Differential Characteristics of Neural Circulatory Control
Early Versus Late After Cardiac Transplantation
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Background—Reappearance of low-frequency (LF) (±0.10 Hz) oscillations in RR interval (RR) after cardiac transplantation is indicative of sympathetic efferent reinnervation. We hypothesized that restored LF oscillations in RR in heart transplant recipients (HTRs) are linked to oscillations in muscle sympathetic nerve traffic (MSNA).

Methods and Results—RR, RR variability, and MSNA were recorded 5±2 months (n=7, short-term HTRs) and 138±8 months (n=7, long-term HTRs) after heart transplantation and compared with matched hypertensive patients (n=7). A coherence function determined the coupling between LF oscillations in MSNA and RR. RR variance did not differ between short-term and long-term HTRs. However, LF variability was only 1±0.5 ms² in the short-term HTRs but was 15±8 ms² in the long-term HTRs (P<0.05). Normalized LF variability was also higher in the long-term HTRs (40±14 normalized unites) versus the short-term HTRs (6±3 normalized united, P<0.05) but did not differ from the LF variability of the hypertensive patients. Long-term HTRs were taking less cyclosporine (P<0.01) but had higher MSNA than the short-term HTRs (62±7 versus 31±7 burst/min, respectively, P<0.05). Coherence between LF oscillations in MSNA and RR was similar in the long-term HTRs (0.59±0.11) and the hypertensive patients (0.60±0.07) and was 3-fold greater than in the short-term HTRs (0.20±0.06, P<0.05).

Conclusions—Cardiac reinnervation after long-term heart transplantation is characterized by a restoration of the coherence between LF oscillations in RR and MSNA. Higher MSNA in long-term than in short-term HTRs suggests that time elapsed after cardiac transplantation may be a major determinant of sympathetic excitation in heart transplant recipients. (Circulation. 2001;104:1809-1813.)

Key Words: transplantation ■ nervous system, sympathetic ■ circulation

Despite complete cardiac denervation after orthotopic transplantation, heart transplant recipients have limited oscillations in RR interval (RR) synchronous with respiration (high-frequency [HF]) attributable to the atrial stretch caused by changes in venous return.1,3 These patients may also manifest slower oscillations in RR (0.03 to 0.15 Hz, low-frequency [LF]), which are not mediated by the mechanical effects of respiration. These LF oscillations are a marker of functional sympathetic reinnervation of the sinus node, because they increase in amplitude during sinusoidal neck suction and are attenuated by β-blockade.3 Reinnervation has been demonstrated mainly for sympathetic efferents1,3-6 and is of clinical importance, because it is accompanied by partial restoration of the heart-rate response to exercise.7

Previous studies in control subjects and patients with cardiac transplants have demonstrated that direct recordings of sympathetic nerve traffic (muscle sympathetic nerve activity [MSNA]) exhibit LF and HF oscillatory components almost identical to those present in heart-rate variability.2,8,9 High sympathetic drive in transplant recipients has been linked to cyclosporine use and cardiac denervation.10 There are no data indicating the effects of duration of transplantation on sympathetic activity. In the present study we tested the hypothesis that the reappearance of LF oscillations in RR in heart transplant recipients is linked to the oscillations present in muscle sympathetic nerve traffic. We additionally evaluated whether an extended duration of transplantation, associated with lower levels of cyclosporine therapy, or cardiac reinnervation could be accompanied by lower levels of sympathetic activation.

Methods

Heart Transplant Patients
Seven patients aged 57±3 years (6 male and 1 female) were studied 5±2 months (mean±SE) after transplantation (range, 1 to 14 months). These patients were recruited according to previous observations that cardiac reinnervation does not occur before 14 months...
after cardiac transplantation. Their body mass index was 26±1 kg/m². These short-term heart transplant recipients were compared with 7 male long-term cardiac transplant patients (time after transplantation, 138±8 months; range, 103 to 163 months), matched for age (59±2 years) and body mass index (26±1 kg/m²). Immunosuppression was achieved by combinations of cyclosporine (n=12), tacrolimus (n=2), azathioprine (n=7), and prednisolone (n=13). Thirteen patients were hypertensive and received angiotensin-converting enzyme inhibitors or angiotensin II antagonists (n=6), diuretics (n=4), β-blockers (n=4), or calcium antagonists (n=3). Severity of rejection was graded histologically, and no patients showed moderate or severe rejection. No patients showed clinical, chest radiographic, or echocardiographic evidence of heart failure.

Control Subjects

Essential Hypertensive Patients

Recordings of 7 untreated patients with essential hypertension (5 male and 2 female) matched for age (59±2 years) and body mass index (25±1 kg/m²) were compared to the cardiac transplant patients. All were in stage I of the World Health Organization classification (systolic blood pressure [SBP] 140 to 159 mm Hg or diastolic blood pressure [DBP] 90 to 99 mm Hg).11

Healthy Subjects

We also compared measurements in transplant patients to those obtained in 7 normotensive healthy subjects matched for age (60±5 years), gender (6 male and 1 female), and body mass index (25±1 kg/m²).

Informed consent was obtained from all patients. The Institutional Human Subjects Review Committee approved the study.

Measurements

SBP and DBP were measured every 3 minutes with a Physiocontrol Colin BP-880 sphygmomanometer. ECG (Siemens) respiration (Respirator) and MSNA were recorded on a MacLab/8i data acquisition system for 20 minutes. Sympathetic nerve activity to the muscle circulation was recorded continuously by obtaining multielectrode recordings of postganglionic sympathetic activity, measured from a nerve fascicle in the peroneal nerve posterior to the fibular head.12

Electrical activity in the nerve fascicle was measured using tungsten microelectrodes (shaft diameter 200 μm, tapering to an uninsulated tip of 1 to 5 μm). A subcutaneous reference electrode was inserted 2 to 3 cm away from the recording electrode, which was inserted into the nerve fascicle. The neural signals were amplified, filtered, rectified, and integrated to obtain a mean voltage display of sympathetic nerve activity.

Data Analysis

Sympathetic bursts were identified by a careful inspection of the mean voltage neurogram by a single experienced observer (P.v.d.B.). Analog-to-digital conversion was performed over 20 minutes at 300 samples per second for the ECG, MSNA, and respiratory signals. The data were then analyzed offline with a personal computer (Siemens Scenic). The principles of the software for data acquisition and autoregressive spectral analysis have been described elsewhere.13–15 The MSNA series were obtained by time integrating the MSNA signal over the RR and by dividing the MSNA time integral by the RR duration. Respiratory activity was sampled once every cardiac cycle. These procedures produced two time series (neurogram and respirogram), which were synchronized with the electrogram. Stationary segments devoid of arrhythmias and artifacts were analyzed with autoregressive algorithms. These algorithms automatically provide the number, center frequency, and power of the oscillatory components. Anderson’s test14 verified that all information contained in the time series had been extracted in the computation, and Akaike’s test14 allowed the determination of the optimal model order fitting the data. Previous studies have shown that two major oscillatory components are detectable in short-term RR and MSNA and SBP variability. One of these oscillatory components is synchronous with respiration and is called the HF oscillation. The other component is described as the LF oscillation and has a center frequency of about 0.10 Hz but can vary considerably (from 0.03 to 0.15 Hz).16,17 In this study, the LF and HF components were expressed in normalized units (nu). The normalized LF and HF units were obtained by calculating the absolute variability of each LF and HF component as a percentage of the total power after subtracting the power of the very LF component (frequencies below nominal 0.03 Hz).17

The amount of linear coupling between variability in MSNA and RR was determined by means of a coherence function.16,17 This function varies between 0 and 1 and can be compared with the squared correlation coefficient in a linear regression equation, because it indicates the amount of variance in RR linearly related to the variance in sympathetic nerve traffic.

Statistical Analysis

Results are expressed as mean±SE. The distribution of all absolute measures of variability and the distribution of the LF/HF ratios were normalized using a logarithmic transformation. Statistical analysis consisted of unpaired (2-tailed) Student’s t tests. Significance was assumed at P<0.05.

Results

Patients With Transplants and Hypertensive Control Subjects

Left ventricular ejection fraction determined by resting radionuclide ventriculogram did not differ between the short-term and long-term heart transplant recipients (58±2% versus 53±4%, respectively, P=0.32). SBP was 133±7 mm Hg in the short-term heart transplant recipients and 139±7 mm Hg in the long-term transplant patients (P=0.54). DBP was also similar in both groups (86±5 and 90±4 mm Hg in the short-term and long-term heart transplant recipients, respectively, P=0.48). These blood pressures did not differ from those of the patients with untreated essential hypertension (SBP 147±2 mm Hg, DBP 88±1 mm Hg, P=0.32 versus long-term heart transplant recipients). MSNA was higher in the long-term heart transplant recipients (62±7 burst/min) than either the short-term transplant patients (31±7 burst/min) or the patients with essential hypertension (37±7 burst/min, P<0.05) (Table and Figure 1). Heart transplantation decreased RR and RR variability (P<0.01) (Table). RR variance and the absolute respiratory variability of RR did not differ between the two groups of heart transplant recipients. However, the LF variability of RR was 1±0.5 ms² in the short-term heart transplant recipients and 15±8 ms² in the long-term transplant patients (P<0.05) (Figure 1). Consequently, long-term heart transplant recipients had a greater normalized LF variability than the short-term transplant patients (40±14 versus 6±3 nu, respectively, P<0.05). The normalized variability of RR did not differ between long-term transplant recipients and the patients with essential hypertension. MSNA variability did not differ between the 3 groups of patients.

Respiratory rate was higher in the short-term cardiac transplant recipients than in the long-term transplant patients (0.33±0.03 versus 0.25±0.01 Hz, P=0.03). However, the proportion of respiratory power located in the HF band did not differ between the groups (short-term, 87±3, versus long-term, 92±2 nu, P>0.17). Breathing frequency also tended to be slower in the hypertensive patients (0.21±0.01 Hz, P=0.06 versus long-term cardiac transplant recipients).
The proportion of respiratory power present in the HF band was also smaller in the patients with hypertension than in the long-term transplant recipients (83 ± 6 nu, *P*, 0.05).

The coherence between the LF oscillations in RR and MSNA was 0.59 ± 0.001 in the long-term cardiac transplant recipients, 0.60 ± 0.07 in the patients with essential hypertension, and 0.20 ± 0.06 in short-term heart transplant patients (*P*<0.05 versus long-term cardiac transplant patients) (Figures 2 and 3). The coherence between HF oscillations in MSNA and RR did not differ between the three groups (Figure 3).

Normotensive Control Subjects
The normotensive subjects disclosed a SBP of 126 ± 2 mm Hg and DBP of 73 ± 2 mm Hg (*P*<0.0001 versus hypertensive patients). Muscle sympathetic nerve activity of 33 ± 5 burst/min in the normotensive control subjects did not differ from the essential untreated hypertensive patients (*P*=0.62) and the short-term transplant patients (*P*=0.82) but was lower than the MSNA of the long-term heart transplant recipients (*P*=0.004). The normotensive subjects disclosed a larger variance in RR (1284 ± 387 ms², *P*=0.03 versus hypertensive controls) and a faster breathing frequency (0.28 ± 0.02 Hz, *P*=0.02 versus hypertensive controls) and, as a result, a tendency toward faster frequencies of the HF components of RR, MSNA, and SBP (*P*=0.11, *P*=0.05, *P*=0.02, respectively, versus hypertensive controls). The coherence between the LF oscillations in RR and MSNA was 0.68 ± 0.07 in the normotensive patients and did not differ from the hypertensive subjects (*P*=0.41) and the long-term heart transplant recipients (*P*=0.50) but was higher than in the short-term transplant patients (*P*=0.0002).

Discussion
One of the novel findings of this study is the reappearance of the link between LF oscillations in the donor heart RR and LF oscillations in direct recordings of sympathetic nerve traffic in the recipient after heart transplantation. In previous studies, we have shown a restoration of LF oscillations in MSNA after heart transplantation.2 Partial cardiac reinnervation was sug-

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**Table:**

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au indicates arbitrary units. Student’s t test. *P*<0.05; †*P*<0.01; ‡*P*<0.0001. Short-term vs long-term HTRs and long-term HTRs vs hypertensive patients, *n*=7.

Figure 1. Electrocardiographic activity, sympathetic nerve activity, and respiratory activity in a short-term (9 months after heart transplantation, left) and a long-term (163 months after transplantation, right) heart transplant recipient (HTR). MSNA is markedly elevated in the long-term transplant patient. Spectral analysis recordings from these subjects are shown in Figure 2. HR indicates heart rate.
gested by limited LF oscillations in RR in half of these patients.\textsuperscript{2} In the present study, the comparison of short-term and long-term heart transplant patients extends these findings by demonstrating that the reappearance of LF oscillations in RR are linked to those of MSNA after heart transplantation. This finding suggests the restoration of a functional link between the sympathetic outflow of the recipient and the sinus node of the donor.

There is some evidence that a limited proportion of LF oscillations in RR might be modulated by the vagus in healthy subjects.\textsuperscript{17} However, it is likely that reappearance of a link between LF oscillations in RR and MSNA is indicative of a cardiac sympathetic reinnervation in our patients, because first, LF oscillations in RR induced by sinusoidal neck suction in heart transplant recipients are attenuated by β-blockade but are not affected by atropine,\textsuperscript{1} and, second, all of our patients had undergone standard cardiac transplantation, and cardiac reinnervation is primarily sympathetic rather than vagal in this condition.\textsuperscript{3,4}

Limited HF oscillations in RR can be induced by nonautonomic mechanisms, such as atrial stretch induced by changes in venous return.\textsuperscript{1} Our observation of comparable HF oscillations in RR after an average of 5 and 138 months after transplantation additionally emphasizes that this component is unlikely to be mediated by the autonomic nervous system in cardiac transplant patients.

Respiration is a major source of fluctuations in autonomic nervous activity.\textsuperscript{15–18} All of our patients with essential hypertension disclosed clear-cut HF oscillations in MSNA, and these oscillations were coherent with those present in RR. Respiratory oscillations in RR are under vagal control, and direct recordings of efferent vagal outflow disclose marked respiratory oscillations in RR in animals.\textsuperscript{19} The link between respiratory oscillations in MSNA and RR in the hypertensive patients is not indicative of a sympathetic influence on respiratory sinus arrhythmia but highlights the importance of respiration as a common regulatory mechanism of HF fluctuations in MSNA and RR. This mechanism explains comparable coherence between HF oscillations in MSNA and RR in patients with essential hypertension and heart transplant recipients.

We recorded respiratory activity in all our transplant patients to avoid the possibility that LF oscillations in RR, induced by low-frequency breathing, would be mistakenly taken as a sign of cardiac reinnervation. Although our analysis revealed that the long-term heart transplant patients breathed at a slightly slower frequency than the short-term cardiac transplant recipients, this did not explain the reappearance of LF oscillations in RR after transplantation, because there was no LF respiratory component to explain the presence of LF oscillations in RR in these patients.

An additional novel finding relates to the potential mechanisms that have been proposed to explain the heightened MSNA after cardiac transplantation. Both cardiac denervation and cyclosporine therapy have been proposed as possible causes for higher MSNA in heart transplant recipients.\textsuperscript{10} However, our data show that sympathetic nerve traffic was comparable in short-term heart transplant patients, patients with essential hypertension, and normotensive control subjects but was markedly elevated in the long-term cardiac transplant recipients. This unexpected finding cannot be
explained by differences in BP, gender,\textsuperscript{20,21} age,\textsuperscript{20,21} or left ventricular function\textsuperscript{22} between the patients.

Reduction of tonic restraint of vagal sensory afferents after cardiac denervation increases sympathetic efferent activity.\textsuperscript{23} This finding might contribute to the maintenance of elevated MSNA after heart transplantation.\textsuperscript{10} There is also evidence that cyclosporine-induced sympathetic activation contributes to hypertension after cardiac transplantation,\textsuperscript{10} but this finding has not been consistent.\textsuperscript{24,25} The 5 short-term heart transplant recipients who received 435 ± 92 mg cyclosporine per day (2 short-term heart transplant recipients received tacrolimus instead of cyclosporine) had MSNA levels of 30 ± 7 burst/min in contrast to the 7 long-term transplant patients who took only 175 ± 16 mg of cyclosporine per day (P<0.01) but had MSNA levels of 62 ± 7 burst/min (P<0.05). We speculate, therefore, that the daily oral dose of cyclosporine is not the primary determinant of sympathetic activity after heart transplantation. However, cumulative effects of longer exposure to cyclosporine in the presence of incomplete cardiac reinnervation could also account for higher MSNA in the long-term heart transplant recipients.

Thus, although our study suggests that time after transplantation may be important factor on MSNA activity, it does not exclude possible contributions from the duration and cumulative dose of cyclosporine therapy.

Our data provide important insights into the controversy regarding whether or not sympathetic nerve traffic is increased after cardiac transplantation. Some studies report near-normal MSNA in cardiac transplant recipients,\textsuperscript{24,25} whereas others demonstrate significantly greater sympathetic drive in these patients.\textsuperscript{2,10} Time after heart transplantation was shorter in the two studies that reported near-normal MSNA in heart transplant recipients (3 and 11 months)\textsuperscript{24,25} than in the two studies where MSNA was clearly increased (14 and 51 months).\textsuperscript{2,10} Our data suggest that time elapsed after transplantation may be an important determinant of sympathetic outflow in heart transplant patients and suggest an explanation for the discrepancy in findings in these earlier studies. Whether a combination of factors, such as prolonged exposure to cyclosporine and cardiac afferent denervation, can additionally explain differences in sympathetic nerve activity is not known and will need additional longitudinal studies.

In conclusion, our study reveals that cardiac reinnervation after heart transplantation is characterized by a normalization of the coherence between reappearing LF oscillations in RR and LF oscillations present in direct intraneural recordings of MSNA. Higher MSNA in long-term than in short-term heart transplant recipients suggests that time elapsed after cardiac transplantation may be a major determinant of sympathetic excitation after cardiac transplantation.

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