Functional and Neurochemical Evidence for Partial Cardiac Sympathetic Reinnervation After Cardiac Transplantation in Humans

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Background. The presence of cardiac reinnervation in humans after cardiac transplantation has been widely debated, based on the application of differing methods for the assessment of neuronal function. Some of these techniques have been rather indirect; consequently, the time course and extent of cardiac reinnervation remains uncertain.

Methods and Results. To test for the presence of cardiac reinnervation after transplantation, we examined neurochemical (radionabeled norepinephrine [NE] kinetics) and functional markers (power spectral analysis, heart rate response to exercise) of cardiac sympathetic nerve integrity in 15 cardiac transplantation recipients and 25 healthy control subjects of similar age. Cardiac transplantation subjects were studied 9 weeks to 8 years after cardiac transplantation (10 “early” patients <18 months and 5 “late” patients >2 years after cardiac transplantation). At rest, cardiac NE spillover was markedly attenuated early after transplantation (11.2±18.3 pmol/min) compared with subjects late after transplantation (105±11 pmol/min, P<.01) or in healthy control subjects (103±15 pmol/min, P<.01). Heart rate variability (measured by total spectral power) was significantly reduced in cardiac transplantation recipients compared with control subjects (59.4±30 vs 1673±516 milliseconds squared; P<.05), with evidence of a trend toward increasing spectral power late after transplantation. During exercise, the cardiac NE spillover was significantly lower in early cardiac transplantation recipients when compared with control subjects (163±50 vs 1876±418 pmol/min, P<.01). Late cardiac transplantation subjects showed a response intermediate (1080±254 pmol/min) between that of the early cardiac transplantation and control groups. However, measurements of the neuronal reuptake process for NE (assessed by the fractional extraction of plasma labeled NE across the heart and tritiated dihydroxyphenylglycol release) were significantly depressed in both early and late cardiac transplantation subjects.

Conclusions. The present study demonstrates a partial restoration of cardiac sympathetic nerve function in humans up to 8 years after heart transplantation. (Circulation. 1993;88:1110-1118.)

KEY WORDS • transplantation • norepinephrine • sympathetic nerves

The cardiac denervation associated with heart transplantation and subsequent potential for reinnervation has been the focus of recent interest among clinicians, physiologists, and pharmacologists. One justification for exploring the cardiovascular effects of cardiac denervation and reinnervation after transplantation is to better understand exercise responses after heart transplantation, particularly the potential for improvement in exercise capacity late after transplantation that may be consequent upon cardiac reinnervation. There are additional areas where posttransplantation cardiac reinnervation has possible clinical relevance. One area concerns the ability of some patients to experience ischemia-like chest pain, which remains unexplained. Another area is the responsiveness of the transplanted heart to adrenergic drugs, which early after transplantation is dictated by denervation supersensitivity. In nontransplanted patients with coronary artery disease, neural mechanisms are commonly of importance in the development of ventricular arrhythmias. The presence or absence of reinnervation in the transplanted heart may therefore also be pertinent in the setting of late coronary artery disease, with its attendant complications such as cardiac arrhythmias.

Recent observations of cardiac reinnervation have principally focused on the return of sympathetic nervous function in the heart. Sympathetic reinnervation has been inferred from progressive normalization of the heart rate response to various stimuli such as exercise after transplantation. Of interest also, resting heart rate has been shown to be significantly lower late after transplantation, possibly reflecting a degree of vagal reinnervation. Power spectral analysis of heart rate variability after heart transplantation has been applied to test for the return of sympathetic and parasympathetic influences on sinus node activity.

In animal models of cardiac autotransplantation, myocardial catecholamine content has been found to be extremely low early after transplantation. The content increases late after surgery but does not reach normal
levels. More recently, cardiac release of norepinephrine in human cardiac transplant recipients has been demonstrated using tyramine stimulation. Paradoxically, despite evidence suggesting a return of myocardial sympathetic fibers, a supersensitivity to β-adrenergic agonists persists, recently being described in cardiac transplant recipients studied an average of 2.4 years after transplantation. With the application of histochemical methods, the reappearance of sympathetic fibers has been described in a number of animal transplant models, with only limited supportive pathological data from human myocardial specimens.

To detect sympathetic reinnervation of the transplanted heart, we used both neurochemical and functional tests of neuronal integrity. Using the isotope dilution method with coronary sinus venous sampling, the release and reuptake of norepinephrine and production of the norepinephrine precursor dihydroxyphenylalanine (DOPA) and the intraneuronal norepinephrine metabolite dihydroxyphenylglycol (DHPG) were assessed in transplant recipients and control subjects at rest and during exercise. These biochemical indices of neuronal function were combined with power spectral analysis of heart rate variability and assessment of the hemodynamic responses to exercise as functional markers of cardiac neural activity.

**Methods**

**Experimental Subjects**

The study subjects comprised 15 orthotopic cardiac transplant recipients and 25 healthy volunteer subjects. The mean age of the transplant recipients was 50±2 years, and their healthy counterparts were of similar age at 55±3 years (P=NS). The transplant recipients were studied an average of 88 weeks after transplantation (range, 9 weeks to 8 years), of whom 10 were arbitrarily classified as “early” (<18 months: mean, 24 weeks; range, 9 to 58 weeks) and 5 as “late” (>2 years: mean, 210 weeks; range, 116 weeks to 8 years) after surgery. At the time of study, the transplant recipients were free from clinical and histological features of cardiac reinnervation and cardiac failure. All subjects were in sinus rhythm as documented by continuous ECG monitoring. Studies were performed in the morning after a standardized light breakfast, with tea, coffee, and cigarettes being withheld for at least 12 hours before testing. Transplant recipients treated with antihypertensive medications ceased these drugs for 72 hours before study participation, and cyclosporine was also withheld on the morning of the study. The control subjects were recruited from the general community by advertisement and underwent a medical examination before participation in the study. No healthy volunteer was receiving any medication. All subjects gave written informed consent, and the study was approved by the Alfred Hospital Ethics Review Committee.

**Study Design**

To detect the presence and extent of cardiac reinnervation in the transplanted heart, we performed both a functional (spectral analysis) and a neurochemical (cardiac catecholamine kinetics) analysis of cardiac activity at rest. Cardiac and whole-body catecholamine kinetics were also assessed during submaximal exercise.

Under local anesthesia, the radial artery was cannulated (3F, 5-cm catheter, Cook, Brisbane, Australia) for arterial pressure monitoring and blood sampling. The heart rate was continuously monitored electrocardiographically. Under fluoroscopic control, a coronary sinus thermodilution catheter (Webster Lab Inc) was inserted via an introducer sheath previously placed in the antecubital fossa or in the right internal jugular vein (at the time of routine endomyocardial biopsy in two transplant recipients). The tip of the coronary sinus catheter was advanced to a stable position in the coronary sinus, confirmed by injection of a small quantity of radiographic contrast.

Tritiated levo-(7-3H)-norepinephrine and epinephrine (New England Nuclear, Boston, Mass) were each infused at a rate of 0.5 to 1.0 μCi/min through a peripheral vein for a minimum period of 60 minutes. This procedure has demonstrated to be of sufficient duration to achieve steady-state plasma concentrations. During this period, subjects were resting quietly in the supine position. At steady state, simultaneous arterial and coronary sinus samples were drawn for the determination of the plasma concentration of norepinephrine, epinephrine, DOPA, and DHPG and for determining the plasma concentrations of tritiated norepinephrine, epinephrine, and DHPG. Coronary sinus blood flow and the hematocrit were measured, allowing calculation of the coronary sinus plasma flow.

To detect the presence of reflex augmentation of cardiac sympathetic nerve function in the transplanted heart, after collection of resting samples, 10 healthy volunteers and 12 transplant (7 early and 5 late) recipients performed 10 minutes of supine cycling at a work load equivalent to 60% of their previously determined maximal work capacity. During exercise, heart rate and blood pressure were recorded at 2-minute intervals. Coronary sinus blood flow measurements and simultaneous arterial and coronary sinus blood samples were obtained at the end of the cycling period.

**Neurochemical Measurements of Sympathetic Nerve Competence**

To directly test the competence of cardiac sympathetic nerves, we measured the cardiac spillover of norepinephrine, DOPA, and DHPG (Fig 1a). The spillover of norepinephrine, DOPA, and DHPG are known to be near zero in the denervated hearts of patients with pure autonomic failure who have degeneration of postganglionic sympathetic nerves. As such, these patients can serve as a nonsurgical reference group of cardiac denervation.

The rate of spillover of norepinephrine from the heart was calculated as

Cardiac norepinephrine spillover = 

\[ \frac{[N_{CS} - N_{A}]}{[N_{A} \cdot N_{CS}]} \cdot CSPF \]

where \( N_{A} \) and \( N_{CS} \) are the arterial and coronary sinus plasma concentrations of norepinephrine, \( N_{EX} \) is the fractional extraction of tracer norepinephrine across the heart, and CSPF is the coronary sinus plasma flow. The net spillover of DOPA from the heart was determined from the product of the coronary sinus arterial difference in plasma DOPA concentration and the coronary sinus plasma flow.
the spillover of endogenous and tritium-labeled DHPG was calculated in a similar fashion, although DHPG is distributed equally between plasma and red blood cells, necessitating the use of the coronary sinus blood flow rather than plasma flow for the quantitation of DHPG release rates\textsuperscript{14}.

The competence of cardiac sympathetic innervation was further examined by measuring the extraction and neuronal processing of infused norepinephrine by the heart (Fig 1b). The removal of tritiated norepinephrine from plasma in transit through the heart and its subsequent intraneuronal processing to tritiated DHPG is largely dependent on an active process of neuronal norepinephrine uptake\textsuperscript{15}. In patients with pure autonomic failure, or after pharmacological inhibition of neuronal norepinephrine uptake, both the extraction of tritiated norepinephrine from plasma by the heart and the cardiac production of tritiated DHPG are significantly reduced\textsuperscript{13}.

As a measurement of the overall sympathetic nervous and adrenal medullary response to exercise, the total spillovers of norepinephrine and epinephrine to plasma were measured by isotope dilution, as previously described\textsuperscript{16}, at rest and during exercise. Using this method, the clearances of norepinephrine and epinephrine from plasma also were determined.

\textbf{Spectral Analysis of Heart Rate Variability}

To further test the functional integrity of cardiac nerves, spectral analysis of heart rate variability was performed in 9 of the transplant recipients (4 early and 5 late; mean age, 50±3 years) and in 8 of the healthy control subjects (mean age, 51±4 years).

The ECG was recorded continuously for a period of 20 minutes from lead II during a period of quiet, supine rest. The ECG signal was digitized on line at 1000 Hz using a 386/25 IBM-compatible PC and a data acquisition package (CVMs, McPherson Scientific) incorporating a 12-bit analog-to-digital converter (Computer Boards Inc). The data acquisition system includes a variable threshold peak detection technique from which the RR interval was determined. Data segments of 128-second duration were sampled at 2 Hz to create 256-point data sets. For each 20-minute recording period, 15 data sets of 256 points that overlapped by 50\% were processed. The linear trend was removed from each data set to avoid its contribution to low frequency power. A Hanning window in the time domain was used to attenuate spectral leakage. Spectral analysis was performed using direct fast Fourier transform. The frequency resolution was 0.0078 Hz, and the highest frequency evaluated was 0.5 Hz. The spectra obtained for different data sets were averaged to reduce variance and to sharpen reproducible spectral peaks.

\textbf{Biochemical Analysis}

Analysis of plasma samples for the catechols norepinephrine, epinephrine, DOPA, and DHPG were performed as previously described by our laboratory\textsuperscript{16}. Briefly, samples were immediately transferred to iced-chilled tubes containing an anticoagulant and an antioxidant (EGTA plus glutathione). The plasma was then separated by centrifugation and then stored at −70°C until assayed. Catecholamine concentrations were measured by high-performance liquid chromatography with electrochemical detection. This process was coupled with a fraction collector, allowing the collection and subsequent counting of portions of the eluant containing tritiated norepinephrine, epinephrine, and DHPG by liquid scintillation spectroscopy.

\textbf{Statistical Methods}

Data are expressed as mean±SEM. The comparison of group means was performed using an unpaired t test or one-way ANOVA when more than two groups were present. The relation between time after transplantation and the spillover of norepinephrine was examined using a logarithmic regression model, and linear regression was used to examine the relation between the fractional extraction of norepinephrine and time after transplantation. Where multiple comparisons were performed, post hoc testing was performed using Scheffe’s test. The comparison of the time course of hemodynamic variables between subject groups during exercise was performed using split-plot ANOVA. The null hypothesis was rejected when the probability value was ≤0.05.

\textbf{Results}

\textbf{Neurochemical Indices of Sympathetic Function at Rest}

The plasma arterial norepinephrine concentration at rest was similar in control subjects and transplant recipients (1583±213 vs 1790±199 pmol/L), with no differences in the total spillover rate to plasma (3658±473 vs 3574±420 pmol/min) or rate of clearance.
Neurochemical Indices of Cardiac Sympathetic Nerve Integrity at Rest

<table>
<thead>
<tr>
<th>Cardiac NE SR (pmol/min)</th>
<th>Control subjects (n=25)</th>
<th>&lt;18 Months (n=10)</th>
<th>&gt;2 Years (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>103±15</td>
<td>11.2±18.3*†</td>
<td>105±11</td>
</tr>
<tr>
<td>Cardiac DOPA SR (pmol/min)</td>
<td>130±20</td>
<td>-17±21*</td>
<td>84±18</td>
</tr>
<tr>
<td>Cardiac DHPG SR (pmol/min)</td>
<td>565±72</td>
<td>-59±18*</td>
<td>108±47*</td>
</tr>
<tr>
<td>Cardiac 3H-DHPG SR (dpm/min)</td>
<td>4025±590</td>
<td>-296±382*</td>
<td>1248±341‡</td>
</tr>
<tr>
<td>Cardiac 3H-NE ExR (%)</td>
<td>73±3</td>
<td>8±3*§</td>
<td>27±8*</td>
</tr>
</tbody>
</table>

NE indicates norepinephrine; SR, spillover rate; DOPA, dihydroxyphenylalanine; DHPG, dihydroxyphenylglycol; 3H-DHPG, tritium-labeled dihydroxyphenylglycol; 3H-NE, tritium-labeled norepinephrine; ExR, fractional extraction.

*P<.01 vs control, †P<.01 vs late transplant group, §P<.05 vs control, ¶P<.05 vs late transplant group.

from plasma (2.41±0.23 vs 2.28±0.18 L/min). The plasma concentration of epinephrine at rest was significantly lower in transplant recipients (control subjects vs transplant patients, 403±63 vs 206±36 pmol/L; P<.01), with a significantly lower rate of release of epinephrine to plasma (983±197 vs 442±93 pmol/min, P=.05) and similar rates of epinephrine plasma clearance (2.39±0.21 vs 2.40±0.26 L/min).

Resting cardiac norepinephrine spillover (Table) was markedly reduced in transplant recipients early after surgery (11.2±18.3 pmol/min) in comparison to recipients late after transplantation (105±18 pmol/min, P<.01) and their healthy counterparts (103±15 pmol/min, P<.01). The cardiac release of the norepinephrine precursor DOPA was also significantly reduced early after transplantation, subsequently rising substantially although not to normal levels (Table). In comparison, the capacity of cardiac sympathetic nerve terminals for norepinephrine reuptake, as measured by the transcadiac fractional extraction of tracer norepinephrine from plasma and the release of tritiated DHPG from the heart, was markedly attenuated in the first 18 months after transplantation and thereafter remained significantly impaired in comparison to that of the healthy control subjects (Table). To examine more specifically the time-dependent process of reinnervation, the relation between time after transplantation and norepinephrine release and the fractional extraction of tracer norepinephrine, individual data are depicted in Figs 2a and 2b. The spillover of norepinephrine shows a logarithmic progression toward control values with time after transplantation (r=.65, P<.01). In contrast, there was a linear relation between time after transplantation and tracer extraction (r=.53, P<.05).

Heart Rate, Blood Pressure, and Heart Rate Variability

At rest, cardiac transplant recipients (<18 months and >2 years after transplantation) had significantly higher heart rates than their aged-matched healthy counterparts (86±3, 87±5, and 66±4 beats per minute, both P<.01 compared with control). Mean arterial pressure was also significantly greater in the transplant group (112±4 vs 100±2 mm Hg, P=.04). Heart rate variability as assessed by total power of the heart period spectrogram (Fig 3) was markedly reduced in cardiac transplant recipients compared with control subjects (59.4±30 vs 1673±516 milliseconds squared; P<.05). Late transplant recipients showed a trend toward increased total power in comparison to their early counterparts (70±30 vs 29.1±11 milliseconds squared, P=NS). Although total spectral power remained very low, some increase in heart rate variability was observed in the late transplant group (Fig 3), with the return of characteristic 0.1- and 0.25-Hz peaks seen in normal subjects.

Exercise Responses in Heart Rate and Catecholamines

The maximum exercise capacity of the transplant recipients was significantly less than that of their age-matched counterparts (108±6 vs 158±17 W, P<.05), with no significant difference between the early and late transplant recipient group. During exercise (Fig 4), the heart rate response of the early transplant recipient...
group was significantly attenuated in comparison to the control group, with the late transplant recipients having an intermediate response.

In response to exercise, the plasma arterial norepinephrine concentration showed a nonsignificant trend toward higher values in transplant recipients (control subjects vs transplant recipients, 6105±691 vs 9279±1868 pmol/L), with no significant difference in the total spillover of norepinephrine to plasma (14 150±2227 vs 17 707±2476 pmol/min). Exercise raised the arterial plasma epinephrine concentration in both control subjects and transplant recipients, although the arterial plasma epinephrine concentration tended to remain lower in the transplant group (803±125 vs 472±108 pmol/L, P=.06). The epinephrine secretion rate during exercise was not significantly different in control subjects and transplant patients (1839±174 vs 1315±322 pmol/min). During exercise, the cardiac norepinephrine spillover rate (Fig 5) rose to 1876±418 pmol/min in control subjects compared with 164±50 pmol/min in the early transplant group (P<.01). Heart transplant recipients in the late group showed an intermediate response (1080±254 pmol/min), which did not differ significantly from either group, with the small number of patients studied.

Discussion

Using a comprehensive functional and neurochemical assessment of sympathetic neuronal integrity, we have demonstrated the presence of a trend toward the restoration of cardiac sympathetic nerve activity after transplantation. This was particularly evident in patients studied 2 years or more after transplantation. Early (<18 months) after transplantation, no cardiac release of the sympathetic neurotransmitter norepinephrine could be demonstrated, which is consistent with denervation. The cardiac production of DOPA (a precursor of norepinephrine) was also markedly reduced, in keeping with the absence or at least a marked reduction in the number of sympathetic nerve fibers within the transplanted heart early after

 FIG 3. Graphs illustrate the total spectral power in control subjects and "early" and "late" transplant recipients. Typical individual spectrograms are also illustrated. The difference in power scale for control subjects and transplant recipients should be noted.

 FIG 4. Graph A shows the heart rate during supine cycling in control subjects (○), "early" (■), and "late" (●) transplant recipients; graph B shows the heart rate increment during exercise (bpm indicates beats per minute).

 FIG 5. Scatterplot demonstrates the increment in cardiac norepinephrine spillover during exercise in control subjects and "early" and "late" transplant recipients, with a significantly (P<.01) attenuated response in the early group.
transplantation. Our group previously has demonstrated a similar degree of reduction in myocardial catecholamine production in patients with primary autonomic failure, which represents a nonsurgical form of cardiac denervation.

In addition to the reduction in norepinephrine and DOPA release, neuronal reuptake of norepinephrine was also shown to be significantly impaired. There was a marked reduction in the fractional extraction of tritiated norepinephrine from plasma across the heart and an attenuated cardiac production of tritiated DHPG, which is a major intraneuronal metabolite of neuronally released norepinephrine. In the heart, both of these processes are particularly dependent on neuronal capture (uptake-1) of norepinephrine rather than nonneuronal (uptake-2) mechanisms. Late after transplantation, there was a return toward normal of resting cardiac spillover of norepinephrine and DOPA, whereas the capacity of the uptake-1 process to recapture norepinephrine from the synaptic cleft remained significantly impaired in contrast to the apparent restoration of neurotransmitter release.

To date, evidence regarding the capacity of the transplanted heart to synthesize and release norepinephrine has been conflicting. In a study by Regitz et al., tissue levels of norepinephrine were severely depressed in right ventricular endomyocardial biopsy specimens from patients 1 to 62 months after transplantation. This was in contradiction with studies performed in animal models of cardiac autotransplantation, where a return of cardiac norepinephrine content to about 50% of baseline was found. As in the present study, Wilson et al. previously demonstrated an increase (albeit to subnormal levels) in net overflow of norepinephrine from the heart in patients 1 or more years after transplantation, using intravenous tyramine administration as a stimulus for cardiac norepinephrine release. Although this study provided compelling evidence for cardiac reinnervation, interpretation of this data is somewhat confounded by the variable effects of tyramine on coronary blood flow, the fact that tyramine displaces norepinephrine from nerve terminals, and the dependence of the tyramine response on the uptake-1 process, which in the present study was shown to be significantly impaired.

Conflicting histological evidence for cardiac reinnervation after transplantation has been reported by a number of authors. In some postmortem studies of human cardiac allografts, nerves and ganglion cells have been demonstrated. Other groups have examined right ventricular endomyocardial biopsy specimens using electron microscopy with limited ability to demonstrate neuronal tissues. In contrast, histological studies performed in canine transplant models have demonstrated considerable reinnervation within the first year after surgery. Recently, Wharton et al. examined myocardial tissue obtained at surgery from patients undergoing retransplantation (predominantly in heart-lung transplant recipients). In their study, using immunohistochemical techniques, viable intrinsic nerves were demonstrated, as previously shown by others, but they were unable to demonstrate the presence of extrinsic nerves in the majority of tissues examined.

The surgical denervation associated with cardiac transplantation is associated with a higher resting sinus node rate, presumably reflecting intrinsic pacemaker cell activity. As documented in our study and by others, exercise in cardiac transplant recipients is associated with a blunted heart rate response, with the rise in heart rate being attributed to the influences of circulating catecholamines (both norepinephrine and epinephrine) as well as other effects such as the effects of rising body temperature, local mechanical changes, and the possible influences of other circulating substances. In our study we have demonstrated a significantly impaired heart rate response to exercise early (<18 months) after transplantation, with an improvement toward normal in subjects 2 years or more after transplantation. This trend toward normalization of the heart rate response to exercise was associated with a greater release of norepinephrine from the heart in recipients late after transplantation.

Spontaneous cyclical changes observed in heart rate recordings have been attributed to the influences of sympathetic and vagal nerve fibers on the sinus node. The activity of these two efferent components of the autonomic nervous system has been characterized in normal subjects with the use of power spectral analysis. This technique also has been applied after cardiac transplantation to test for the presence of functional reinnervation. Using power spectral analysis, Fallen et al. were able to provide evidence of sympathetic and vagal reinnervation in only 1 of 9 subjects at 2 to 37 months after transplantation. In the present study, power spectral analysis indicated a marked reduction in heart rate variability after heart transplantation. Whereas there was some increase in heart rate variability late (2 years or more) after transplantation, total power was significantly reduced in comparison to control subjects. In addition to a reduction in overall heart rate variability, the distinct peaks characteristic of the innervated heart were markedly attenuated. These peaks represent nonrandom variation in heart rate caused by neural modulation and typically occur at 0.1 Hz (corresponding to fluctuations in sympathetic activity and sympathovagal interactions) and at the respiratory frequency of approximately 0.25 Hz, corresponding to fluctuations in parasympathetic activity. Although neurochemical markers indicate substantial sympathetic reinnervation of the heart late after transplantation, it appears that functional neural activity at rest is present to a much lesser degree. These contradictory findings may, perhaps, be explained by “patchy” reinnervation, particularly in the region of the sinoatrial node, or by an inability of the ingrowing nerves to reform the complex interaction of cardiac afferent and efferent fiber pathways that must underlie the reflex mechanisms responsible for heart rate variability in normal subjects. Using graded lower-body negative pressure and the cold pressor test, Mohanty et al. confirmed the presence of ventricular deafferentation early (<12 months) after cardiac transplantation. Later after transplantation, cardiac afferent function is unclear in humans, although reafferentation has been observed in a canine auto-transplant model.

Our neurochemical finding in transplanted hearts of a persisting impairment of neuronal uptake of norepinephrine despite the apparent return of norepinephrine provides a potential explanation for the phenomenon of cardiac supersensitivity to catecholamines seen in ani-
mal models of cardiac denervation.\textsuperscript{30,31} This phenomenon also has been described in humans after cardiac transplantation,\textsuperscript{8,32,33} based on the demonstration of enhanced inotropic and chronotropic responses to a number of \( \beta \)-adrenergic agonists. Conversely, however, Quigg et al\textsuperscript{11} compared the chronotropic response to isoproterenol in transplant recipients and were unable to show a difference in response to that in healthy control subjects after the blockade of vagal influences with atropine. A number of potential presynaptic and postsynaptic mechanisms have been invoked to explain this phenomenon. Some authors have described changes in \( \beta \)-receptor density or adenylate cyclase activity after stimulation with isoproterenol or forskolin when compared with myocardium from normal hearts. The latter group did, however, find that the response of adenylate cyclase to Gpp(NH)p was significantly impaired, suggesting the presence of an abnormality of \( G \) protein function in this setting. Alternatively, other authors have proposed that a presynaptic process explains the phenomenon of denervation supersensitivity. Gilbert and coworkers\textsuperscript{34} and von Schiedt et al\textsuperscript{41} demonstrated an enhanced sensitivity to the inotropic and chronotropic properties of epinephrine, which undergoes neuronal reuptake, in comparison to isoproterenol, which is not affected by the uptake-1 process. These findings thus provided indirect evidence for an abnormality of the neuronal reuptake process. Furthermore, in the human heart, neuronal uptake-1 activity has been shown to be responsible for the removal of the majority of norepinephrine released.\textsuperscript{15} In the present study, we have shown that norepinephrine extraction from plasma is significantly attenuated for up to 8 years after transplantation. This conclusion is based on our findings of a reduction in the fractional extraction of tritiated norepinephrine across the heart and a reduction in the cardiac production of tritiated DHPG, which is synthesized in the sympathetic nerves after neuronal uptake.\textsuperscript{15} Although a chronic effect of cyclosporine, particularly in the long-term posttransplant subjects, could not be absolutely excluded, the impairment of uptake-1 activity after cardiac transplantation does appear to be specific to the heart, which tends to mitigate against a general effect of immunosuppressive therapy. This view is supported by our observation that the total plasma norepinephrine clearance rate, which is dependent in part on neuronal uptake of norepinephrine, was similar in control subjects and transplant recipients. More importantly, we recently reported that the fractional extraction of tritiated norepinephrine across the kidney is normal after heart transplantation,\textsuperscript{29} indicating that neuronal uptake of norepinephrine is not suppressed by cyclosporine in this organ. The return of the heart rate response and increase in cardiac norepinephrine spillover during exercise toward normal late after transplantation may therefore reflect a combination of some degree of reinnervation and a persistent abnormality of the uptake-1 process.

Whereas the heart rate response to exercise improves with time after transplantation, exercise capacity tends to remain depressed\textsuperscript{22} (also noted in the present study). This persistent limitation probably reflects the contribution of multiple processes. These include the dependence of the exercise-mediated rise in cardiac output of the transplanted heart on the capacity to increase preload rather than an elevation in heart rate,\textsuperscript{38} as opposed to the converse situation that occurs in normal subjects. In transplant recipients, the rise in ventricular filling pressures during exercise is greater than that observed in normal subjects, suggesting that transplanted hearts may rely heavily on the Frank-Starling curve to increase cardiac output from a resting point on the curve, where any increment in diastolic volume is associated with a disparate rise in diastolic pressure.\textsuperscript{39}

The genesis of this phenomenon may reflect mechanical factors including donor-recipient heart size mismatch,\textsuperscript{38} the effects of cardiac hypertrophy (secondary to hypertension), or myocardial edema and scarring caused by cardiac rejection. Persistent ventricular deafferentation also may be implicated in this form of exercise intolerance, as ventricular afferents probably play an important role in the control of peripheral vascular tone and neurohumoral responses to changes in central blood volume, as recently reviewed by Hainsworth.\textsuperscript{40} Of interest, resting plasma epinephrine was significantly lower in transplant recipients compared with control subjects, although the adrenal response to exercise was similar on the basis of a comparable increment in plasma epinephrine and a similar increase in epinephrine secretion during exercise. The finding of significantly lower plasma epinephrine levels at rest in the transplant group possibly may be explained by ventricular deafferentation resulting in inhibition of adrenal catecholamine release but may also be related to hypertension and extracellular fluid volume expansion.

**Study Limitations**

The spillover of released norepinephrine from the synaptic cleft to plasma is dependent on a number of factors, which include the capacity for neuronal reuptake of norepinephrine, diffusional concentration gradients, capillary permeability to the neurotransmitter, and the surface area for exchange in the microcirculation.\textsuperscript{41-43} As a corollary of these influencing factors, norepinephrine spillover measurements represent but a small fraction of norepinephrine release, perhaps constituting 10\% to 20\% of the total turnover.\textsuperscript{44} Therefore, these neurochemical methods possibly may not be sensitive enough to detect very low levels of sympathetic activity in regional vascular beds such as might be seen in the very early phase of cardiac reinnervation, particularly if there has been any change in microcirculatory dynamics affecting the diffusibility of norepinephrine. Despite these theoretical limitations, this technique is sensitive enough to detect a linear rise in norepinephrine spillover during electrical stimulation of cardiac sympathetic nerves.\textsuperscript{45,46} Furthermore, the method allows an assessment of the competence of cardiac norepinephrine uptake to be made, although not providing a precise quantification, and documents a persistent impairment in reuptake capacity to levels seen during pharmacological uptake-1 blockade with agents such as desipramine.\textsuperscript{13,41}
Conclusions

In this cross-sectional study of cardiac sympathetic activity in heart transplant recipients, we have confirmed the presence of an apparent restoration of cardiac sympathetic nerve function late (2 years or more) after transplantation, using comprehensive neurochemical indices of sympathetic neuronal integrity. That this reinnervation was, in part, functional was evident from a return of the heart rate response to exercise toward normal in the late transplant group. Despite these findings indicating substantial reinnervation, major abnormalities persist late after transplantation, including an impairment of the uptake-1 process for neuronally released norepinephrine and a marked attenuation of heart rate variability as demonstrated by power spectral analysis. Definitive confirmation that this process of reinnervation is progressive requires further longitudinal study.

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