Evidence for Structural Sympathetic Reinnervation After Orthotopic Cardiac Transplantation in Humans

Robert F. Wilson, MD; Betsy V. Christensen, BSN; Maria Teresa Olivari, MD; Ada Simon, PhD; Carl W. White, MD; and David D. Laxson, MD

Background. Cardiac transplantation (CT) causes total cardiac denervation. Methods and Results. To test directly for sympathetic reinnervation in humans, we measured the cardiac release of norepinephrine (NE) in response to tyramine (an agent that causes NE release from intact sympathetic nerve terminals) and sustained handgrip exercise (a reflex sympathetic stimulus) in 12 patients less than 5 months after CT, in 50 patients 1 year or more after CT, and in eight patients without CT. Plasma [NE] was measured in the aorta ([NE]o) and coronary sinus ([NE]s) at rest, after tyramine administration (55 μg/kg, i.v.), and during sustained handgrip exercise. Cardiac NE release was determined by subtracting [NE]o from [NE]s ([NE]o−[NE]s). NE release was defined as [NE]s−[NE]o during the intervention−[NE]s−[NE]o at rest ([Δ]NEo−[Δ]NEs at rest). In patients studied within 5 months of CT, no significant NE release occurred after tyramine administration ([Δ]NEo 33±18 pg/ml; range, −98 to 117 pg/ml) or handgrip exercise ([Δ]NEo 58±11 pg/ml; range, −46 to 8 pg/ml; n=10). Conversely, in 39 of 50 patients studied 1 year or more after CT, tyramine administration caused a significant cardiac NE release ([Δ]NEo 500±59 pg/ml; range, −11 to 1,918 pg/ml), and handgrip exercise caused a significant NE release in 17 of 41 patients ([Δ]NEo 189±34 pg/ml; range, −211 to 949 pg/ml). In normally innervated patients, tyramine caused an even larger NE release ([Δ]NEo 1,943±210 pg/ml; range, 1,152 to 2,977 pg/ml), and handgrip exercise caused a significant NE release in two of seven patients ([Δ]NEo 143±51 pg/ml; range, −15 to 338 pg/ml).

Conclusions. Early after CT, neither tyramine nor handgrip exercise caused a significant cardiac release of NE, suggesting sympathetic denervation. Late after CT, most patients had a significant, but subnormal, NE release in response to pharmacological or reflex stimuli, suggesting that limited sympathetic reinnervation occurs in most patients after orthotopic CT.

In normal humans, the myocardium and coronary vessels are richly innervated by the sympathetic nervous system. Cardiac transplantation severs sympathetic nerve fibers and causes total cardiac denervation. In animal models, limited reinnervation by the sympathetic nervous system usually occurs within the first year after either homotopic or autotopic cardiac transplantation. Studies using microscopy have documented regrowth of sympathetic nerves along coronary blood vessels and over the anastomoses between the donor heart and recipient atria and great vessels. Myocardial norepinephrine content, nearly absent immediately after transplantation, later increases to subnormal levels. Moreover, studies in physiology have shown that heart rate and myocardial contractility in reinnervated animals can be increased by reflex sympathetic maneuvers and direct sympathetic neural stimulation.

In contrast to the extensive evidence for sympathetic reinnervation in animals, evidence for reinnervation after orthotopic transplantation in humans is lacking. Although anatomic studies of transplanted hearts rarely demonstrated nerve fibers, they have been interpreted as postganglionic parasympathetic neurons (where the ganglion was transplanted with the heart). Sympathetic neurally mediated changes in heart rate that normally occur during exercise, Valsalva maneuver, and orthostasis have been reported to be
A single study using a spectral analysis of heart rate has demonstrated in one patient small changes in heart rate with respiration, suggesting possible neural reinnervation.20

It is curious that reinnervation would so frequently occur in animal models and yet be absent in humans, most of whom received close attention to immunosuppressive therapy and metabolic factors. In this study, we hypothesized that sympathetic reinnervation does occur after orthotopic cardiac transplantation in humans. We viewed the heart as a neuroendocrine organ in which sympathetic stimulation can cause the release of norepinephrine from sympathetic nerve terminals within the myocardium and coronary vasculature. For a nerve terminal to contain vesicular stores of norepinephrine, the terminal must be connected to the cell nucleus.21,22 Because the nuclei of cardiac sympathetic nerves reside outside the transplanted portion of the heart, the presence of norepinephrine within cardiac nerve terminals implies reinnervation.2

To test directly for reinnervation, we measured the cardiac release of norepinephrine in response to two stimuli: tyramine and sustained handgrip exercise. Tyramine causes degranulation of large and, possibly, small neuronal vesicles containing norepinephrine and is, therefore, a pharmacological method of detecting norepinephrine within nerve terminals.23–25 Sustained handgrip exercise reflexively augments sympathetic tone. For handgrip exercise to cause the release of norepinephrine from the heart, the heart must be operative in a reflex arc that includes sympathetic reinnervation.

**Methods**

**Patient Selection**

Three groups of patients were evaluated. The first group was composed of 12 consecutive patients who had undergone orthotopic cardiac transplantation within 5 months (mean, 3.1 ±0.4 months; range, 2–5 months) of the study. Although not selected for these traits, all patients underwent uncomplicated orthotopic cardiac transplantation (seven for ischemic coronary artery disease, three for dilated cardiomyopathy of uncertain origin, and two for valvular heart disease). Each patient was treated with cyclosporine A, azathioprine, and glucocorticoid immunosuppression, and five patients had prior allograft rejection, as assessed by serial endomyocardial biopsies and clinical course. The protocol is described elsewhere. Three patients were treated with antihypertensive drugs (in one, a β-adrenergic receptor antagonist and in two, a calcium receptor antagonist). Two patients received low-dose aspirin therapy. This group comprised the patients studied early after cardiac transplantation.

Fifty consecutive patients were studied 1 or more years after orthotopic cardiac transplantation (mean, 37 ±3 months; range, 12–102 months). Twenty-six patients underwent transplantation for ischemic heart disease; 17 for dilated cardiomyopathy of uncertain origin; two for valvular heart disease; one each for viral, hypertrophic, valvular, and familial cardiomyopathy; and one for congenital heart disease. Each patient was treated with long-term immunosuppressive therapy similar to that described above. Seven patients had at least one prior episode of rejection. Thirty-three patients received antihypertensive drugs (in 19, a β-adrenoreceptor antagonist; in 21, a calcium channel antagonist; and in eight, an angiotensin converting enzyme inhibitor). In addition, 16 patients received low-dose aspirin therapy (≤650 mg daily), and all received oral calcium supplements. These patients made up the group studied later after cardiac transplantation.

Eight patients undergoing coronary angiography for the diagnosis of a chest pain syndrome were studied as controls in whom we presumed normal cardiac innervation. Two patients had mild-to-moderate atherosclerotic coronary artery disease, and one had small-vessel vasodilator insufficiency (syndrome X). Six patients received antianginal or antihypertensive therapy (in one, a β-adrenoreceptor antagonist; in four, a calcium channel antagonist; in three, an angiotensin converting enzyme inhibitor).

Patients were excluded from any of the groups if they had diabetes mellitus, amyloidosis, or other diseases that can impair peripheral neural function. All studies were approved by the University of Minnesota Institutional Review Board.

**Catheterization Protocol**

Patients were brought to the catheterization laboratory in a fasting state after premedication with diazepam (10 mg, orally). Right heart catheterization, measurement of cardiac output (thermodilution method), and right ventricular endomyocardial biopsies were performed in all transplant recipients. The coronary sinus and ascending aorta were then cannulated with 5–8F catheters. Aortic blood pressure and electrocardiogram were continuously recorded. Paired blood samples for norepinephrine analysis were then obtained from the aorta and coronary sinus. The coronary sinus sample was obtained 5–7 seconds after the aortic sample was drawn to allow for the transit time of blood through coronary circulation. In 17 patients, a repeated set of paired samples was obtained within 1 minute to assess the measurement variability of norepinephrine.

**Sustained handgrip.** Next, a subgroup (10 patients early after transplantation, 41 patients late after transplantation, and seven normal patients) performed sustained 50–75% maximal handgrip exercise (with the aid of a dynamometer) for 1 minute. Paired aortic and coronary sinus blood samples to assess norepinephrine release were obtained after 1 minute of handgrip exercise.

**Intravenous tyramine.** After a 5-minute rest period, paired aortic and coronary sinus blood samples were again obtained as a control. Tyramine, 55 μg/kg (Sigma Chemical Co., St. Louis, Mo.), was infused
intravenously as a bolus. This dose was chosen because previous dose–response studies indicated that 50–60 μg/kg caused a 15–20 mm Hg rise in mean arterial pressure, suggesting that significant peripheral neuronal norepinephrine release was elicited. The method has been described elsewhere by Forman et al.21 In the first five patients in each group, paired aortic and coronary sinus blood samples were obtained at 30 seconds and at 1, 2, 3, 4, 5, and 7 minutes after tyramine bolus administration. These initial studies demonstrated that peak cardiac norepinephrine release occurred in each group within 1 minute of tyramine administration. In the remaining patients, samples were obtained at 30 seconds and at 1 and 2 minutes after tyramine injection.

Intracoronary tyramine. In a subgroup of three patients, a bolus of tyramine (10 μg/kg in 10 ml 0.9% saline) was given into the left coronary artery to elicit a cardiac norepinephrine release without changing systemic arterial pressure. This dose of tyramine was chosen because studies in dogs suggested that 10 μg/kg or less intracoronary tyramine produced a large cardiac release of norepinephrine without altering systemic hemodynamics. Paired blood samples from the aorta and coronary sinus were drawn before and at 30 seconds, 1 minute, and 2 minutes after intracoronary administration of tyramine.

Measurement of Plasma Norepinephrine Concentration

Blood samples obtained from the coronary sinus and aorta were immediately centrifuged at 2,000 rpm for 12 minutes. The plasma was decanted and frozen at −70°C. Plasma norepinephrine concentration was subsequently determined by a radioenzymatic method that has been described elsewhere (Cat-a-kit, Amersham, Corp. Arlington Heights, Ill.).26

Assessment of Cardiac Norepinephrine Release (Uptake)

Cardiac release or uptake of norepinephrine was assessed by subtracting the norepinephrine concentration in the blood entering the heart through the aorta from the norepinephrine concentration in the blood leaving the heart through the coronary sinus ([NE]CS-Ao). The effect of an intervention on cardiac norepinephrine release (uptake) was assessed by subtracting the cardiac norepinephrine release (uptake) during control conditions from the cardiac norepinephrine release (uptake) during the intervention (∆[NE]CS-Ao).

Electrocardiography

Electrocardiograms were recorded in each patient within 24 hours of catheterization. The QT interval was measured as the longest time in any precordial lead from the onset of the QRS complex to the end of the T wave. The QT interval was corrected for heart rate by use of a previously defined formula: QTc =QT/(RR interval)^-1.27 None of the patients received drugs that are known to directly alter the duration of the QT interval.

Statistical Analysis

All data are presented as mean±SEM, except where noted otherwise. Differences between the group means were assessed with analysis of variance (STATVIEW II). Paired differences were assessed with a paired t test. Changes in heart rate and arterial pressure after an intervention were compared with changes in heart rate and arterial pressure measured during a similar time interval in which no intervention occurred. Statistical significance was defined as a probability value of 0.05 or less.

Results

Measurement Variability

Plasma norepinephrine concentration. The average difference in plasma norepinephrine concentration of 34 paired samples (17 aortic and 17 coronary sinus pairs) obtained within 1 minute of each other was 12±53 pg/ml (mean±SD). The values were normally distributed.

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Plot of plasma concentration of norepinephrine ([NE]) in the coronary sinus and aorta in each patient group. There were no significant differences between groups. CT, cardiac transplantation.
TABLE 1. Plasma Norepinephrine Concentrations During Basal Conditions

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>[NE]_{Ao}</th>
<th>[NE]_{Cs}</th>
<th>[NE]_{Cs-Ao}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplant recipients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early (&lt;5 mo)</td>
<td>12</td>
<td>233±31</td>
<td>243±23</td>
<td>11±12</td>
</tr>
<tr>
<td>Late (≥1 yr)</td>
<td>50</td>
<td>320±20</td>
<td>343±24</td>
<td>22±10</td>
</tr>
<tr>
<td>Normal controls</td>
<td>8</td>
<td>294±21</td>
<td>330±39</td>
<td>36±34</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

[NE]_{Ao}, plasma concentration of norepinephrine in the aorta; [NE]_{Cs}, plasma concentration of norepinephrine in the coronary sinus; [NE]_{Cs-Ao}, cardiac norepinephrine release or uptake ([NE]_{Cs}-[NE]_{Ao}).

Cardiac norepinephrine release (uptake). The mean difference in 17 paired measurements of cardiac norepinephrine release obtained within 1 minute of each other was 5±46 pg/ml (mean±SD). The mean difference between paired measurements obtained at rest and 5 minutes after sustained handgrip exercise (the second "control" period, n=58) was 7±57 pg/ml. The likelihood that an intervention in a single patient would spuriously cause a 178-pg/ml or more rise in norepinephrine concentration between the coronary sinus and aorta (3 SD change of the largest variability measurement) was less than 1%. Hence, a measured cardiac norepinephrine release of more than 178 pg/ml in response to an intervention was taken as evidence of a significant norepinephrine release.

Plasma Norepinephrine Concentrations Under Basal Conditions

Under basal conditions, the average concentration of norepinephrine in the aorta and coronary sinus was similar in all patient groups (Figure 1, Table 1). In addition, the range of values was similar between groups. Under basal conditions, the magnitude of cardiac norepinephrine release (uptake) was also similar between patient groups (Table 1). Hence, during basal conditions, the plasma norepinephrine concentration in blood entering the myocardium and in blood exiting the myocardium was similar early and late after transplantation, and concentrations in transplanted hearts were similar to those in normal hearts.

Effects of Intravenous Tyramine

Hemodynamics. Intravenous tyramine administration caused a prompt rise in aortic blood pressure in each group (Table 2). The magnitude of the rise in systolic and mean aortic blood pressures was not significantly different between groups. Tyramine elicited no significant change in heart rate in patients studied early after transplantation (Table 2). Patients studied late after transplantation had a small, but significant, rise in heart rate (4±1 beats/min, p<0.05). Normal patients had a more marked increase in heart rate (10±6 beats/min), but the response was variable (−5 to 50 beats/min).

Cardiac norepinephrine release or uptake. In normal patients, there was a rapid and striking release of norepinephrine after intravenous tyramine injection (Table 2, Figures 2 and 3). Within 30 seconds of tyramine administration, the transcadiac norepinephrine concentration gradient (Δ[NE]_{Cs-Ao}) rose by an average of 1,943±210 pg/ml and then rapidly fell to basal levels. Twelve of 12 patients who were studied within 3 months of transplantation had no significant norepinephrine release after tyramine injection. Late after transplantation, however, 39 of 50 patients had a norepinephrine release that exceeded the 99% confidence limits for repeated measurements (i.e., >178 pg/ml). On average, however, the magnitude of norepinephrine release was significantly less than that observed in normal patients. Hence, in most patients, hearts late after transplantation secreted significant quantities of norepinephrine in response to tyramine but less than the amount generated by nontransplanted hearts.

Effects of Intracoronary Tyramine

Hemodynamics. Neither arterial blood pressure nor heart rate changed after intracoronary tyramine administration. In each patient, the sinus node artery arose from the right coronary artery.

<table>
<thead>
<tr>
<th></th>
<th>Δ Aortic pressure</th>
<th>Δ Heart rate</th>
<th>Δ[NE]_{Ao}</th>
<th>Δ[NE]_{Cs}</th>
<th>Δ[NE]_{Cs-Ao}</th>
<th>Δ[NE]_{Cs-Ao}</th>
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<tr>
<td></td>
<td>n</td>
<td>Systolic (mm Hg)</td>
<td>Diastolic (mm Hg)</td>
<td>(beats/min)</td>
<td>(pg/ml)</td>
<td>(pg/ml)</td>
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<td>Intravenous tyramine</td>
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<td></td>
<td></td>
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<td>Transplant recipients</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Early (&lt;5 mo)</td>
<td>12</td>
<td>25±3</td>
<td>13±2</td>
<td>2±1</td>
<td>91±18</td>
<td>99±16</td>
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<tr>
<td>Late (≥1 yr)</td>
<td>50</td>
<td>20±1</td>
<td>12±1</td>
<td>4±1*</td>
<td>111±10</td>
<td>565±59†</td>
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<td>Normal controls</td>
<td>8</td>
<td>25±5</td>
<td>8±1</td>
<td>10±6*</td>
<td>217±81‡</td>
<td>2,054±205‡</td>
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<td></td>
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<td>Late transplant recipients</td>
<td>3</td>
<td>2±1</td>
<td>2±1</td>
<td>1±1</td>
<td>9±37</td>
<td>1,014±121</td>
</tr>
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</table>

Data are mean±SEM.

Δ[NE]_{Ao}, peak change in plasma concentration of norepinephrine in the aorta; Δ[NE]_{Cs}, peak change in plasma concentration of norepinephrine in the coronary sinus; Δ[NE]_{Cs-Ao}, peak cardiac norepinephrine release (uptake) after tyramine.

*p<0.05 vs. repeated measurement without intervention; †p<0.05 vs. early after transplantation; ‡p<0.05 vs. early and late after transplantation.
Cardiac norepinephrine release (uptake). In the three late transplant recipients who received intracoronary tyramine, there was a rapid and striking cardiac norepinephrine release (Δ[NE]CS-Ao, Table 2). Within 60 seconds of drug administration, the transcardiac norepinephrine gradient increased by 1,004±121 pg/ml. There was no significant change in aortic plasma norepinephrine concentration in the same time interval.

Effects of Sustained Handgrip Exercise

Hemodynamics. In response to sustained handgrip exercise, patients studied early and late after transplantation and normal patients had a significant rise in systolic, diastolic, and mean aortic blood pressures (Table 3). There was no significant difference in the magnitude of blood pressure rise between groups. The magnitude of arterial pressure rise in response to handgrip exercise was similar to the change in arterial pressure elicited by tyramine. Heart rate was unchanged during sustained handgrip exercise in early transplant recipients, increased slightly in late transplant recipients (4±1 beats/min), and rose significantly in normal patients (14±5 beats/min).

Cardiac norepinephrine release (uptake). None of the 10 patients studied within 5 months of transplantation had a significant cardiac norepinephrine release during handgrip exercise (Table 3, Figure 4). In contrast, 17 of 41 patients studied late after transplantation had a significant cardiac norepinephrine release, and the average norepinephrine release measured in all patients studied late after transplan-
**Table 3. Effects of Sustained Handgrip Exercise on Hemodynamics and Cardiac Norepinephrine Release**

<table>
<thead>
<tr>
<th>Transplant recipients</th>
<th>Systolic (mm Hg)</th>
<th>Diastolic (mm Hg)</th>
<th>Δ Aortic pressure</th>
<th>Δ Heart rate (beats/min)</th>
<th>Δ[NE]_{CS-Ao} (pg/ml)</th>
<th>Δ[NE]_{CS} (pg/ml)</th>
<th>Mean (pg/ml)</th>
<th>Range (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (≤ 5 mo)</td>
<td>10</td>
<td>20±3</td>
<td>15±2</td>
<td>2±1</td>
<td>74±16</td>
<td>39±11</td>
<td>−34±10</td>
<td>−46−8</td>
</tr>
<tr>
<td>Late (≥ 1 yr)</td>
<td>41</td>
<td>23±2</td>
<td>18±1</td>
<td>4±1*</td>
<td>77±13</td>
<td>267±37†</td>
<td>189±34†</td>
<td>−211−949</td>
</tr>
<tr>
<td>Normal controls</td>
<td>7</td>
<td>24±3</td>
<td>15±4</td>
<td>14±5‡</td>
<td>47±18</td>
<td>191±59</td>
<td>143±51</td>
<td>−15−338</td>
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</tbody>
</table>

Data are mean±SEM.
Δ[NE]_{CS-Ao} peak change in plasma concentration of norepinephrine in the aorta; Δ[NE]_{CS} peak change in plasma concentration of norepinephrine in the coronary sinus; Δ[NE]_{CS-Ao} peak cardiac norepinephrine release (uptake) during handgrip exercise.

*p<0.05 vs. repeated measurement without intervention; †p<0.05 vs. early after transplantation; ‡p<0.05 vs. early and late after transplantation.

Norepinephrine release was significantly higher than norepinephrine release at rest ([Δ[NE]_{CS-Ao} p<0.01]) and greater than that measured in patients early after transplantation (p<0.05). Each of the 17 patients who released significant quantities of norepinephrine during handgrip exercise also released norepinephrine after tyramine administration. None of those who failed to release norepinephrine after tyramine administration had a significant norepinephrine release after handgrip exercise. Two of seven normal patients had a significant cardiac release of norepinephrine at peak sustained handgrip exercise.

There was no significant relation in any group between norepinephrine release and the change in arterial blood pressure during handgrip exercise.

**Relation of Tyramine-Induced Cardiac Norepinephrine Release to Demographic, Hemodynamic, and Electrocardiographic Characteristics (Table 4)**

The likelihood and magnitude of cardiac norepinephrine release increased with the time interval between the transplantation and the study (Figure 5). The maximal release was observed at 4 years or more after transplantation.

Resting heart rate was significantly higher in early transplant recipients than in late recipients who had a significant norepinephrine release. The reduction in heart rate, however, was only seen in patients who had a large cardiac norepinephrine release after tyramine administration (>500 pg/ml peak Δ[NE]_{CS-Ao}). In those patients, the mean heart rate was 77±3 compared with 85±4 beats/min in patients with a small release (i.e., 179−499 pg/ml peak Δ[NE]_{CS-Ao}).

There was no significant difference in right atrial, right ventricular, pulmonary artery, or pulmonary capillary wedge pressures between patients studied early and late after transplantation and in patients with and without a significant norepinephrine release after tyramine administration. Furthermore, the cardiac index, stroke index, heart rate, and aortic blood pressure were not significantly different between the patients studied early and late after transplantation and in patients who had evidence of delayed reinnervation and those who did not.

Drug treatment (β-receptor antagonists, calcium channel antagonists, angiotensin converting enzyme inhibitors, or immunosuppressive regimens) was not significantly different between those with and without a significant norepinephrine release after tyramine.

**Discussion**

This study demonstrates that early after cardiac transplantation there was an absence of norepinephrine release.

**Figure 4. Plot of peak difference in plasma norepinephrine concentration between the coronary sinus and aorta (Δ[NE]_{CS-Ao}) after sustained handgrip exercise.**

Shaded area shows the 99% confidence limits for repeated measurements of Δ[NE]_{CS-Ao}. None of the patients studied within 5 months of cardiac transplantation (CT) had a significant norepinephrine release. Of patients studied 1 year or more after cardiac transplantation, however, 17 of 41 had a significant cardiac norepinephrine release. Only two of seven normally innervated controls had a significant increase in Δ[NE]_{CS-Ao}, but the mean change was similar to that measured in patients 1 year or more after cardiac transplantation and was significantly different from no change.
rine stores within the heart, suggesting sympathetic denervation. Late after transplantation, most patients released cardiac norepinephrine in response to either pharmacological or reflex physiological stimuli, indicating sympathetic reinnervation. The likelihood of cardiac norepinephrine release increased over time, reaching maximal levels 4 or more years after surgery. The magnitude of norepinephrine release from transplanted aortas, however, was less than that elicited in normally innervated patients, suggesting that only partial reinnervation occurred and that the degree of reinnervation varied widely among patients.

Potential Methodological Problems

Before accepting these findings as proof of sympathetic reinnervation after transplantation, several methodological factors should be considered. Is it possible that the norepinephrine released late after transplantation was from a remnant of the recipient's atrium? Two factors make this unlikely. First, the coronary sinus of the transplanted heart does not drain the recipient's atrial remnant; consequently, the norepinephrine release measured in the coronary sinus could not have been from the atrial remnant. Second, if the norepinephrine originated in the atrial remnant or other site, its release should have been detected in the patients studied early after transplantation. Because no cardiac norepinephrine release was measured in patients studied early after transplantation, the atrial remnant could not have accounted for the cardiac norepinephrine release observed late after transplantation.

Is it possible that intact sympathetic nerve terminals existed within the heart in the absence of reinnervation? Anatomic studies demonstrate that the sympathetic ganglia lie outside the transplanted portion of the heart, although a few parasympathetic ganglia may be included. In peripheral adrenergic nerves, vesicles and enzymes needed for synthesis of norepinephrine must be shuttled from the cell body to the nerve terminal by an intact axon. Hence, logic dictates that in order for the nerve terminal to contain norepinephrine, it must be connected to the ganglia outside the heart. This is borne out by the failure of patients studied early after transplantation to release norepinephrine. Furthermore, our studies show that cardiac norepinephrine release can also be brought about by reflex sympathetic stimulation (sustained handgrip exercise). This could only occur if the sympathetic nerve terminals in the heart were connected to the spinal or central nervous systems. These findings are overwhelming evidence that sympathetic reinnervation occurs after cardiac transplantation in humans.

The sensitivity of the provocative maneuvers we used to detect reinnervation (tyramine administration and sustained handgrip exercise) is uncertain but is probably far less than perfect. Although tyramine is a stimulus for degranulation of large vesicles within the sympathetic nerve terminal, the fraction of vesicular norepinephrine released after a

<table>
<thead>
<tr>
<th>Table 4. Relation of Innervation to Demographics, Hemodynamics, and the Electrocardiogram</th>
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<tr>
<td>Patients (time after cardiac transplantation)</td>
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<tr>
<td>------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Months after transplantation (mo)</td>
</tr>
<tr>
<td>Prior rejection</td>
</tr>
<tr>
<td>CMV+</td>
</tr>
<tr>
<td>Heart rate (beats/min)†</td>
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<tr>
<td>Aortic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Systolic</td>
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<tr>
<td>Diastolic</td>
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<tr>
<td>Intracardiac pressure (mm Hg)</td>
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<tr>
<td>Right atrium (mean)</td>
</tr>
<tr>
<td>Right ventricle</td>
</tr>
<tr>
<td>Systolic</td>
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<tr>
<td>End diastolic</td>
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<td>Pulmonary artery wedge (mean)</td>
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<td>Cardiac index (l/min/m²)</td>
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<tr>
<td>Stroke volume index (ml/m²)</td>
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<tr>
<td>QTc interval (msec)</td>
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</tbody>
</table>

Data are mean±SEM.
CMV, cytomegalovirus antibody.
*Denervated, Δ[NE]c–a >178 pg/ml; innervated, Δ[NE]c–a <178 pg/ml.
†Only patients not taking β-adrenergic or calcium channel antagonists.
§p<0.05 vs. early after transplantation.
A single dose of tyramine may vary among patients. Handgrip exercise (which releases the contents of neuronal small vesicle) is also a submaximal and somewhat variable stimulus for sympathetic discharge. To the converse, presynaptic norepinephrine reuptake may be defective after denervation, causing a greater fraction of the norepinephrine released by reinnervating neurons to be absorbed by the blood and exaggerating the measured magnitude of norepinephrine release. Thus, the sensitivity of the "tyramine test" has not been defined precisely.

The failure of normally innervated patients to release norepinephrine in response to handgrip exercise is curious, particularly because 41% of late transplant recipients did release norepinephrine in response to a similar stimulus. Cardiac norepinephrine release in normal patients might have been modulated either at the level of the peripheral nerve terminal or more centrally (central nervous system or ganglion) from an inhibitory vascular or ventricular afferent response brought about by the increased blood pressure associated with handgrip exercise. Prior investigators have shown that acetylcholine release can reduce synaptic norepinephrine release, and parasympathetic activation from handgrip-induced hypertension might, therefore, have reduced the cardiac norepinephrine release in normal patients. The same parasympathetic efferent arm might have been absent in late transplant recipients.

In the absence of reinnervation, coronary blood flow might have been altered by changes in aortic plasma norepinephrine concentration, affecting the magnitude of the norepinephrine concentration difference between the aorta and coronary sinus. Two factors suggest that changes in blood flow did not significantly alter our assessment of norepinephrine release. First, without cardiac release of norepinephrine, the concentration of norepinephrine leaving the heart should have been the same as that entering the heart. Although a change in blood flow could alter the magnitude of concentration change for a given quantity of norepinephrine release, it could not reverse the direction of the concentration change (e.g., Δ[NE]cs-Ao > Δ[NE]ao).

Second, we found that aortic plasma norepinephrine concentration rose similarly in patients studied early and late after transplantation, but only in patients studied late after transplantation was there a significant change in Δ[NE]cs-Ao. Third, coronary blood flow would have had to fall by 90% to cause the 10-fold rise in Δ[NE]cs-Ao seen in most patients. This would have had to occur despite augmented metabolic oxygen demand from increased blood pressure and in the absence of electrocardiographic changes. Last, in a prior study, we found that handgrip exercise in late transplant recipients increases coronary blood flow. Consequently, changes in coronary blood flow associated with these interventions (except intracoronary tyramine administration) probably reduced the sensitivity of the method for detecting a norepinephrine release. Although the presence of myocardial norepinephrine release is
clear, these studies should be viewed as qualitative or at best semiquantitative indexes of reinnervation.

Although we were careful to determine the accuracy of our measurement techniques, there was range in which norepinephrine concentrations could not be distinguished with confidence. The decision to define a significant norepinephrine release as an increase in transcardiac norepinephrine concentration of more than 3 SDs from the mean of repeated measurements was arbitrary. Smaller increases in the transcardiac norepinephrine concentration gradient (Δ[NE]CS-Ao) after tyramine might have been caused by reinnervation rather than chance. In addition, reinnervation is probably a spatially heterogeneous process. Lesser amounts of reinnervation, in which the released norepinephrine would be diluted by drainage from the noninnervated heart, may be difficult to detect by this method. Furthermore, it may be difficult to detect reinnervation in the atria or right ventricle, where venous drainage may occur primarily through the Thebesian system into the cardiac chambers. In the future, several portions of the coronary sinus could be selectively cannulated to determine the degree of spatial heterogeneity of reinnervation.

Comparison With Prior Studies

Studies in animal models clearly demonstrate that sympathetic, and occasionally parasympathetic, reinnervation occurs after cardiac transplantation.6-13 Early after autotransplantation or homotransplantation, efferent and afferent denervation can be demonstrated by the absence of appropriate responses to reflex stimuli. Later, sympathetic reinnervation occurs in a base-to-apex sequence.13 Changes in heart rate and contractility in response to stellate ganglion stimulation or tyramine can be detected in reinnervated animals, suggesting that reinnervation can have a physiological impact.

The timing of reinnervation may be somewhat similar in animals and in humans. From studies in dogs, reinnervation appears to occur more slowly after homotransplantation than after autotransplantation.6,9,13 After homotransplantation, evidence of reinnervation surfaces within 4–9 months. We found evidence of reinnervation in three of six patients studied during the second year after transplantation. Maximal norepinephrine excretion, however, was not observed until 4 years after transplantation. Reinnervation in humans may take longer than that in dogs, and reinnervation in humans may be less complete than that in dogs because of the larger size of the human heart.

The magnitude of reinnervation cannot be precisely calculated from our data, although norepinephrine excretion after a standardized dose of tyramine was less in the transplanted hearts of patients than in normal controls. These findings are similar to those reported in animals. Cardiac norepinephrine stores plummet to negligible levels immediately after transplantation.9,11-13 Late after transplantation, tissue norepinephrine concentration rises but usually not to the concentration present before transplantation.7,9,11,13 Consecutive studies will be required to determine if the magnitude of norepinephrine excretion continues to increase with time late after transplantation.

Implications of Sympathetic Reinnervation

Our studies demonstrate that reinnervation responsive to central neural control occurs after cardiac transplantation in humans. The physiological consequences of reinnervation have not been defined but could include the return of sympathetic neural modulation of heart rate, ventricular contractility, and modulation of coronary vasomotor tone.

Although, sympathetic reinnervation may be anticipated to increase the basal heart rate, we found that the resting heart rate was lower in late transplant recipients who released large amounts of norepinephrine in response to tyramine. Basal heart rate after transplantation, however, may be influenced by many factors, including time (with attendant sinus node dysfunction11) and parasympathetic reinnervation. We found also that, in reinnervated late transplant recipients, heart rate significantly increased after tyramine administration and handgrip exercise. The heart rate response to these stimuli, however, was subject to considerable variability that might have resulted from several factors. First, the magnitude of reinnervation probably varies substantially among subjects. Second, if reinnervation is spatially heterogeneous, then its specific effects would also be heterogeneous (e.g., different in the sinoatrial node and coronary arteries) and different from patient to patient. In some patients, reinnervation of the sinus node could take place independent of reinnervation of the left ventricle. Third, Kaye et al13 showed in dogs with reinnervation after autotopic transplantation that myocardial catecholamine concentration is lowest in right atrial tissue (compared with the other chambers). Consequently, the sinus node may normally be less reinnervated than the rest of the heart. Sinus node dysfunction has also been demonstrated late after transplantation.31 Hence, assessing reinnervation by examining the effect of stimuli on heart rate is likely to have a low sensitivity.

Many factors also affect ventricular function after transplantation, and reinnervation may only be one, inconstant factor. Prior investigators showed in animal models that denervation does not alter ventricular function or myocardial blood flow,32 and we showed that coronary flow reserve is normal in humans late after uncomplicated orthotopic transplantation.30 Moreover, the compensatory mechanisms that occur as a result of denervation may fade when reinnervation occurs, leaving no net change in hemodynamics with reinnervation. Further studies examining specific responses to provocative maneuvers (e.g., exercise) may, however, uncover important physiological effects of reinnervation on hemodynamics. Presently, however, our findings suggest that prior studies of ventricular function using trans-
planted hearts as “denervated controls” will need to be rethought.19

The coronary arteries are normally well innervated by the sympathetic and parasympathetic nervous systems.1 Sympathetic reinnervation in humans may proceed preferentially down vascular pathways as it does in animal models of transplantation.10 Moreover, sympathetic reinnervation may frequently occur in the absence of parasympathetic reinnervation, leaving the possibility of “unbalanced” neural input and a propensity for vasoconstriction. Spontaneous and catheter-induced coronary vasospasm have been reported in transplant recipients, and we have observe one episode of spontaneous vasospasm in a distal coronary vessel during routine coronary arteriography.33,34 Furthermore, because most patients studied late after transplantation are not totally denervated, studies of the coronary circulation using late transplant recipients as “denervated” controls will need to be reexamined.35–36 In our opinion, only patients studied very early after transplantation (e.g., within 3–4 months) can be considered totally denervated.

Last, it is unclear why some patients appear to have substantial reinnervation over time while others do not. Further studies will perhaps identify the factors or mechanisms by which reinnervation occurs and determine the physiological consequences (good or bad) of this phenomenon.

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