Epicardial Coronary Artery Responses to Acetylcholine Are Impaired in Hypertensive Patients

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Hypertension is a risk factor for coronary atherosclerosis possibly via an adverse effect on the vascular endothelium. Endothelium-mediated relaxation is impaired in animal models of hypertension. However, the effects of hypertension on human coronary artery endothelial cell function are unknown. To test whether endothelium-mediated relaxation is impaired in the coronary arteries of patients with hypertension, we studied 14 patients with essential hypertension requiring therapy and 15 nonhypertensive control patients undergoing cardiac catheterization. All had angiographically normal, smooth-appearing coronary arteries. Patients were matched for age and other coronary atherosclerosis risk factors. To assess endothelial cell function, the endothelium-dependent vasodilator acetylcholine (ACh, 0.01, 0.1, and 1.0 μM) and the endothelium-independent vasodilator nitroglycerin (40 μg) were selectively infused into the left anterior descending or circumflex coronary artery. Diameter change (expressed as percent) was assessed using quantitative angiography. There was a marked vasoconstrictor response to serial doses of ACh in hypertensive patients (−7%, −21%, and −27%) compared with control patients (−4%, −5%, and −7%) (p < 0.02). The vasodilator response to nitroglycerin was preserved in hypertensive patients (+29%) and control patients (+25%) (p = NS), suggesting that endothelial cell dysfunction accounted for the differences in response to ACh. Thus, patients with hypertension have an accentuated coronary vasoconstrictor response to ACh, suggesting that endothelium-mediated regulation of coronary vascular tone is impaired by essential hypertension. This may reflect more generalized coronary endothelial changes contributing to the pathogenesis of atherosclerosis as well as hypertension. (Circulation Research 1992;71:776–781)

KEY WORDS • endothelium • hypertension • acetylcholine • coronary artery

Of all the currently recognized risk factors, hypertension is one of the major contributors to cardiovascular morbidity and mortality.1 Hypertensive patients from the Framingham study2 have been reported to have an incidence of sudden death, myocardial infarction, and coronary heart disease twice that of nonhypertensive subjects. Even mild blood pressure elevation is associated with an increased risk of cardiovascular events. Surprisingly, mechanisms of vascular damage by hypertension are poorly understood.

In addition to accelerating the atherogenic process, hypertension has several direct structural and functional effects on the coronary circulation. Adaptive responses to elevated arterial pressure in the epicardial coronary arteries include medial hypertrophy/hyperplasia and intimal thickening.3 Vessel wall permeability is increased,4 and vascular responsiveness to vasoconstrictor stimuli may be augmented.5,6 The endothelium, an important regulator of vascular tone via release of relaxing and constricting substances, is damaged in certain models of experimental hypertension. The endothelial cell increases in size and height,7 and normal endothelium-mediated relaxation is impaired.8,9 Although previous human studies have suggested that hypercholesterolemia, a family history of premature atherosclerosis,10 and overt atherosclerosis11 are associated with an impairment of endothelium-mediated relaxation in the large coronary arteries, the isolated effects of hypertension on epicardial coronary artery endothelial cell function have not been evaluated in humans.

We hypothesized that epicardial coronary artery endothelium–mediated relaxation is impaired in patients with hypertension. We investigated this hypothesis by evaluating coronary artery responses to infusions of the endothelium-dependent vasodilator acetylcholine and the smooth muscle dilator nitroglycerin in hypertensive and nonhypertensive patients.

Materials and Methods

Patient Population

From January 1985 through October 1990, epicardial coronary artery responses to intracoronary infusions of
acetylcholine were studied in 117 patients at Brigham and Women’s Hospital, Boston; the University of Michigan Hospital, Ann Arbor; and Grady Memorial Hospital, Atlanta, Ga. To assess the isolated effects of hypertension on the human coronary endothelium (while avoiding the effects of atherosclerosis on the endothelium), only patients with angiographically normal, smooth-appearing coronary arteries were selected from this population of 117 patients. Forty-one of these 117 patients had entirely smooth epicardial coronary arteries (no luminal irregularities), as judged by two experienced coronary angiographers who were unaware of the patients’ status. Hypertensive and nonhypertensive patient populations were then matched for all other coronary atherosclerosis risk factors by 1) excluding all patients with insulin-dependent diabetes mellitus and 2) excluding all patients younger than 36 years or older than 60 years. Twenty-nine patients (14 patients with a history of hypertension [HTN group] and 15 nonhypertensive control patients) met these entry criteria for comparison and are the subjects of this study. All selection was performed without knowledge of responses to drug infusions. All patients were undergoing diagnostic catheterization for evaluation of chest pain. No patient had a history suggestive of variant angina. The responses of 23 patients (only eight of whom were hypertensive) have been presented in a previous report.10

Definition of Coronary Risk Factors
The following information was collected from all patients: age, gender, history of cigarette smoking (defined as cigarette smoking within the 3 months before study), history of hypertension (defined as blood pressure elevation requiring treatment), total cholesterol level at the time of catheterization, family history of premature coronary atherosclerosis (defined as first degree relative at age <60 years with clinical evidence of coronary atherosclerosis), history of insulin-dependent diabetes mellitus, and total number of coronary atherosclerosis risk factors (calculated using cholesterol level >210 mg/dl, age >40 years, male sex, positive family history of coronary disease, and cigarette smoking).10

Protocol
Written informed consent was obtained from all patients before the diagnostic catheterization, in accordance with guidelines established by the Committee for the Protection of Human Subjects at Brigham and Women’s Hospital, the Human Investigations Committee at Emory University, and the Committee to Review Grants for Clinical Research and Investigation Involving Human Beings at the University of Michigan Medical School. Vasoactive medications were discontinued 12–24 hours before catheterization. The protocol for infusion of pharmacological agents has previously been described in detail.10,11

Diagnostic right and left heart catheterization and coronary angiography were performed by a standard percutaneous femoral approach. After completion of the diagnostic catheterization, 5,000–10,000 units heparin was given intravenously, and an 8F guiding catheter was positioned in the ostium of the left coronary artery. Each patient then underwent the following study protocol. A 2.5F coronary infusion catheter was advanced through the guiding catheter into the proximal segment of the left anterior descending (n=28) or circumflex (n=1) coronary artery. Using a Harvard infusion pump, serial intracoronary infusions of acetylcholine chloride (Miochol, Iolab Pharmaceuticals, Inc., Claremont, Calif.) and nitroglycerin (Tridil, Dupont Pharmaceuticals, Inc., Wilmington, Del.) were administered at 0.8 ml/min via the central lumen of the infusion catheter in the following sequence: 2.5-minute control infusion (5% dextrose with 1 unit/ml heparin); three 2.5-minute infusions of acetylcholine to achieve estimated final blood concentrations of 0.01, 0.1, and 1.0 µM (based on assumed left anterior descending coronary blood flow of 80 ml/min12); a 5-minute repeat control infusion (5% dextrose with 1 unit/ml heparin); and one infusion of nitroglycerin (40 µg) over 3.0 minutes. Just before the end of each infusion, coronary arteriography was performed with the use of a power injection of nonionic contrast medium (Omnipaque, Winthrop-Breon Laboratories, New York). Throughout each infusion the heart rate, arterial pressure, and electrocardiogram (lead I) were monitored continuously, and all measurements were recorded in steady-state conditions. If marked coronary constriction was observed during acetylcholine infusion, the higher doses of acetylcholine were omitted.

Quantitative Coronary Angiography
Quantitative coronary angiography was performed by previously validated techniques.13,14 Nonionic contrast medium was injected into the left coronary artery at the rate of 5–10 ml/sec to a total of 7–12 ml with the use of a power injector (Meadrad, Pittsburgh, Pa.) to optimize the quality and reproducibility of the injections.15 A biplane system (Polydiagnost-C, Philips Medical Systems, Inc., Shelton, Conn., or BICOR, Siemens, Inc., Erlangen, FRG) was used for cineangiography.

Analysis of Arterial Dimensions
Quantitative angiographic analysis was similar to that described in previous studies.10,11 Technically suitable single-plane angiograms were selected from the biplane views for analysis. In each patient, two or three available segments in the left anterior descending or circumflex vessels, 10–25 mm in length, were analyzed. Each segment was centered, and the single-frame cine image was digitized (20–40 µm/pixel) with the use of a video camera (Cohu, San Diego, Calif.) connected to a video interface (Recognition Concepts, Incline Village, Nev.) and a Microvax II computer (Digital Equipment Corp., Maynard, Mass.). Two-line profile averaging was used to minimize anatomic noise, and 16 video images were summed to minimize video noise. Four cine frames in end diastole were scanned and averaged. Calibrated grids or guiding catheter dimensions in the field of view were used to scale the data from pixels to millimeters. A series of measurements of diameter were recorded for the length of the arterial segment. Two anatomic features were used to reproduce the segment of interest after each drug infusion and to assess serial changes in vessel diameter. The 2-mm segment that showed the greatest mean diameter change at peak acetylcholine (either dilation or constriction) was identified by the computer, and the response of this segment was used
for the calculation of the acetylcholine response of each patient. In each patient, the response of the 2-mm segment had the same directional change as the entire 10–25-mm segment. In four patients, a 6–8-mm segment distal to the catheter infusion site was analyzed. In these patients, the changes in vessel diameter were determined with a previously described and validated method.14

Statistical Analysis

To ensure matching of patient populations, HTN and control groups were compared using an unpaired Student’s t test for continuous variables (age, cholesterol level, blood pressure at time of study, number of coronary risk factors, baseline coronary diameter, and left ventricular end-diastolic pressure) and χ² analysis for categorical variables (gender, family history of premature atherosclerosis, and history of smoking). Because all patients did not receive all three acetylcholine doses, epicardial coronary artery responses to acetylcholine infusions were assessed in two ways. First, HTN and control patients were compared using a repeated-measures analysis of variance. Second, responses to individual doses of acetylcholine were compared between HTN and control patients by using Student’s t test. Responses to nitroglycerin were compared by using Student’s t test. Statistical significance was assumed if the null (two-tailed) hypothesis could be rejected at the 0.05 probability level. All data are expressed as mean±SEM.16

Results

Clinical Data

The clinical characteristics of HTN and control patients were similar (Table 1). The mean ages of HTN and control patients were 50±2 (range, 38–59) and 46±2 (range, 37–57) years, respectively (p=0.11). Nine of 14 HTN and 10 of 15 control patients were male (p=0.89). Three of 14 HTN and seven of 15 control patients had a history of cigarette smoking (p=0.15). Eight of 14 HTN and five of 15 control patients had a family history of premature coronary atherosclerosis (p=0.20). Mean total cholesterol level at the time of catheterization was 225±10 (range, 164–310) mg/dl in HTN patients and 207±14 (range, 124–322) mg/dl in control patients (p=0.30). The total number of coronary risk factors (excluding hypertension) was similar in the two groups (1.7±0.2 in HTN patients and 1.3±0.2 in control patients, p=0.31). No patient had insulin-dependent diabetes mellitus or a history of myocardial infarction.

Hemodynamic and Angiographic Data

As shown in Table 1, the mean arterial pressure at the time of catheterization was significantly elevated in HTN patients (105±3 mm Hg) versus control patients (89±2 mm Hg) (p=0.0002). Left ventricular end-diastolic pressure was similar in the two groups (11±1 mm Hg in HTN patients and 11±2 mm Hg in control patients, p=0.75). Baseline epicardial coronary diameters were similar in the two groups (2.16±0.19 mm in HTN patients versus 2.04±0.21 mm in control patients, p=0.67).

Epicardial Responses to Acetylcholine

Because of marked constriction at the lower acetylcholine doses, the 1.0 μM dose was omitted in three (one control and two HTN) patients. In five (one HTN and four control) patients, the 0.01 μM dose of acetylcholine was omitted to limit contrast load. One control patient did not receive acetylcholine at a dose of 0.1 μM.

Comparison within groups. In control patients, coronary artery diameter did not change significantly over the acetylcholine dose–response curve (2.04±0.21 mm at baseline versus 1.81±0.21 mm at peak [1.0 μM] acetylcholine, p=0.18). In HTN patients, coronary artery diameter did decrease significantly over the acetylcholine dose–response curve (2.16±0.19 mm at baseline versus 1.59±0.22 mm at 1.0 μM acetylcholine, p=0.0001). Pearson χ² analysis revealed no relation of the acetylcholine response to the nitroglycerin response (r=0.25, p=0.34) or baseline diameter (r=0.06, p=0.77), which is consistent with a lack of effect of basal tone or intrinsic vascular smooth muscle abnormalities on the acetylcholine response.

Comparison between groups. Because of missing data, responses to acetylcholine were analyzed in two ways: a comparison of dose–response curves using analysis of variance for repeated measures and comparison of responses at individual doses using Student’s t test. The results were similar regardless of the analysis used.

HTN patients demonstrated a marked epicardial vasconstrictor response to acetylcholine compared with control patients (Figure 1). In HTN patients, epicardial coronary diameter decreased −7±2%,-21±4%, and
Epicardial Responses to Nitroglycerin

To control for differences in baseline coronary arterial tone, a single intracoronary dose of nitroglycerin (40 μg) was given. HTN and control patients had similar epicardial coronary diameter responses to nitroglycerin (29±6% in HTN patients and 25±4% in control patients, p=0.96) (Figure 2).

Discussion

We have demonstrated that epicardial coronary arteries of HTN patients constrict markedly in response to the endothelium-dependent agent acetylcholine, whereas responses to the smooth muscle dilator nitroglycerin are preserved. These findings suggest a primary defect in endothelium-mediated regulation of epicardial coronary artery tone in hypertensive humans. A relation between some risk factors for coronary atherosclerosis (total cholesterol, family history of premature atherosclerosis, and total number of risk factors)\textsuperscript{10} and overt atherosclerosis\textsuperscript{11} with the epicardial coronary artery response to acetylcholine in humans has been demonstrated previously. This study is the first to demonstrate the isolated effects of hypertension on endothelium-mediated regulation of epicardial coronary vascular tone in patients with no angiographically detectable atherosclerosis.

Vascular Changes in Hypertension

Hypertension is associated with many structural and functional changes in the coronary arteries. Vascular smooth muscle cells undergo hypertrophy and hyperplasia, eventually reducing lumen size.\textsuperscript{3} Collagen and elastin accumulate,\textsuperscript{17} and the intima thickens. Endothelial cell size and height increase.\textsuperscript{7} The progression of atherosclerotic changes is accelerated, especially in the presence of lipid abnormalities.\textsuperscript{18} Functional changes include increased vascular permeability and decreased arterial compliance.\textsuperscript{19} The sensitivity of the vessel wall to vasoconstrictor stimuli is increased.\textsuperscript{5,6,20} This may be related to increased vascular smooth muscle sensitivity to circulating vasoconstrictors\textsuperscript{8} or to defective endothelium-mediated regulation of coronary tone.

Importance of the Endothelium

The endothelium is an important regulator of vascular tone and growth, exerting paracrine influences on vascular smooth muscle via a multitude of vasoconstricting, vasorelaxing, and growth-regulating factors. It synthesizes and releases endothelium-derived relaxing factor, which stimulates relaxation of underlying vascular smooth muscle. Endothelium-derived relaxing factor is thought to be nitric oxide\textsuperscript{21,22} or a closely related nitrosothiol.\textsuperscript{23} Continuous production and release of endothelium-derived relaxing factor by normal endothelium probably plays an important role in regulation of blood pressure and resting coronary tone.\textsuperscript{24} In addition, endothelial stimulation with acetylcholine, substance P, or other endothelium-dependent vasodilators will elicit vasodilation via this mechanism.\textsuperscript{25} This has been documented in both large\textsuperscript{11} and small\textsuperscript{26} coronary arteries of humans.

Endothelium-derived constricting factors have been less thoroughly studied. In animal models, several endothelium-derived constricting factors have been described.\textsuperscript{27-31} Endothelin-1, the only endothelium-derived constricting factor known to be present in human endothelium, produces a slow-onset sustained constriction in both large and small coronary arteries.\textsuperscript{32} Al-
Although preliminary human studies indicate that plasma levels of endothelin-1 may be elevated in essential hypertension, acetylcholine does not appear to cause release of endothelin-1 in human coronary arteries in vivo (authors’ unpublished observations). Therefore, it is unlikely that increased release of endothelin-1 in hypertensive patients accounts for the results in the present study.

**Endothelial Abnormalities in Hypertension**

The endothelium, a sensor of physical forces such as pressure, shear stress, and stretch, is an obvious target tissue for hypertension. Changes in endothelial cell function occur in response to these stimuli. Experimental hypertension is associated with impaired endothelium-dependent relaxation to acetylcholine, ADP, and thrombin in large vessels. In the microcirculation of the hypertensive rat and human, endothelial vasodilator function is also impaired. In humans, increases in forearm blood flow during acetylcholine infusion are impaired in patients with essential hypertension and may be related to decreased endothelium-derived relaxing factor release. In a group of patients with no angiographic evidence of atherosclerosis, Vita et al suggested that risk factors for coronary atherosclerosis are associated with the loss of endothelium-dependent vasodilation in the epicardial coronary arteries. In that study, hypertension was not an independent predictor of the coronary artery response to acetylcholine. Few (only 10) of those patients were hypertensive, and no attempt was made to compare two populations matched for all risk factors other than hypertension. This study is the first to demonstrate the isolated effects of hypertension on epicardial coronary artery endothelial function in humans.

**Potential Limitations**

Nonhypertensive (control) patients demonstrated no significant change in epicardial dimensions to acetylcholine, although a trend toward mild vasoconstriction exists. One might expect angiographically smooth coronary arteries to dilate in response to acetylcholine. However, as previously noted, the acetylcholine response in the intact human coronary artery relates to risk factors for coronary atherosclerosis, and our control patients had a mean number of 1.3 risk factors for atherosclerosis.

Because angiography is an insensitive method of detecting early atherosclerosis, we cannot exclude the possibility that HTN patients had more epicardial atherosclerosis undetected by angiography, accounting for the observed differences in response to acetylcholine. This distinction may be moot, however, because hypertension is an important risk factor for the development of atherosclerosis, and the two conditions are likely to coexist in the clinical setting.

**Implications**

This study demonstrates that endothelium-mediated relaxation is impaired in the epicardial coronary arteries of hypertensive patients. These results are consistent with defective endothelial cell regulation of coronary arterial tone in hypertension. This defect may partially explain the abnormalities of vascular function seen in hypertension and may reflect broader endothelial regulatory abnormalities that form the substrate for hypertensive and atherosclerotic heart disease.

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**References**

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