cardiac image analysis system (Cardio 500, Kontron Instruments). Automated counter detection was performed by a geometric edge differentiation technique similar to the method described by Reiber et al. (21). The diameter of the segment of interest was measured, and the averaged value from triplicate measurements was used for later analysis. A 7F Judkins catheter was used to calibrate the arterial diameter in millimeters. The arterial diameter measurements were done without knowledge of the clinical characteristics of the patients.

Measurements of coronary blood flow velocity and estimation of coronary blood flow. The Doppler guide wire with a 12-MHz piezoelectric transducer at the tip (FloWire, Cardiometrics) was used to measure the coronary artery blood flow velocity. The use of the Doppler flow guide wire for this purpose has been validated previously (22). Continuous-flow velocity profiles using the 12-MHz pulsed Doppler velocimeter (FloMap, Cardiometrics), along with simultaneous ECGs and aortic pressures, were displayed on the video monitor and recorded continuously on 0.5-in. VHS (12.7-mm) videotape. Changes in coronary blood flow evoked by the vasoactive agents were estimated from the product of the mean coronary blood flow velocity and the cross-sectional area 2 to 3 mm distal to the tip of the Doppler flow guide wire. This distance was selected because the Doppler transducer of this device has a range gate depth of 4.2 mm.

Preparation of drugs. Acetylcholine (Daiichi Pharmaceutical Corp.) was dissolved in physiologic saline at a concentration of 3 or 30 μg/ml. Acetylcholine was dissolved immediately before use because it is unstable in solution. Adenosine (Sigma Chemical Co.) was dissolved in physiologic saline at a concentration of 100 μg/ml. The L-NMMA (Sigma) was dissolved in physiologic saline at a concentration of 15 or 25 μmol/ml. Nitroglycerin (Nihonkayaku Corp.) was dissolved in physiologic saline and infused at a concentration of 200 μg/min. The infusion of each drug was performed with an infusion pump (CFV 3000, Nihonkoden) at a rate of 1 ml/min.

Statistical analysis. All data are expressed as mean value ± SEM. Changes in lumen diameter are expressed as the percent change from the baseline control value. Two-way repeated-measures analysis of variance (ANOVA) was used to compare the effects of adenosine and acetylcholine on the systemic hemodynamic and coronary vascular responses before and after the infusion of L-NMMA. One-way repeated-measures ANOVA was used to assess the effects of the L-NMMA infusions on the systemic hemodynamic and coronary vascular responses. If the ANOVA showed a significant difference between the means, the level of significance was determined by contrast analysis using the GLM procedure of the SAS statistical software. Paired data were compared by paired *t* tests. A value of $p < 0.05$ was considered significant.

Results

Hemodynamic variables. There were no significant changes in heart rate, mean arterial pressure or rate–pressure product during the infusions of adenosine or acetylcholine before or after L-NMMA (Table 1). Nitroglycerin increased heart rate ($p < 0.01$) and decreased mean arterial pressure ($p < 0.01$) compared with baseline values before L-NMMA.

Effect of L-NMMA on baseline coronary blood flow and coronary diameter. Infusion of L-NMMA (15 μmol/min) produced no significant change in coronary blood flow. The higher dose of L-NMMA (25 μmol/min) caused a decrease in baseline coronary blood flow, from 54.3 ± 6.1 to 45.5 ± 5.1 ml/min ($p < 0.01$) (Table 2).

The diameters of the proximal and distal left anterior descending coronary artery and the proximal left circumflex artery decreased significantly from baseline values before L-NMMA by 5.3 ± 1.3%, 3.9 ± 1.2% and 7.2 ± 1.6%, respectively (Fig. 3).

Coronary blood flow changes during serial infusions of adenosine and acetylcholine. Infusion of adenosine into the distal left anterior descending coronary artery produced significant increases in coronary blood flow before and after L-NMMA (Table 2). However, the percent increase after L-NMMA was greater than that before L-NMMA ($p < 0.01$).

Table 2. Changes in Coronary Blood Flow

	Before L-NMMA		After L-NMMA	
	CBF (ml/min)	Change (%)	CBF (ml/min)	Change (%)
Baseline 1	54.3 ± 6.1		45.5 ± 5.1†	
Adenosine	272.7 ± 34.7*	399.5 ± 27.5	278.4 ± 36.8*	511.9 ± 33.3†
Baseline 2	54.3 ± 5.9	0.8 ± 1.3	45.2 ± 5.0	−0.1 ± 1.3
ACh 3 μg/min	103.2 ± 66.2*	78.7 ± 13.2	77.6 ± 11.4*	70.7 ± 12.3
ACh 30 μg/min	170.8 ± 26.1*	204.5 ± 20.8	131.6 ± 18.5*	171.8 ± 19.0

*$p < 0.01$ versus baseline control diameter (Baseline 1). †$p < 0.01$ versus before L-NMMA. Data presented are mean value ± SEM. CBF = coronary blood flow; other abbreviations as in Table 1.

Serial infusions of acetylcholine caused significant increases in coronary blood flow before and after L-NMMA.

Paired response of proximal left anterior descending coronary artery to adenosine and acetylcholine before and after L-NMMA. Before L-NMMA, the proximal left anterior descending coronary artery dilated in response to increases in blood flow during the infusion of adenosine (9.2 ± 1.5%, $p < 0.01$) (Fig. 4A). Acetylcholine infusion (3 μg/min) also caused a significant dilation in the proximal segment of the artery (7.6 ± 1.0%, $p < 0.01$).

Flow-mediated dilation during adenosine infusion after L-NMMA was similar to that before L-NMMA. In contrast, after L-NMMA, the coronary diameter response to serial infusions of acetylcholine was significantly different from that before L-NMMA. Acetylcholine infusion (3 μg/min) did not produce significant dilation of the proximal segment of the left anterior descending coronary artery ($p = 0.3445$). Thus, L-NMMA attenuated acetylcholine-induced endothelium-dependent dilation but had no effect on flow-mediated vasodilation.

Direct drug-induced dilation in distal left anterior descending coronary artery and proximal left circumflex artery. The segment of left anterior descending coronary artery distal to the tip of the infusion catheter dilated in response to adenosine by 20.5 ± 3.6% ($p < 0.01$) before L-NMMA and by 21.0 ±

Figure 3. Effect of intracoronary infusion of N^G-monomethyl-L-arginine (L-NMMA) (15 and 25 μmol/min) and nitroglycerin (TNG) (200 μg/min) on baseline coronary diameter of **(A)** proximal left anterior descending coronary artery (LAD), **(B)** distal left anterior descending coronary artery and **(C)** proximal left circumflex coronary artery (LCx). C = baseline (before L-NMMA). *$p < 0.01$ versus baseline diameters before L-NMMA. Data shown are mean value ± SEM.

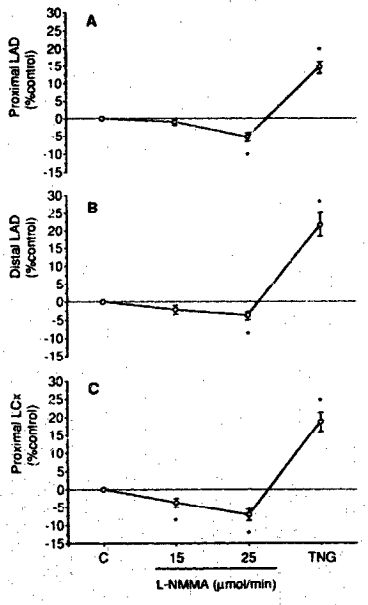
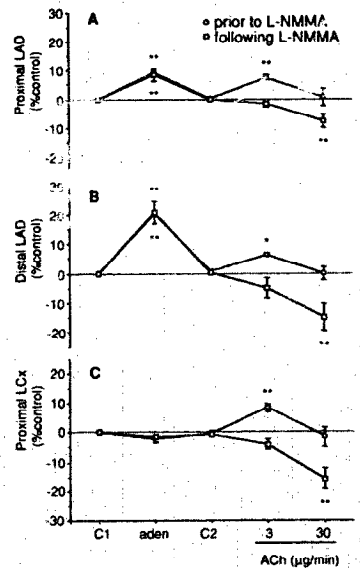


Figure 4. Sequential coronary vasomotor response of **(A)** proximal left anterior descending coronary artery (LAD), **(B)** distal LAD and **(C)** proximal left circumflex coronary artery (LCx) to serial infusions of adenosine (aden) (100 μg/min) and acetylcholine (ACh) (3 and 30 μg/min) before and after N^G-monomethyl-L-arginine (L-NMMA). C1 = baseline control diameter; C2 = repeat control diameter. *$p < 0.05$, **$p < 0.01$ versus baseline control diameter. Data shown are mean value ± SEM.



3.5% ($p < 0.01$) after L-NMMA (Fig. 4B). There was no significant dilation of the proximal segment of the left circumflex artery during adenosine infusion before or after L-NMMA (Fig. 4C), thus excluding a significant direct second-pass effect of adenosine on the proximal segment of the left anterior descending coronary artery.

Acetylcholine infusion (3 μg/min) caused significant dilation in the distal segment of left anterior descending coronary artery and the proximal segment of left circumflex artery before L-NMMA. However, this endothelium-dependent, acetylcholine-induced dilation was attenuated after infusions of L-NMMA. The higher dose acetylcholine infusion (30 μg/min) produced no significant changes in coronary artery diameter before L-NMMA but caused decreases in the diameter of the distal left anterior descending coronary artery and the proximal left circumflex artery after L-NMMA.

Response to endothelium-independent vasodilator nitroglycerin. Nitroglycerin infusion induced significant dilation of the proximal (14.6 ± 1.8%) and distal left anterior descending coronary artery (21.7 ± 3.3%) and the proximal left circumflex artery (18.6 ± 2.6%) (Fig. 3).

Discussion

The major findings of the present study are that L-NMMA, a specific inhibitor of nitric oxide synthesis, caused a decrease in baseline coronary diameter and attenuated acetylcholine-induced endothelium-dependent coronary dilation. However, L-NMMA had no effect on flow-mediated coronary dilation. These findings suggest that there is basal release of nitric oxide in human epicardial coronary arteries but that nitric oxide does not regulate flow-mediated vasodilation. Flow-mediated dilation may be mediated by endothelium-derived relaxing factors other than nitric oxide.

Effect of inhibition of nitric oxide synthesis on flow-mediated vasodilation: comparison with other studies. To our knowledge, this is the first study to report the effects of a nitric oxide synthesis inhibitor on flow-mediated vasodilation in human epicardial coronary arteries. A limited number of in vivo studies have examined the effects of the inhibition of nitric oxide synthesis on flow-mediated epicardial coronary vasodilation in animals. Canty and Schwartz (23) reported that in conscious dogs, inhibiting nitric oxide production by N-nitro-L-arginine methyl ester (L-NAME) had no effect on epicardial coronary dilation in response to increases in blood flow induced by adenosine at a constant heart rate but that the epicardial artery dilation in response to increases in blood flow induced by pacing was attenuated by L-NAME. Recently, Smith et al. (24) reported that L-NAME fails to attenuate flow-mediated dilation in canine epicardial coronary arteries. Our results with L-NMMA are similar to the findings of Canty and Schwartz (23) and Smith et al. (24) and indicate that flow-mediated vasodilation is not dependent on nitric oxide production in epicardial coronary arteries.

In contrast to the results of our study, Kuo et al. (25) showed that flow-mediated vasodilation in isolated subepicardial porcine arterioles (~80-μm rest diameter) is abolished by inhibiting nitric oxide production with L-NMMA. A number of studies have demonstrated differential responses of coronary conduit and resistance vessels to pharmacologic stimuli, such as thrombin, vasopressin and nitrate (26,27). The mechanisms responsible for flow-mediated vasodilation in each class of vessel may be different, and nitric oxide–dependent mechanisms may predominate in coronary resistance vessels.

Lieberman et al. (28) reported that L-NMMA reduces flow-mediated vasodilation and acetylcholine-induced vasodilation in human brachial arteries. They suggested that flow-mediated dilation of human brachial artery is partially mediated by nitric oxide. However, endothelium-dependent vasodilator mechanisms may differ among different vascular beds in humans (29). Therefore, vasodilator mechanisms induced by increasing flow may differ between the coronary and brachial artery beds.

Mechanisms of flow-mediated vasodilation in human epicardial coronary arteries. Although our results indicate that nitric oxide production is not required for the flow-mediated vasodilation of epicardial coronary arteries, we did not identify the other mediators that may be responsible for these responses. In previous studies, it has been demonstrated that flow-mediated effects are abolished by denuding the endothelium in vitro (30) and in vivo (5). Although prostacyclins or other prostanoids may affect basal tone, previous studies have shown that they are unlikely to be mediators of flow-mediated vasodilation (4,6,31). Several investigators have reported that a soluble hyperpolarizing factor is released in response to a variety of endothelium-dependent agonists (32,33). Although this factor has not been identified, it could explain the flow-induced dilation observed in the present study. Another possibility is that the flow-mediated vasodilation of epicardial coronary arteries is mediated by direct hyperpolarization of the endothelial cell by luminal shear stress (34). It has been proposed that activation of a hyperpolarizing potassium current within the endothelium may produce dilation in response to increases in flow (35). This mechanism has been demonstrated in cultured endothelial cells, but its importance in vivo remains to be defined.

Basal release of nitric oxide. Several studies support the concept that the intact coronary vascular bed is maintained under a constant dilating tone by basal, agonist-independent release of endothelium-derived relaxing factor (36–38). Lefroy et al. (39) demonstrated that there is basal release of nitric oxide in human epicardial coronary arteries and resistance vessels. In our study, L-NMMA caused decreases in the basal diameter of the proximal and distal epicardial coronary arteries and decreases in basal coronary blood flow. This finding suggests that there is basal release of nitric oxide in the proximal and distal epicardial arteries and resistance vessels in humans.

Study limitations. We used 15 and 25 μmol/min infusions of L-NMMA for 5 min. Lefroy et al. (39) reported that 4, 10 and 25 μmol/min infusions of L-NMMA for 5 min abolish acetylcholine-induced dilation of human epicardial coronary arteries. We also observed that endothelium-dependent vaso-

dilation induced by a 3 μg/min infusion of acetylcholine was attenuated after L-NMMA. Thus, at the dose we used, L-NMMA-attenuated epicardial coronary dilation caused by agonist-induced nitric oxide production. In contrast, acetylcholine-induced increases in coronary blood flow were not attenuated after L-NMMA infusions. Recent studies in humans (39) and in dogs (40) have shown that acetylcholine-induced dilation of resistance vessels is not abolished by nitric oxide synthesis inhibitors. This finding is in keeping with the results of our study, in which L-NMMA did not attenuate acetylcholine-induced increases in blood flow.

Although previous studies have suggested that the coronary artery flow–diameter relation is linear (4,10), the effects of inhibiting nitric oxide production on this relation are unknown. High levels of flow may overcome the blockade of nitric oxide production produced by L-NMMA. Studies of flow–diameter relation performed over a wide range of flows will be required to address this question.

Finally, L-NMMA caused a decrease in the basal coronary artery diameter, indicating that there is a basal release of nitric oxide in human epicardial coronary arteries. However, L-NMMA caused a significant decrease in basal coronary artery blood flow. This finding suggests that the decrease in basal diameter after L-NMMA may be caused by reductions in shear stress and not by the inhibition of the basal release of nitric oxide in the arterial beds. Smith et al. (24) have demonstrated that in conscious dogs, inhibiting nitric oxide production with L-NAME reduces coronary artery diameter under rest conditions, although coronary blood flow is not changed. It was impossible in our study in patients to keep basal coronary blood flow at a constant rate both before and after L-NMMA.

Conclusions. Inhibition of nitric oxide synthesis by the intracoronary infusion of L-NMMA decreased the basal epicardial coronary artery diameter but did not attenuate flow-mediated vasodilation. These findings indicate that nitric oxide modulates basal tone but that mediators other than nitric oxide may be responsible for the flow-mediated vasodilation of human epicardial coronary arteries.

We thank Koichi Tanaka, MD, Yuji Yasunobu, MD, Masaya Kato, MD, Akito Hiraoka, MD, Hitoshi Fujiwara, MD, Shinji Karakawa, MD and Masahiro Kawanishi, MD for offering technical assistance and many helpful comments. Furthermore, we gratefully acknowledge the assistance of the nurses and technicians of the catheterization laboratory at Hiroshima University Hospital.

References

1. Schretzenmayr A. Uber kreislaufregulatorische Vorgange an den grossen Arterien bei der Musclearbeit. Pflugers Arch 1933;232:743–8.
2. Gerova M, Gero J, Barta E, Dolezel S, Smiesko V, Levicky V. Neurogenic and myogenic control of conduit coronary artery: a possible interference. Basic Res Cardiol 1981;76:503–7.
3. Hintze TH, Vatner SF. Reactive dilation of large coronary arteries in conscious dogs. Circ Res 1984;54:50–7.
4. Holtz J, Forstermann U, Pohl U, Giesler M, Bassenge E. Flow-dependent, endothelium-mediated dilation of epicardial coronary arteries in conscious dogs: effects of cyclooxygenase inhibition. J Cardiovasc Pharmacol 1984;6:1161–9.
5. Inoue T, Tomoike H, Hisano K, Nakamura M. Endothelium determines flow-dependent dilation of the epicardial coronary artery in dogs. J Am Coll Cardiol 1988;11:187–91.
6. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250:H1145–9.
7. Liao JK, Bettmann MA, Sandor T, Tucker JI, Coleman SM, Creager MA. Differential impairment of vasodilator responsiveness of peripheral resistance and conduit vessels in humans with atherosclerosis. Circ Res 1991;68:1027–34.
8. Sinoway LI, Hendrickson C, Davidson WR Jr, Prophet S, Zelis R. Characteristics of flow-mediated brachial artery vasodilation in human subjects. Circ Res 1989;64:32–42.
9. Celermajer DS, Sorensen KE, Grooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1993;340:1111–5.
10. Cox DA, Vita JA, Treasure CB, et al. Atherosclerosis impairs flow-mediated dilation of coronary arteries in humans. Circulation 1989;80:458–65.
11. Nabel EG, Selwyn AP, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. J Am Coll Cardiol 1990;16:349–56.
12. Drexler H, Zeiher AM, Wollschlager H, Meinertz T, Just H, Eanzei T. Flow-dependent coronary artery dilation in humans. Circulation 1989;80:466–74.
13. Gordon JB, Ganz P, Nabel EG, et al. Atherosclerosis and endothelial function influence the coronary response to exercise. J Clin Invest 1989;83:1946–52.
14. Nabel EG, Ganz P, Gordon JB, Alexander RW, Selwyn AP. Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. Circulation 1988;77:43–52.
15. Zeiher AM, Drexler H, Wollschlager H, Saubier BHJ. Coronary vasomotion in response to sympathetic stimulation in humans: importance of the functional integrity of the endothelium. J Am Coll Cardiol 1989;325:1181–90.
16. Yeung AC, Vekshtein VI, Krantz DS, et al. The effect of atherosclerosis on the vasomotor response of coronary arteries to mental stress. N Engl J Med 1991;325:1151–6.
17. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–6.
18. Palmer RMJ, Ashton D, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988;333:664–6.
19. Gardiner GA, Meyerovitz MF, Bext LM, et al. Selective coronary angiography using a power injector. Am J Radiol 1986;146:831–3.
20. Mancini GBJ, Simon SB, McGillen J, LeFree M, Friedman H, Vogel RA. Automated quantitative coronary arteriography: morphologic and physiologic validation in vivo of a rapid digital angiographic method. Circulation 1987;75:452–60.
21. Reiber JHC, Serruys PW, Kooijman CJ, et al. Assessment of short-, medium-, and long-term variations in arterial dimensions from computer-assisted quantification of coronary cineangiograms. Circulation 1985;71:280–8.
22. Doucette JW, Corl PD, Payne HM, et al. Validation of a Doppler guidewire for intravascular measurement of coronary artery flow velocity. Circulation 1992;85:1899–911.
23. Canty JM, Schwartz JS. Nitric oxide mediates flow-dependent epicardial coronary vasodilation to changes in pulse frequency but not mean flow in conscious dogs. Circulation 1994;89:375–84.
24. Smith TP, Grant BJB, Schwartz JS, Canty JM. Contrasting role of nitric oxide as a mechanism of flow dependent epicardial artery dilation at reduced vs. elevated level of blood flow in conscious dogs. Circulation 1994;90:I-103.
25. Kuo L, Chilian WM, Davis MJ. Interaction of pressure- and flow-induced responses in porcine coronary resistance vessels. Am J Physiol 1991;261:H1706–15.
26. Myers PR, Beyitt PF, Guerra R Jr, Harrison DG. Characteristics of canine coronary resistance arteries: importance of endothelium. Am J Physiol 1989;257:H603–10.
27. Selke FW, Myers PR, Bates JN, Harrison DG. Influence of vessel size on the sensitivity of porcine coronary microvessels to nitroglycerin. Am J Physiol 1990;258:H515–20.
28. Lieberman EH, Knab ST, Creager MA. Nitric oxide mediates the vasodilator response to flow in humans. Circulation 1994;90:I-138.

29. Hirooka Y, Egashira K, Imaizumi T, et al. Effect of L-arginine on acetylcholine-induced endothelium-dependent vasodilation differs between the coronary and forearm vasculatures in humans. J Am Coll Cardiol 1994;24:948–55.
30. Pohl U, Busse R, Kuon E, Bassenge E. Pulsatile perfusion stimulates the release of endothelial autacoids. J Hypertens Cardiol 1986;1:215–35.
31. Hull SS Jr, Kaiser L, Jaffe MD, Sparks HV Jr. Endothelium-dependent flow-induced dilation of canine femoral and saphenous arteries. Blood Vessels 1986;23:183–98.
32. Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br J Pharmacol 1988;93:515–24.
33. Brayden JE. Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. Am J Physiol 1990;259:H668–73.
34. Kauser K, Stekiel WM, Rubanyi G, Harder DR. Mechanism of action of EDRF on pressurized arteries effect on K^+ conductance. Circulation 1989;65:199–204.
35. Olesen SP, Clapham DE, Davies PF. Haemodynamic shear stress activates a K^+ current in vascular endothelial cells. Nature 1988;331:168–70.
36. Kelm M, Schrader J. Nitric oxide release from the isolated guinea pig heart. Eur J Pharmacol 1988;155:313–6.
37. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. Circ Res 1990;66:1561–75.
38. Pohl U, Busse R. EDRF increases cyclic GMP in platelets during passage through the coronary vascular bed. Circ Res 1989;65:1798–803.
39. Lefroy DC, Crake T, Uren NG, Davies GJ, Maseri A. Effect of inhibition of nitric oxide synthesis on epicardial coronary artery caliper and coronary blood flow in humans. Circulation 1994;88:43–54.
40. Sudhir K, MacGregor JS, Amidon TM, Gupta M, Yock PG, Chatterjee K. Differential contribution of nitric oxide to regulation of vascular tone in coronary conductance and resistance arteries: intravascular ultrasound studies. Am Heart J 1994;127:858–65.