OBJECTIVES
To evaluate the effects of exogenous bradykinin on coronary epicardial and microcirculatory tone in transplant patients (HTXs), and to compare them with the effects of acetylcholine.

BACKGROUND
Coronary endothelial dysfunction has been reported to occur early after heart transplantation, most notably when acetylcholine was the endothelium-function marker used. The effects of bradykinin on coronary vasomotion are unknown in HTXs.

METHODS
Sixteen HTXs were compared 3.6 ± 1.7 months after transplantation to seven control subjects. Coronary flow velocity was measured using guide-wire Doppler. Diameters (D) of three segments of the left coronary artery and coronary blood flow (CBF) were assessed at baseline, after 3-min infusions of increasing bradykinin doses (50, 150 and 250 ng/min) then of increasing acetylcholine doses (estimated blood concentrations of 10⁻⁸, 10⁻⁷ and 10⁻⁶ M).

RESULTS
Bradykinin induced similar dose-dependent increases in D and CBF in both groups: D was 11 ± 12%, 19 ± 14% and 22 ± 16% (all p < 0.0001), and CBF was 50 ± 40%, 130 ± 68% and 186 ± 77% (all p < 0.0001). Acetylcholine induced significant epicardial vasodilation in control subjects and vasoconstriction in HTX, as well as a marked increase in CBF in both groups. Acute allograft rejection, present in 8 of the 16 HTXs, did not modify responses to bradykinin, but was associated with a smaller CBF increase in response to acetylcholine (p < 0.05).

CONCLUSIONS
The coronary vasodilating effects of bradykinin are preserved early after heart transplantation, even in the presence of acute allograft rejection. Although there is an abnormal vasoconstricting response to acetylcholine reflecting endothelium dysfunction, the endothelium remains a functionally active organ in heart transplant recipients. (J Am Coll Cardiol 2000; 35:1607–15) © 2000 by the American College of Cardiology

Coronary endothelial dysfunction has been shown to occur early after surgery in heart transplant patients, before the development of graft atherosclerosis detectable by angiography (1–3). Several potentially injuring factors present starting at the time of heart explantation may explain the impaired endothelium-mediated vasodilating responses, most notably in response to acetylcholine, reported as early as one month after transplantation (4). However, although an abnormal response to acetylcholine is a hallmark of impaired endothelial function (5), normal coronary vasomotor responses in transplants have been found in studies using other pharmacological (6,7) and nonpharmacological tests (8,9).

Bradykinin is an endogenous vasoactive substance involved in a wide range of biological processes (10). Bradykinin is a potent endothelial-dependent vasodilator, whose effects are mediated through the nitric oxide and prostacyclin pathways, and probably also through an endothelium-derived hyperpolarizing factor (EDHF), as shown by in vitro and animal model experiments (11–15). Few data exist on the effects of bradykinin on the human coronary circulation. Kuga et al. (16) showed for the first time that intracoronary administration of bradykinin dilates normal human epicardial coronary arteries in vivo. In contrast, in the same study, bradykinin-induced vasodilation was impaired in atheromatous stenotic segments. The effects of bradykinin on the coronary circulation of transplanted hearts are unknown.

The aim of this study was to assess the effects of exogenous bradykinin on coronary blood flow and vasomotion in heart transplant patients, and to compare them with the response to acetylcholine. Results in transplant recipients were compared with those in a control group.
Abbreviations and Acronyms:
ACE = angiotensin-converting enzyme
EDHF = endothelium-derived hyperpolarizing factor
HTX = transplant patients
Sim-1 = linsidomine

METHODS

Patient selection. CONTROL SUBJECTS. Seven young adults were selected as control subjects (group 1, controls). They were referred for diagnostic coronary arteriography because of repetitive atypical chest pain with equivocal bicycle exercise test and/or thallium scan findings. All were found to have angiographically normal coronary arteries without lumen irregularities. They were normotensive and free of diabetes mellitus and hypercholesterolemia. All had normal left ventricular systolic function as assessed using two-dimensional and M-mode echocardiography (Table 1), and none had echocardiography evidence of cardiac hypertrophy according to American Society of Echocardiography criteria (diastolic septal and posterior wall thickness less than 10 mm, measured at end-diastole). At the time of the investigations, none of the controls were on cardiovascular medications, and four were taking aspirin on a regular basis.

HEART TRANSPLANT RECIPIENTS. Sixteen heart transplant patients were included in the study (group 2, HTXs), 3.6 ± 1.7 months after the transplant procedure. All had angiographically normal coronary arteries, without luminal stenoses or irregularities, as judged by two experienced observers. All had normal left ventricular two-dimensional and M-mode echocardiography parameters (Table 1). Post-transplantation immunosuppressive therapy included prednisone and cyclosporine in all patients, and azathioprine in 10. Cyclosporine was titrated to maintain serum levels between 150 and 200 ng/mL, as measured by non-specific radioimmunoassay (Sandoz). Right ventricular endomyocardial biopsy was performed on the day of the investigation. Potential risk factors for graft vasculopathy evaluated during the study included recipient age, donor age, number of previous rejection episodes, incidence of hypertension and diabetes, lipid profile and cytomegalovirus infection.

Study protocol was approved by the local Ethical Committee. Informed consent was obtained from each patient.

Study protocol. All patients fasted for at least 12 h before the investigation. Vasoactive drugs including calcium channel blockers, beta-adrenergic receptor antagonists, alpha-adrenergic receptor antagonists and angiotensin-converting enzyme inhibitors were discontinued 24 h before catheterization. None of the HTXs were on aspirin. No premedication was administered. One percent lidocaine was used for local anesthesia, and 5,000 U heparin was administered intravenously at the time of percutaneous femoral cannulation. After completion of diagnostic right and left heart catheterization, an additional 5,000-U heparin dose was given. A 7.0 F guide catheter was positioned in the left main coronary artery. A 6.0 F bipolar pacing wire was placed in the right ventricle through the femoral vein. A 0.014-inch tip guide wire Doppler (FLOWIRE, 12 MHz; Cardiometrics, Inc., Mountain View, California) was advanced through the guiding catheter into the proximal segment of the left anterior descending coronary artery distal to any large branch.

Intracoronary drugs. After an appropriate spectral Doppler signal and a baseline left coronary angiogram were obtained, the following studies were performed, according to an established protocol. First, bradykinin (Clinalfa AG, Basel, Switzerland) was infused into the left coronary artery through the guide catheter by pump. After a 3-min infusion of warm 0.9% saline solution at a rate of 1 mL/min, incremental doses of bradykinin were infused, namely 50, 150 and 250 ng/min, at a rate of 1 mL/min, each for 3 min. A 3-min recovery period was observed between the successive bradykinin doses. Second, a bradykinin bolus (400 ng/3 mL) was injected through the guiding catheter into the left coronary artery, preceded by a 3-mL bolus of warm 0.9% saline solution, both for 10 s. Third, acetylcholine (Phar- macie Centrale des Hôpitaux, Assistance Publique, Paris, France) was administered into the left coronary artery through the guide catheter by pump. After a 3-min infusion

Table 1. Study Population Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 7)</th>
<th>Transplant Recipients (n = 16)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>44 ± 4</td>
<td>52 ± 6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Male/Female</td>
<td>4/3</td>
<td>11/5</td>
<td>NS</td>
</tr>
<tr>
<td>Time from HTX (months)</td>
<td>—</td>
<td>3.6 ± 1.7</td>
<td>—</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>—</td>
<td>39 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of ischemia (min)</td>
<td>—</td>
<td>152 ± 45</td>
<td>—</td>
</tr>
<tr>
<td>No. of rejection episodes</td>
<td>—</td>
<td>1.75 ± 0.8</td>
<td>—</td>
</tr>
<tr>
<td>Rejection the day of study</td>
<td>—</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Total cholesterol (g/L)</td>
<td>1.96 ± 0.52</td>
<td>2.05 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (g/L)</td>
<td>0.96 ± 0.42</td>
<td>1.12 ± 0.69</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic hypertension</td>
<td>0</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Cytomegalovirus infection</td>
<td>?</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>48 ± 10</td>
<td>49 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>FS (%)</td>
<td>41 ± 4</td>
<td>39 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>LV mass (g/m²)</td>
<td>84 ± 12</td>
<td>88 ± 14</td>
<td>NS</td>
</tr>
</tbody>
</table>

FS = fractional shortening; HTX = transplantation; LVEDD = left ventricular end-diastolic diameter; LV mass = left ventricular mass; NS = not significant.
of warm 0.9% saline solution at a rate of 1 mL/min, increasing doses of acetylcholine were infused, in order to achieve the estimated blood concentrations of $10^{-8}$, $10^{-7}$ and $10^{-6}$ M in the left coronary circulation assuming a blood flow of 120 mL/min for a dominating left coronary artery and of 100 mL/min for a dominating right coronary artery. Infusion rate was 1 mL/min for 3 min for each concentration. A 3-min recovery period was observed between the successive acetylcholine doses. Lastly, a linsidomine bolus (Sin-1) (1 mg/3 mL) was injected into the left coronary artery to assess the vasodilatory response of the coronary arteries to a nonendothelium-dependent vasodilator.

Serial manual injections of a nonionic contrast agent (Iopamidol, Schering, France) into the left main stem were performed at baseline, immediately after each drug dose (10 to 15 s after infusion termination), at the end of each postinfusion recovery period, 30 s after the warm saline and bradykinin boluses and 3 min after Sin-1 injection. Special care was taken to quickly withdraw the fluid contained into the catheter and coronary device before each contrast agent injection. Because velocity variations after contrast agent injection can be anticipated, the velocity reflecting the effects of the various tests was measured before each contrast agent injection. Systolic, diastolic and mean aortic pressure, heart rate and the electrocardiogram were monitored continuously throughout the investigation.

Doppler flow wire and calculation of coronary blood flow. The Doppler guide wire position in the proximal left anterior descending was the same throughout the study. Spectral Doppler frequency analysis was recorded continuously throughout the experiment. Time-average peak velocity was used as a measure of mean coronary blood flow velocity (17). Coronary blood flow was calculated by multiplying one-half this value by the calculated coronary artery area at the tip of the Doppler flow wire on the corresponding angiogram, and by using a 0.6 conversion factor for mm$^2$/cm$^2$ and min/s. Coronary blood flow variations during the different tests are reported as the percent change versus baseline.

Quantitative coronary angiography. A CGR (General Electric, Issy les Moulineaux, France) X-ray unit connected to a DPS PLUS system (ADAC Laboratories, Milpitas, CA) was used in all studies. The size of the image intensifier was 6 in. The digital system and software analysis program (ARTREK; ADAC Laboratories) used in this study, which allow a fully automatic edge-detection and quantitation of coronary segments, have been validated previously (18). For each patient, the angiographic system was set up in the right anterior oblique position, with adequate cranial or caudal angulation allowing analysis of the left arterial tree on end-diastole frames without overlap of the selected segments. The proximal and middle portion of the left anterior descending coronary artery and the proximal portion of the left circumflex artery were positioned near the isocenter. Using this view, pincushion distortion was found to be small because measurements made at the periphery of the zone of interest varied by only 5% from those made at the center (19). Because the position was kept the same throughout the experiment, and relations between focal spot, patient and image intensifier height remained unchanged, no correction for distortion was introduced. Digitally acquired images coupled to an electrocardiograph monitor were recorded at the rate of 25 frames/s. End-diastolic frames were analyzed using 4× magnification to help provide adequate pixel resolution for small vessels and catheters. The distal end of the injection catheter served for calibration with automatic tracking for edge determination. Fixed anatomic coordinates were used to reproduce the same regions of interest to assess serial changes. Three defined regions of the proximal and middle left anterior descending coronary artery and of the proximal left circumflex artery were selected in both control subjects and HTXs. In both groups, coronary segments analyzed were approximately 10 mm in length (mean 9.7 ± 2.7 mm). Automatic vessel segment contour detection was performed and the averaged diameter of each segment was obtained directly from the computer. Additionally, measurements were made by two operators blinded to the conditions under study. Interobserver (standard error of estimate for repeated measurements in percent of mean vessel diameter) and intraobserver variabilities using this system were found to be low (4.3 and 4.7%, respectively). Responses of coronary arteries to the various stimuli are reported as the percent change versus the control value.

Statistical analysis. All data are reported as mean ± SDs. When applicable, comparisons between groups were performed using unpaired $t$ tests. Two-way analyses of variance (ANOVAs) with repeated measurements were performed. The effects of drugs on luminal diameter, coronary blood flow velocity and estimated coronary blood flow were compared in both groups, testing for group effect, dose effect and interaction. When applicable, the Fisher protected least significant difference test or the Dunnett’s test for comparisons with control values were used. Statistical difference was assumed if the null hypothesis could be rejected at the 0.05 probability level.

RESULTS

Study population characteristics. The age of transplant donors was similar to the age of control subjects. The two groups were similar with respect to left ventricular dimensions, fractional shortening and left ventricular mass. Lipid profile was normal and similar in the two groups. Eight transplant patients had histological signs of rejection justifying augmented immunosuppressive therapy (two patients had rejection grade 2, and six rejection grade 3, according to the ISHLT classification); none of these eight patients had hemodynamic compromise (Table 1).

Systemic hemodynamic responses. The data are summarized in Table 2. All patients were normotensive at the time of investigation. At baseline, heart rate and the rate-pressure
Effects of Bradykinin in Transplant Patients

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Systolic Aortic Pressure (mm Hg)

Heart Rate (beats/min)

Table 2. Systemic Hemodynamics

<table>
<thead>
<tr>
<th>Rate-Pressure Product</th>
<th>Control Subjects</th>
<th>HTX Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability Value</td>
<td>Between Groups</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systolic Aortic Pressure (mm Hg)</th>
<th>Control Subjects</th>
<th>HTX Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability Value</td>
<td>Between Groups</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heart Rate (beats/min)</th>
<th>Control</th>
<th>HTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability Value</td>
<td>Between Groups</td>
<td></td>
</tr>
</tbody>
</table>

Product were significantly higher in HTXs than in control subjects. Bradykinin in graded doses and as a bolus did not alter systemic hemodynamics, except for the 150-ng/min dose, which induced a slight but significant decrease in systolic aortic pressure (-5 ± 3%, p < 0.01) and rate-pressure product (-8 ± 9%, p < 0.05) in the control subjects but not the HTXs. Acetylcholine did not modify systemic hemodynamics in either group. Linsidomine induced a significant fall in systolic aortic pressure (-8 ± 9%, p < 0.01) and in the rate-pressure product (-7 ± 10%, p < 0.01) in the HTXs but not the controls.

**Effects of bradykinin on coronary epicardial vasomotion and blood flow.** The effects of drugs on coronary epicardial vasomotion and blood flow are summarized in Table 3. At baseline, the average mean coronary diameter of the three segments assessed in each patient was higher in HTXs than in control subjects: 2.7 ± 0.8 mm versus 2.3 ± 0.7 mm, p < 0.05. Bradykinin induced significant and similar vasodilation of all measured epicardial segments in both groups, in a dose-dependent manner: 11 ± 12%, 19 ± 14% and 22 ± 16% (all p < 0.0001) with bradykinin doses of 50, 150 and 250 ng/min, respectively (Figs. 1 and 2). Bradykinin-induced vasodilation was comparable across the three coronary artery segments assessed. Vasodilation in response to the bradykinin bolus was similar to that observed with the lowest-dose bradykinin infusion: 10 ± 16% (p < 0.0001).

The average peak velocity of coronary blood flow was slightly but nonsignificantly higher at baseline in the HTXs (23 ± 8 cm/s) than in the control subjects (17 ± 4 cm/s). It also increased in a dose-dependent manner during bradykinin infusion in both groups: 22 ± 24% (p < 0.001), 72 ± 40% and 111 ± 50% (both p < 0.0001) (see Figs. 1 and 2). In contrast, the bolus of bradykinin induced a small, nonsignificant, transient increase (less than 10 s) in average peak velocity, which was no longer present at the time angiography was done (10 ± 13% in control subjects, 5 ± 13% in HTXs).

At baseline, estimated coronary blood flow was higher in HTXs (68 ± 28 mL/min) than in control subjects (33 ± 13 mL/min), although the difference was not statistically significant. As a result of the effects of bradykinin, estimated coronary blood flow increased in a dose-dependent manner in both groups: 50 ± 40%, 130 ± 68% and 186 ± 77% (all p < 0.0001) (Fig. 1). The bradykinin bolus induced a small increase in coronary blood flow in both groups (49 ± 44%, p = NS in control subjects, 21 ± 21%, p < 0.01 in HTXs).

**Effects of acetylcholine and Sin–1 on coronary epicardial vasomotion and blood flow.** The effects of acetylcholine on the epicardial vasomotor response were strikingly different in both groups of patients (two-way ANOVA, p < 0.0001 for interaction). In control subjects, the increasing-dose acetylcholine infusions produced increasing degrees of epicardial vasodilation at estimated concentrations of 10⁻⁸ to 10⁻⁶. ACH: 1 ± 7% (p = NS), 7 ± 9% (p < 0.001) and 12 ±
Table 3. Coronary Diameter and Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Bradykinin</th>
<th>Acetylcholine</th>
<th>Sin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base 1</td>
<td>50 ng/min</td>
<td>150 ng/min</td>
</tr>
<tr>
<td>Mean CD, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>2.3 ± 0.7</td>
<td>2.6 ± 0.8</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>p value vs. base</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>HTX</td>
<td>2.7 ± 0.8*</td>
<td>3.0 ± 0.8*</td>
<td>3.2 ± 0.9*</td>
</tr>
<tr>
<td>p value vs. base</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>APV, cm/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>17 ± 4</td>
<td>22 ± 5</td>
<td>34 ± 11</td>
</tr>
<tr>
<td>p value vs. base</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CBF, mL/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>33 ± 13</td>
<td>57 ± 29</td>
<td>93 ± 36</td>
</tr>
<tr>
<td>p value vs. base</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

APV = average peak velocity of the coronary blood flow; CBF = estimated coronary blood flow; HTX = transplant patients; Mean CD = average diameter value of the three coronary segments assessed; Sin-1 = Linsidomine.

*p < 0.05 HTX vs. control subjects.

Influence of acute allograft rejection on vasomotor responses to bradykinin and acetylcholine. Because of our 16 HTXs had evidence of rejection (two-way ANOVA, p = 0.0001) and 141 ± 57% (p = 0.001), 59 ± 73% (p = 0.0001), 1611 ± 638% (p = 0.0001) and 138 ± 90% (p = 0.0001). In all, Linsidomine induced marked and similar epicardial vasodilation in both groups (Fig. 1 and D). 25 ± 16% (p < 0.0001). The effects of acetylcholine on average peak velocity and blood flow did not change significantly in either group.

DISCUSSION

The main finding of this study is that epicardial and microvascular responses to acetylcholine and bradykinin infusion are preserved in recently transplanted donor hearts. In addition, the normal coronary vasomotor mechanisms are intact in recently transplanted hearts.

Heart transplant patients; Mean CD = average diameter value of the three coronary segments assessed; Sin-1 = Linsidomine.

*p < 0.05 HTX vs. control subjects.
Bradykinin-induced coronary vasodilation. Bradykinin is a potent vasodilator whose effects on the systemic and coronary circulations were described in animal models many years ago (20). However, the effects of exogenous bradykinin on the human coronary circulation were documented only recently by Kuga et al. (16). These authors reported that intracoronary administration of bradykinin dilated normal human epicardial coronary arteries in vivo. Our results confirm and extend this finding, because in our control subjects bradykinin elicited not only epicardial but also significant coronary microcirculatory dilation, in a dose-dependent manner. The coronary blood flow increase was independent of hemodynamic conditions, as demonstrated by the absence of heart rate or aortic pressure variations during bradykinin infusion. Because of the marked effect of bradykinin on coronary resistance vessels, it could be argued that epicardial vasodilation was due to a flow-dependent, endothelium-mediated mechanism. Nevertheless, when bradykinin was infused as a bolus, the coronary blood flow velocity increase was extremely short-lived, having disappeared at the time angiography was performed. These data indicate that epicardial vasodilation was due to a direct effect of bradykinin.

Mechanisms of bradykinin-mediated vasodilation. Bradykinin is an endothelium-dependent vasodilator. In vitro and in vivo studies have shown that the effects of bradykinin are mediated through nitric oxide and prostacyclin pathways (11–13), as well as through the release of an endothelial hyperpolarizing factor (13–15). The relative contribution of these three factors in mediating epicardial and microcirculatory dilation in vivo are not yet well defined (21,22). Nevertheless, blockade of bradykinin B2 receptors with the selective antagonist HOE 140 has been shown to decrease basal epicardial luminal area and to increase coronary vascular resistance, producing a fall in coronary blood flow. Moreover, flow-dependent vasodilation is blunted after blockade of bradykinin B2 receptors (23). These findings underline the important role of endogenous bradykinin in the control of epicardial and microcirculatory vasomotor tone in humans.

Bradykinin-, acetylcholine- and Sin-1-mediated effects in transplant patients. The vasodilator response to exogenous bradykinin is blunted in epicardial atherosclerotic segments in humans (16). Also, pharmacological or mechanical removal of endothelial cells in coronary microvessels blunts vascular relaxation to bradykinin (24,25). Thus, the vasomotor response to exogenous bradykinin may be considered a marker of a functionally active endothelium, both in conduit and in resistance coronary vessels.

The results of our study show that endothelium-mediated responses to intracoronary bradykinin are preserved early after heart transplantation. Heart transplantation is associated with many factors potentially responsible for endothelial dysfunction, including cardiac denervation (26), ischemia during transplant harvesting (27), use of cardioplegic solutions (28), immunologically mediated injury to the endothelium (29) and effects of cyclosporine (30). Moreover, although the pathogenesis of transplant atherosclerosis is not yet well understood, any injury to the endothelium and subsequent impairment in vascular response may be capable of initiating this form of accelerated atherosclerosis (31).

Paradoxical epicardial vasoconstriction in response to acetylcholine is thought to be a hallmark of endothelial dysfunction and has been demonstrated even before the development of angiographically visible atherosclerosis in nontransplant patients (5,32). Several studies (1–3) have shown that the response of transplant epicardial coronary arteries to intracoronary acetylcholine is abnormal in most transplant recipients, even early after transplantation. Although this abnormality is generally ascribed to decreased activity of endothelium-derived relaxing factors, other mechanisms deserve to be considered.
such as acetylcholine-mediated release of cyclooxygenase-dependent vasoconstricting factor, and alterations in endothelial receptor responses to acetylcholine or disequilibrium between endothelial and vascular myocyte receptors due to denervation.

In our study, the same epicardial coronary segments that dilated in response to bradykinin in HTXs exhibited paradoxical vasoconstriction to stepwise acetylcholine infusions. In contrast, the microcirculatory vasodilator response was similar with the two drugs, as shown by the comparable increase in the average peak velocity of coronary blood flow after bradykinin and acetylcholine. These differential effects of acetylcholine on large epicardial compared with smaller resistance coronary vessels have been described previously, but remain incompletely explained (6). In HTXs, the coronary blood flow increase associated with the effects of acetylcholine on coronary resistance vessels has been reported to decrease over time after transplantation (33). Because transplant atherosclerosis is a progressive, diffuse, concentric process that is marked in the distal coronary tree (34), endothelial dysfunction should be present simultaneously at the epicardial and at the microcirculatory levels, even before the development of angiographically visible lesions.

Bradykinin shares with acetylcholine one of the main endothelium-dependent vasodilator pathways: both compounds elicit nitric oxide release by the endothelial cell (11,12,35). Thus, although the vasoconstriction observed with acetylcholine at the epicardial level reflects endothelial impairment, our findings, in agreement with previous studies, imply that some endothelium-dependent vasodilator pathways are preserved early after transplantation, the nitric oxide pathway probably being among them.

Linsidomine, the active metabolite of molsidomine, is a direct nitric oxide donor responsible for selective vasodilation of epicardial conductance vessels via mechanisms independent from the endothelium and from cysteine (36). Coronary blood does not change or diminish after intracoronary Sin-1 infusion (9), because of epicardial vasodilation without concomitant microcirculatory effects. Moreover, in our HTXs but not our control subjects, 1 mg of Sin-1 induced systemic hemodynamic changes with significant decrease in the rate-pressure product.

Vasomotor responses in the presence of acute graft rejection. An additional finding from our study is that the coronary vasomotor responses to bradykinin are similar in the presence and absence of acute allograft rejection. Acute rejection is an inflammatory process, which involves the
coronary bed of the graft. Vasoactive substances are released during rejection, most notably cytokines (37), which can cause endothelial injury and dysfunction but also act directly on vascular smooth muscle cells. In a recent study of a swine model of retroperitoneal heterotopic heart transplantation, Perrault et al. (38) found that endothelial dysfunction in untreated acute rejection developed after five days, initially involved G-proteins, and worsened over time, ultimately affecting all endothelial mechanisms and vascular smooth muscle. Thus, multiple abnormalities may explain the impairment in myocardial perfusion observed during severe rejection in animal models (39). In our study, the estimated coronary blood flow at rest and the rate-pressure product were similar in patients with and without rejection. Endothelial dysfunction, as evidenced by the abnormal vasomotor responses to acetylcholine, was significantly more marked in patients with rejection, because acetylcholine at estimated concentrations of 10^{-7} and 10^{-6} M induced more epicardial vasoconstriction and a smaller increase in the coronary blood flow velocity in HTXs with rejection. Conversely, exogenous bradykinin induced a potent vasodilator response at the epicardial and microvascular levels even in patients with rejection, suggesting that endothelium-dependent vasodilator mechanisms may compensate for endothelium impairment during rejection. Our findings suggest a protective role of endogenous bradykinin in rejection, consisting in preservation or even augmentation of coronary perfusion via upregulation of B_2 and/or B_1 receptor subtypes. Confirmation of this mechanism would support use of angiotensin-converting enzyme (ACE) inhibitors after cardiac transplantation, because ACE inhibition potentiates local effects of bradykinin through the nitric oxide and/or prostaglandins pathways (40,41). In addition, chronic ACE inhibition has been shown to reduce significantly myointimal proliferation in a rat heterotopic transplant model, suggesting that angiotensin II may mediate the myointimal proliferative response in transplant coronary artery disease (42). Interestingly, in the same model, chronic ACE inhibition reduced cellular rejection and perivascular edema, indicating that angiotensin II modulates the cellular population during the rejection process.

**Study limitations.** First, neither the relative contribution of the various endothelium-mediated effects of bradykinin nor the effects of selective stimulation of B_1 and B_2 bradykinin receptors were addressed in our study. Second, it is possible that some of our HTXs had already developed some degree of angiographically silent intimal thickening that could have been detected using intravascular ultrasound. However, endothelial dysfunction, as evidenced by a paradoxical response to acetylcholine, has been shown to occur in HTXs even in the absence of intimal thickening (43). It is not known whether the vasodilator response to bradykinin is blunted in the presence of transplant atherosclerosis.

**Conclusions.** We found evidence that the endothelium-mediated vasodilating effects of bradykinin are preserved early after heart transplantation. Although there was an abnormal vasoconstricting response to acetylcholine reflecting endothelial impairment, our data indicate that the endothelium remains a functionally active organ in heart transplant recipients. Moreover, bradykinin may play a protective role in acute rejection, preserving or even increasing myocardial blood flow. Further investigations are necessary to determine whether ACE inhibitor therapy after heart transplantation is a useful therapeutic approach.

**REFERENCES**

is impaired at the atherosclerotic site but is preserved at the spastic site of human coronary arteries in vivo. Circulation 1995;92:183–9.


