Abnormal Neuroendocrine Responses During Exercise in Heart Transplant Recipients

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Background. Osmotic and neural factors stimulate neuroendocrine activity during exercise. In contrast to excitatory mechanisms, afferent information from cardiac mechanoreceptors inhibits integrative centers in the hypothalamus and medulla oblongata, which serves to buffer neuroendocrine activity. Orthotopic cardiac transplantation results in the loss of afferent information from cardiac mechanoreceptors. Thus, transplantation possibly results in exaggerated neuroendocrine responses when patients are physically active.

Methods and Results. We measured the neuroendocrine response to moderate and strenuous exercise performed at the same relative intensity in 11 heart transplant recipients (50±14 years old) 18±12 months after transplantation and 11 control subjects matched with respect to sex, age, and body size. Plasma levels of norepinephrine, vasopressin, renin activity, atrial natriuretic peptide, angiotensin II, and aldosterone were measured at rest, during a maximal graded exercise test, and during submaximal exercise at 40% and 70% of peak power output on a cycle ergometer (W). Plasma renin activity and atrial natriuretic peptide were elevated at rest in heart transplant recipients (p≤0.05). Heart rate (%HRmax reserve), rating of perceived exertion, and reductions in plasma volume (%Δ from rest) at the conclusion of the three exercise conditions did not differ between heart transplant recipients and control (p≥0.05). Relative changes in neuroendocrine hormones were similar (p≥0.05) in heart transplant recipients and control during exercise at 40% of peak power output. Relative changes in plasma norepinephrine, vasopressin, atrial natriuretic peptide, and plasma renin activity were greater (p≤0.05) in heart transplant recipients during exercise at 70% of peak power output and the graded exercise test.

Conclusions. We interpret these data as a possible indication of ablation of cardiac mechanoreceptor afferents and unopposed neuroendocrine stimulation in heart transplant recipients. Furthermore, chronic neuroendocrine hyperactivity is likely in ambulatory heart transplant recipients. Although cyclosporine nephrotoxicity is implicated in the development of hypertension, our data suggest that chronic neuroendocrine hyperactivity, which alters renal volume regulation, also contributes to the incidence and severity of hypertension in heart transplant recipients. (Circulation 1992;86:1453–1463)

Key Words • cardiac denervation • hypertension • cyclosporine • transplantation, heart • exercise

Orthotopic heart transplantation restores cardiac function and reverses congestive heart failure (CHF). However, transplantation results in a high incidence of resistant hypertension that increases progressively with time.1,2 Cyclosporine immunosuppression is implicated in the development of hypertension secondary to increased vascular tone and nephrotoxicity both in heart transplant recipients and in other transplant populations.2,3 However, transplant hypertension does not appear to be directly related to cyclosporine nephrotoxicity.1,2 Reductions in cyclosporine dose, serum concentration, and length of therapy fail to reduce the incidence of hypertension in heart transplant recipients despite improved renal function.3,4 Additionally, it was recently reported that the incidence of hypertension in a group of heart transplant patients receiving only prednisone and azathioprine immunosuppression was also high by 3 years after transplantation.1

Hypertension in heart transplant recipients may be mediated by the effects of cardiac denervation. Cardiac denervation is one consequence of heart transplantation.5 This results in a loss of afferent information from stretch receptors in the heart. When innervated, stretch receptors in the atria and ventricles respond to volume loading by decreasing sympathetic nerve traffic to the kidney, thus increasing renal blood flow and diuresis.6,7 Cardiac mechanoreceptors also inhibit renin, arginine vasopressin (AVP), and thirst when volume is expanded.6,7 Although the native atria remain intact after transplantation, there is evidence of afferent neural disconnection between the heart and brain of heart transplant recipients. These patients demonstrate an impaired ability to regulate volume, frequently requir-
ing diuretics in the presence of normal glomerular function and cardiac hemodynamics.

Increased plasma renin activity (PRA) and renal vascular resistance occur in heart transplant patients during water immersion. Plasma AVP is elevated when the patient is in the supine position. The normal nocturnal decline in blood pressure is lost after transplantation and may be related to cardiac denervation.

We postulated that if heart transplant recipients are denervated, then the neuroendocrine response to the same relative exercise stress would be exaggerated in these patients when compared with innervated matched controls. Exercise alters a variety of osmotic and neural factors that stimulate release of AVP and norepinephrine (NE) and activate the renin-angiotensin-aldosterone (RAA) system. The release of AVP, renin, and NE is mediated by input from the hypothalamus, hypothalamic drive, in turn, is modulated both by a central mechanism (i.e., central command) and by pressor reflexes originating in the contracting skeletal muscles.

In contrast to these excitatory mechanisms, cardiac volume receptors inhibit integrative centers in the hypothalamus and medulla oblongata, which serves to buffer neuroendocrine activity. Cardiac volume receptors are activated by mechanical stimuli, including increases in cardiac preload, contractility, and afterload. Because exercise increases cardiac preload, contractility, and afterload, it is reasonable to postulate that cardiac vagal afferent traffic and central inhibition of neuroendocrine activity are augmented during exercise. Thus, removal of cardiac baroreflex inhibition through orthotopic transplantation might result in exaggerated neuroendocrine responses when patients are physically active.

Because physical activity is widely recommended for patients after heart transplantation and because chronic cardiac denervation may alter neuroendocrine function during physical activity, determination of the neuroendocrine response during various levels of exercise is critical to the resolution of this issue. The present study was designed to characterize the neuroendocrine response to moderate and strenuous dynamic exercise performed at the same relative intensity in heart transplant recipients and matched control subjects.

Methods

Heart Transplant Recipients

Eleven patients (10 men and one woman) who underwent orthotopic cardiac transplantation at Shands Hospital at the University of Florida, Gainesville, Fla., volunteered to participate in the study. Transplantation was performed according to the techniques described by Baumgartner et al. The indications for transplantation were idiopathic cardiomyopathy (seven patients), ischemic cardiomyopathy (three), and retransplantation for refractory rejection (one). The heart transplant recipients were an average of 50±14 (mean±SD) years old (range, 21–63 years), and they were studied 18±12 (mean±SD) months after transplantation (range, 7–41 months).

All heart transplant recipients were clinically stable and free from significant rejection, infection, or other major illness. Nine patients received triple-drug immunosuppressive therapy with cyclosporine, prednisone, and azathioprine, and two patients were treated with cyclosporine and prednisone. Four heart transplant patients required furosemide for fluid retention, and all required antihypertensive drugs for mild-to-moderate hypertension: clonidine (three patients), nifedipine (six), captopril (three), and enalapril (eight). No β-blockers or other cardiac medications were used by the heart transplant recipients at the time of the study. All patients followed their usual protocol of daily medications during the study.

Control Subjects

The control group consisted of 11 subjects who were selected to match the heart transplant recipients, as closely as possible, with respect to age, sex, and body composition. They were sedentary and untrained and had no evidence of cardiac or pulmonary disease as determined by clinical examination, pulmonary screening, and graded exercise testing (GXT). None of the control subjects received medication at the time of the study.

The subjects were tested on two different days separated by a minimum of 72 hours. All subjects restricted strenuous physical activity for 24 hours before experiments and reported to the laboratory 2–3 hours postprandial. The protocol was approved by the institutional review board for the protection of human subjects at the University of Florida College of Medicine, and all subjects provided their written informed consent to participate in the study.

Graded Exercise Test

On the morning of the first day, each subject underwent a physical and cardiovascular examination. A 20-gauge catheter was inserted into an antecubital vein in subjects deemed suitable to undergo a GXT. After 30 minutes of seated rest, a 15-ml blood sample was drawn for hormone, hematocrit, and hemoglobin analysis. Before the GXT, heart rate, blood pressure, and an ECG were recorded with the subject supine and immediately upon returning to an erect seated position. The GXT was a continuous, multistage protocol on an electromagnetically braked cycle ergometer. Initial power output was 20 W for heart transplant recipients and 40 W for the controls. Power output increased every minute by 10 or 20 W for the transplant or control subjects, respectively, until the subject reached voluntary maximal exertion and could not maintain 60 rpm (i.e., 100% of peak power output) or became symptomatic with positive hemodynamic or medical indexes. Immediately on completion of the test, another 15-ml blood sample was drawn. A 12-lead ECG and rating of perceived exertion were recorded at the end of each minute. Blood pressure was measured by auscultation at 2-minute intervals. Expired air samples were collected in meteorological balloons during the final 3–4 minutes of exercise. Expired gases were analyzed by a mass spectrometer (Perkin-Elmer, MGA model 1100) for fractional concentrations of oxygen and carbon dioxide. Ventilatory volumes were measured with a 120-l Tissot spirometer (Collins).

Body Composition Analysis

Body composition was assessed anthropometrically from the sum of seven skinfold sites. Body density was
predicted for men and women with the equations of Jackson and Pollock\textsuperscript{16} and Jackson et al.,\textsuperscript{17} respectively. The Siri equation\textsuperscript{18} was used to estimate percent fat and lean body mass.

**Submaximal Exercise Test**

Submaximal exercise tests on day 2 were performed at the same time of day (12:00-3:00 PM) as the GXT on day 1 to control for a possible effect of circadian rhythms.\textsuperscript{19} Environmental conditions within the laboratory were maintained relatively constant at 22–23\degreeCelsius, 56–62\% relative humidity, and 760–765 mm Hg barometric pressure.

Each subject performed a 10-minute bout of constant-load square wave cycle exercise at 40\% and 70\% of the peak power output (W) achieved during the GXT. An injection of 1\% xylcaine was administered for local anesthesia, and a 20-gauge catheter with three-way stopcock and 3-in. T-connector was placed in the radial artery of the nondominant arm. Each submaximal exercise test was preceded by 10 minutes of quiet rest with the subject seated on the cycle. After collection of blood samples for baseline hematocrit, hemoglobin, and neuroendocrine hormones, the subject pedaled at 60 rpm at 40\% of peak power output. Blood samples for neuroendocrine hormone analysis were drawn at minutes 2, 4, 6, 8, and 10 during exercise. At the end of the first 10-minute cycle test, the subject stopped pedaling and rested quietly in a chair for 10 minutes. The second submaximal cycle test at 70\% of peak power also began with the subject seated quietly for 10 minutes on the cycle, providing a total of 20 minutes of seated recovery between tests. The sample collection described above was repeated during the second ride at 70\% of peak power.

**Blood Sample Collection**

A total of 30 ml of venous blood was obtained during a GXT, and 75–80 ml of arterial blood was drawn during each submaximal 10-minute cycle test. Arterial blood gases were obtained, and those data have been reported.\textsuperscript{20} Blood samples for hormone analysis were withdrawn into a plastic syringe containing no additives and immediately separated into individual aliquots. Blood for NE assay was added to chilled heparinized vacutainers containing 40 \mu l EGTA (90 mg/ml) and reduced glutathione (75 mg/ml) (Sigma Chemical, St. Louis, Mo.). Blood for AVP, PRA, angiotensin II (Ang II), aldosterone, and atrial natriuretic peptide (ANP) assays was added to a chilled vacutainer containing EDTA. The blood samples were immediately mixed, and plasma was separated by centrifugation at 2,500g at 4\degreeCelsius for 20 minutes. The plasma samples were frozen at either −40\degreeCelsius (AVP, PRA, and ANP) or −80\degreeCelsius (NE, Ang II, and aldosterone) until the end of the study so that all samples for each subject could be run in the same assay.

**Blood Sample Analysis**

Plasma AVP was measured by radioimmunoassay (RIA) as previously described.\textsuperscript{21} PRA was determined by RIA using a modification of the method of Haber et al.\textsuperscript{22} Plasma ANP levels were measured by RIA as described by Phillips et al.\textsuperscript{23}

Plasma aldosterone concentration was measured by RIA in unextracted serum using a kit from Diagnostics Products Corp., Los Angeles. Plasma samples (0.200 ml) were added to polypropylene tubes coated with antibodies to aldosterone. Then, 1.0 ml of 125I-aldosterone tracer was added to the tubes; after a 3-hour incubation at 37\degreeCelsius, the supernatant was decanted, and the tubes were counted on a gamma counter. Aldosterone concentration was determined from a standard curve. Intra-assay coefficient of variation (CV) for this procedure is 2.7–8.3\%, and interassay CV is 3.6–10.4\%. The detection limit is approximately 16 pg/ml.

Plasma Ang II concentrations were determined by RIA. Antibody raised in rabbits against Ang II (Sigma Chemical) conjugated to bovine thyroglobulin via glutaraldehyde was used for RIA. The cross-reactivity of this antiserum with Ang III was <27\%. Cross-reactivity with Ang I, renin substrate tetradecapeptide, (Sar\textsuperscript{1},Ala\textsuperscript{8})-ang II, (Sar\textsuperscript{1},Ile\textsuperscript{8})-ang II, ACTH, and AVP were all <0.1\%. Ang II was extracted from plasma by adsorption to dentonite and reconstituted in 0.05 M Tris buffer (0.3\% bovine serum albumin, 0.01\% sodium azide; pH 7.4) as described by Reid.\textsuperscript{24} 125I-Ang II (Dupont, Wilmington, Del.) was used as the tracer. The range of the standard curve was from 0.38 to 25 pg/tube with 50\% displacement of 125I-Ang II with 2.4 pg/tube. The intra-assay CV for a pool of 7.0 pg/tube was 10.5\%, and the interassay CV for the same pool was 10.3\%.

Plasma concentrations of NE were measured by high-performance liquid chromatography (HPLC) (ESA Coulomb, Bedford, Mass.). NE was extracted by adsorbing plasma samples onto alumina. After washing the adsorbed alumina with buffer solution, NE was eluted from the alumina by treating it with an acid solution. 3,4-Dihydroxybenzylamine (DHBA) was used as an internal standard, and extraction efficiency of NE and DHBA was based on the extraction of known standards. All NE samples were corrected for percent recovery. After extraction, the NE samples were assayed by injecting the samples onto a reverse-phase C\textsuperscript{8} column. An ESA 16-channel Coulomb photometric array detector was used to determine the concentration of NE in the samples. The within-assay CV was 1.2\%, and the between-assay CV was 3.2\%.

Hematocrit and hemoglobin determinations were made with a QBC II Centrifugal Hematology system (Becton Dickinson). Layer measurements were used to compute hematocrit. Hemoglobin concentration was derived from the hematocrit and measurements of red blood cell density.\textsuperscript{25} The percent change in plasma volume during exercise was calculated from the preexercise and postexercise hematocrit values.\textsuperscript{26}

**Statistical Analysis**

Descriptive characteristics and GXT measures were compared between groups using ANOVA. ANCOVA for repeated measures was used to analyze the temporal pattern of all cardioodynamic variables and neuroendocrine hormones. When a statistically significant time effect or group by time interaction was observed, within-group comparisons between discrete time points and/or between-group comparisons at each time point were done using ANCOVA with contrast analysis for obtaining appropriate post-hoc custom hypotheses tests. All statistical analyses were completed using the SAS statis-
Results

Descriptive Characteristics

The physical characteristics of the heart transplant and control groups are presented in Table 1. The two groups did not differ \((p \geq 0.05)\) with respect to age, height, weight, and body composition.

Serum Creatinine

Serum creatinine levels in the heart transplant recipients are also shown in Table 1. Criterion creatinine values for each transplant patient were an average of three creatinine measurements taken during a period of 8 months spanning the time of the investigation. Serum creatinine levels in the heart transplant patients were normal or mildly elevated. Serum creatinine in the transplant patients was not significantly \((p \geq 0.05)\) correlated with neuroendocrine hormone levels at rest or during exercise, suggesting that renal function is not a mechanism responsible for elevated plasma NE, PRA, and AVP.

Postural Influence on Heart Rate

Heart rate responses recorded during a postural shift from a supine to an erect seated position are shown in Table 2. Heart rate significantly \((p \leq 0.05)\) increased with the change in posture in the control group. Heart rate did not change \((p \geq 0.05)\) during the postural shift in heart transplant recipients.

Hemodynamic Changes

Rest and peak exercise values for heart rate and blood pressure during the GXT are listed in Table 3. Resting heart rate in the heart transplant patients was 38\% higher \((p \leq 0.05)\) than in the control group. Peak exercise heart rate in the transplant group was 57\% higher than at rest compared with a 149\% increase from rest in the control group \((p \leq 0.05)\). Mean chronotropic reserve for the heart transplant recipient was 53 beats per minute compared with 100 beats per minute for the control group \((p \leq 0.05)\).

Resting systolic blood pressure did not differ between the two groups \((p \geq 0.05)\). Peak systolic blood pressure was 15\% higher \((p \leq 0.05)\) in the control group. Diastolic blood pressure at rest and at peak exercise was significantly higher in the transplant patients \((p \leq 0.05)\). The rate-pressure product (heart rate multiplied by peak systolic blood pressure multiplied by \(10^{-2}\)) at peak exercise was 31\% greater in the control group \((p \leq 0.05)\).

Cardiorespiratory Responses

Peak cardiorespiratory responses during the GXT are summarized in Table 4. All GXTs were terminated due to volitional exhaustion. The peak \(V_{\text{O2}}\) achieved by the heart transplant recipients was 57\% of the peak \(V_{\text{O2}}\) values attained by the control group \((p \leq 0.05)\). The dissimilarity in peak \(V_{\text{O2}}\) was further reflected in the peak power output \(W\) at termination of the GXT with the transplant patients achieving a peak power output value that was 55\% of that attained by the control group \((p \leq 0.05)\).

Peak respiratory exchange ratio (RER) averaged 1.16 \pm 0.06 in all subjects, indicating a high degree of hyperventilation during the GXT. However, peak RER was significantly higher \((p \leq 0.05)\) in the control group. Peak expired carbon dioxide \(\left(V_{\text{CO2}}\right)\) and peak pulmonary ventilation \(\left(V_{\text{E}}\right)\) were significantly higher \((p \leq 0.05)\) in the control group. The heart transplant recipients achieved mean \(V_{\text{E}}\) values that were 71\% of the control. Peak ventilatory equivalent for \(V_{\text{O2}}\) \(\left(V_{\text{E}}/V_{\text{O2}}\right)\) was significantly greater \((p \leq 0.05)\) in the heart transplant recipients, indicating that they ventilated more for a given level of oxygen uptake than did the controls. These data...
confirm that exercise capacity is significantly reduced in clinically stable heart transplant recipients several years after surgery and emphasize that there is a substantial attenuation in the peak heart rate response to a maximal exercise effort.

Relative Exercise Intensity

The mean±SD values of physiological variables considered valid indicators of differences in relative exercise intensity between the two groups are shown in Table 5. There were nonsignificant (p > 0.05) differences between heart transplant and control groups for heart rate (percent of heart rate reserve), rating of perceived exertion, and reductions in plasma volume (percent change from rest) at the conclusion of each of the three exercise conditions. These data indicate that the relative exercise stimulus during the submaximal exercise tests was comparable between groups.

Neuroendocrine Hormones

Resting concentrations of AVP, NE, ANP, and PRA over the three testing conditions did not vary significantly (p > 0.05) within groups. Therefore, the mean of three resting values for each hormone was used as the criterion value for comparisons between groups. No significant correlations were found between the number of months after transplantation and plasma levels of neuroendocrine hormones at rest or during exercise.

TABLE 4. Peak Cardiorespiratory Responses of the Control and Transplant Groups During the Symptom-Limited Graded Exercise Test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Transplant group</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_2$ (l·min$^{-1}$)*</td>
<td>2.69±0.74</td>
<td>1.52±0.24</td>
</tr>
<tr>
<td>VO$_2$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>32.0±8.7</td>
<td>18.3±3.3</td>
</tr>
<tr>
<td>VCO$_2$ (l·min$^{-1}$)†</td>
<td>3.10±0.81</td>
<td>1.73±0.28</td>
</tr>
<tr>
<td>VE (I·min$^{-1}$)‡</td>
<td>97.67±19.80</td>
<td>69.50±16.80</td>
</tr>
<tr>
<td>VE/VCO$_2$</td>
<td>37.55±8.97</td>
<td>45.74±9.43</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>1.17±0.06</td>
<td>1.15±0.05</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>198±47</td>
<td>109±20</td>
</tr>
<tr>
<td>Rating of perceived exertion§</td>
<td>19.5±0.8</td>
<td>19.4±0.8</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*Peak systemic oxygen consumption.
†Peak expired carbon dioxide.
‡Peak expired pulmonary ventilation.
§Borg (1962) scale.
*p<0.05 transplant vs. control.

Plasma aldosterone at rest was not significantly different (p > 0.05) between heart transplant recipients (165 pg/ml) and control subjects (120 pg/ml) (Figure 1). The increase in aldosterone during the GXT was similar (p > 0.05) in the transplant patients (300 pg/ml) and control group (241 pg/ml). Baseline plasma ANG II was not significantly different (p > 0.05) between groups (4.1 versus 2.7 pg/ml), but the heart transplant recipients exhibited a trend toward higher ANG II levels at rest (p = 0.07) (Figure 1). The increase in ANG II during the GXT was similar (p > 0.05) in the transplant (12 pg/ml) and control groups (13 pg/ml).

Baseline PRA (8.8 versus 1.3 ng/ml/hr) and ANP concentrations (43.1 versus 19.9 pg/ml) were significantly higher (p < 0.05) in the heart transplant recipients. During exercise at 40% of peak power, PRA (Figure 2) became significantly greater than baseline after 6 minutes and continued to increase, but the relative changes were similar (p > 0.05) for both groups. Exercise at 70% of peak power output evoked PRA levels that were significantly greater (p < 0.05) than baseline at all measurement periods in both groups. The relative increase in PRA after 4 minutes was significantly greater (p < 0.05) in the heart transplant patients. ANP (Figure 3) increased during exercise at 40% of peak power and was significantly (p < 0.05) greater than baseline by 8 minutes in both groups. The group by time interaction was not significant (p > 0.05). Exercise at 70% of peak power elicited ANP values that were significantly greater (p < 0.05) than those at baseline at all measurement periods in both groups, but the magnitude of increase was significantly greater (p < 0.05) in the heart transplant patients. During the GXT, increases in PRA (200% versus 87%) and ANP (221% versus 163%) were greater (p < 0.05) in the heart transplant recipients.

FIGURE 1. Bar graphs of plasma angiotensin II (ANG II) (top panel) and aldosterone (ALDO) (bottom panel) at rest and immediately after peak exercise during a graded exercise test (GXT). Values represent mean±SEM. *p<0.05 rest vs. peak GXT.

TABLE 5. Heart Rate, Rating of Perceived Exertion, and Plasma Volume Changes During Submaximal Exercise Tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>% Peak power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40%</td>
</tr>
<tr>
<td>Heart rate (% HRR)*</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Transplant</td>
</tr>
<tr>
<td>Perceived exertion†</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Transplant</td>
</tr>
<tr>
<td>%Δ Plasma volume‡</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Transplant</td>
</tr>
</tbody>
</table>

HRR, heart rate reserve. Values are mean±SD.
*Heart rate expressed as percentage of HRR.
†Borg (1962) scale.
‡Percentage loss in plasma volume (rest-exercise).
Mean baseline plasma AVP (Figure 4) was elevated in the transplant patients (3.8 versus 3.0 pg/ml) compared with the control group, but the difference was not significant (p=0.06). During exercise at 40% of peak power, AVP increased to levels significantly greater (p≤0.05) than baseline by 4 minutes in both groups. The group by time interaction was not significant (p≥0.05). During exercise at 70% of peak power, AVP increased significantly (p≤0.05) above baseline within 2 minutes in both groups. AVP continued to increase in both groups, but the increase was greater (p≤0.05) in the heart transplant group. The increase in AVP during the GXT was significantly greater (p≤0.05) in heart transplant recipients (27.3 versus 16.7 pg/ml).

Plasma NE (Figure 5) values at rest were not significantly different between the two groups (0.476 versus 0.394 ng/ml), but the heart transplant patients demonstrated a trend toward higher NE (p=0.08). NE significantly (p≤0.05) increased above baseline in both groups after 4 minutes of exercise at 40% of peak power, but the group by time interaction was not significant (p≥0.05). Both groups increased (p≤0.05) NE levels above baseline after 2 minutes of exercise at 70% of peak power. The magnitude of NE elevation was greater (p≤0.05) in the heart transplant patients at each measurement period. Peak NE during the GXT was significantly higher (p≤0.05) in the heart transplant group (sevenfold versus fivefold increase) (Figure 5).

Discussion

Our data show significantly exaggerated neuroendocrine responses to the same relative exercise stress in heart transplant recipients compared with matched control subjects. Baseline PRA and ANP were significantly higher in the heart transplant group. Heart transplant patients also demonstrated a strong trend...
toward higher baseline AVP and NE. The relative increase (percent change from baseline) in PRA, ANP, AVP, and NE was significantly greater in heart transplant recipients at exercise intensities of 70% and 100% of peak power output. A threshold exercise intensity of >40% of peak power was required to evoke significant neuroendocrine hyperactivity in the heart transplant patients. We interpret these data as a possible indication of ablation of cardiac mechanoreceptor afferents. However, we cannot exclude the possible influence of cyclosporine, antihypertensive drugs, and skeletal muscle deconditioning on our findings. With these limitations in mind, the following discussion will address only those neuroendocrine responses to exercise in which the cardiac atria play a central role: the release of ANP from atrial myocytes and the reflex modulation of circulating PRA, AVP, and NE evoked by atrial neural receptors in response to stretch.

**RAA System**

Baseline PRA was markedly elevated in heart transplant recipients (Figure 2). This finding is not unusual considering that all heart transplant patients required antihypertensive medications, which were not withheld during the study. Four transplant patients required furosemide, and PRA increases after plasma volume depletion by furosemide. All heart transplant patients required angiotensin converting enzyme (ACE) inhibitors (three, captopril; eight, enalapril), which increase PRA by blocking the conversion of Ang I to Ang II, thereby opening the RAA system feedback loop. Additionally, all transplant patients were immunosuppressed with cyclosporine, which is a stimulus of PRA.

The interesting finding, however, was that the relative increase in PRA during strenuous exercise (70% and 100% of peak power output) was greater in the heart transplant group than in matched controls. Although
elevated resting PRA in our heart transplant recipients may be explained by the medication regimen, the explanation for hypersecretion of renin during exercise is less clear. Increased angiotensin substrate could result in increased PRA during exercise, but angiotensinogen concentrations in heart transplant patients treated with ACE antagonists are normal.

Of the mechanisms known to promote secretion of renin (i.e., decreased renal perfusion pressure, reduced sodium in distal tubules, and neural reflex mechanisms), sympathetic stimulation of β-adrenergic receptors located on the renin secreting cells is a particularly important factor during exercise. Cardiac stretch receptors with vagal afferents tonically inhibit renin secretion. Cardiac afferent denervation, however, eliminates the reflex inhibition of renin secretion in animals and humans, suggesting that renin secretion is possibly exaggerated when cardiac receptors are unable to initiate inhibitory mechanisms after cardiac transplantation.

ANP

Resting ANP in the heart transplant group was 2.4 times greater than in the control group. This finding is consistent with others who have measured baseline ANP in heart transplant recipients. The ANP response to exercise at 40% of peak power was similar between groups. During exercise at 70% and 100% of peak power, both absolute and relative increases in ANP were greater in the heart transplant group.

ANP responses during submaximal exercise have not been previously reported in heart transplant recipients. Singer et al reported that plasma ANP levels at peak exercise were elevated in heart transplant patients compared with matched controls (204% versus 151% increase), but the difference between groups was not significant. The discrepancy between our findings and those of Singer et al could be explained, at least in part, by the fact that more atrial tissue was present in our transplant patients. Their surgical procedure increased atrial tissue by approximately 25%. The surgical technique used in the present study resulted in the approximate doubling of right and left atrial tissue in most transplant patients, and that atrial tissue was sectioned at the level of the atrioventricular valves and nearly the entire donor heart was anastomosed.

Atrial stretch is the most widely recognized stimulus for ANP release. Thus, volume expansion and increased atrial pressure may influence ANP release in heart transplant patients. However, elevated ANP is reported in heart transplant recipients in the absence of increased right atrial pressure or systemic hypertension. Hypoxia is also a stimulus for ANP release in animals. We previously reported that five heart transplant recipients included in the present study experienced mild (three patients) to moderate (two patients) hypoxemia during exercise at 70% of peak power. However, in our heart transplant patients who became hypoxic, ANP levels were similar to those found in heart transplant patients who remained normoxic during exercise. Corticosteroids could increase plasma ANP in heart transplant recipients. Gardner et al demonstrated (in vitro) stimulation of ANP gene expression in rat cardiac myocytes through corticosteroid treatment. Elevated plasma NE could stimulate ANP release during exercise, and it is also possible that ANP release from atrial myocytes is under neural control. Further research is necessary to explore the mechanisms and significance of ANP hypersecretion in heart transplant patients.

Vasopressin

Resting plasma AVP was elevated in the heart transplant group (3.8 pg/ml), although not significantly (p = 0.06). Elevated AVP has been reported in heart transplant patients at rest and during supine upright cycle exercise at a fixed load. However, we are unaware of previous studies that have compared the plasma AVP response to upright exercise at the same relative intensities in heart transplant recipients and normal controls. Exercise at 40%, 70%, and 100% of peak power output elicited a dose–response increase in plasma AVP for both groups. These results concur with the established phenomenon that AVP secretion during exercise is proportional to relative exercise intensity. The unique finding was that the magnitude of the AVP response to the same relative exercise stress was greater in heart transplant patients than in the control group. Furthermore, hypersecretion of AVP may have been greater if the transplant patients had not been receiving prednisone therapy because glucocorticoids inhibit AVP secretion.

Factors modulating the release of AVP during exercise are not fully understood. Acute reduction of plasma volume stimulates AVP release via carotid and aortic baroreceptor reflexes. In the present study, exercise-induced reductions in plasma volume (percent change from baseline) were similar for both groups. It is unlikely, therefore, that volume reduction is responsible for hypersecretion of AVP in heart transplant recipients. Hyperosmolality also mediates AVP release via hypothalamic osmoreceptors. We did not measure plasma osmolality, but it is doubtful that heart transplant patients experienced greater hemoconcentration because volume shifts were similar for both groups.

A proportional relation between Ang II and plasma AVP has been reported during Ang II infusions and exercise. However, baseline and exercise Ang II levels were similar in both groups, suggesting that Ang II was not an important factor in the AVP response. Renal insufficiency could also be the cause of the high baseline AVP levels in the transplant group by decreasing AVP clearance via the kidneys. However, plasma AVP levels are normal in patients with mild-to-moderate renal insufficiency, such as those included in the present study (Table 1).

In addition to arterial baroreflexes, osmoregulation, and Ang II levels, relatively minor shifts in left atrial pressure elicit marked alterations of plasma AVP. The AVP response to atrial stretch, however, appears to be abolished in cardiac denervated animals and humans. These data suggest that vagal afferent denervation is responsible for the higher AVP levels at rest and during exercise in heart transplant recipients because the tonic inhibition of AVP cannot be transmitted by atrial stretch receptors. Furthermore, elevated plasma AVP appears to be one possible mechanism for the unexplained volume expansion observed in heart transplant recipients with normal RAA levels.
NE

Baseline plasma NE was not significantly different between the two groups (0.478 versus 0.394 ng/ml), but the heart transplant recipients demonstrated a trend toward elevated NE (p=0.08). These data are in agreement with other reports in heart transplant patients late (>3 months) after transplantation.28–30 Exercise at 40% of peak power evoked comparable increases in NE in each group. The relative NE response during exercise at 70% and 100% of peak power, however, was significantly greater in the heart transplant group.

To our knowledge, this is the first study to compare NE responses in heart transplant recipients and matched controls during exercise at the same relative intensity. Younis et al32 measured plasma NE in heart transplant patients during cycle exercise at 50%, 70%, and 90% of peak power output but did not report NE data for the control group. Others have reported that NE is elevated at the same absolute exercise intensity in heart transplant recipients compared with controls.53,54 It is difficult to infer from the previous data, however, that sympathetic hyperactivity is a consequence of transplantation. Considering the fact that peak VO2 achieved by heart transplant recipients in those earlier investigations was approximately 60% of that in the control group, each absolute exercise stage required a higher relative percentage of maximal effort in the heart transplant group. Therefore, the increases in plasma NE during exercise were simply indicative of the well-documented proportional relation between relative exercise intensity and plasma NE.55

Greater sympathetic activity may be required to regulate blood pressure in heart transplant recipients. Sinoway et al56 studied basal and hyperemic forearm blood flow in 10 patients before and after transplantation. Peripheral vasodilator capacity remained impaired in some heart transplant patients at 4 months after transplant, although blood pressure and cardiac function had returned to normal. The authors speculate that structural abnormalities in the arteries secondary to the low-flow state of CHF cause the peripheral vascular system to become "intrinsically stiff." Peripheral vasoconstrictor responsiveness may also be impaired after CHF. Borow et al57 reported that higher doses (68% higher than control) of the α1-agonist methoxamine were required to produce the same increments in blood pressure (30–60 mm Hg) in heart transplant patients compared with matched control subjects.

Muscle deconditioning in heart transplant recipients may contribute to exaggerated sympathetic tone during exercise. Recent studies using 31P nuclear magnetic resonance (NMR) spectroscopy have demonstrated abnormal muscle metabolism in CHF patients during exercise in the absence of reduced peripheral blood flow.38 Drexler et al39 addressed this issue more directly by taking skeletal muscle biopsy samples from CHF patients and found intrinsic ultrastructural and cytochemical abnormalities. The most prominent findings were reduced volume density, cristae surface density, and cytochrome oxidase activity of skeletal muscle mitochondria. Taken together, these data indicate decreased oxidative capacity of working muscle in CHF patients before heart transplantation. The time course for spontaneous recovery of ultrastructural and cytochemical abnormalities after cardiac transplantation has not been ascertained.

Cyclosporine may also contribute to increased plasma NE in heart transplant recipients during exercise. Scherrer et al1 performed microneurography in heart transplant recipients treated with cyclosporine and reported that muscle sympathetic discharge and plasma NE were higher than in heart transplant patients not receiving cyclosporine. Sympathetic hyperactivity also occurred in myasthenia gravis patients receiving cyclosporine. However, both arterial pressure and sympathetic activity were significantly higher in heart transplant recipients than in myasthenia gravis patients taking similar doses of cyclosporine. Thus, it is possible that cardiac denervation exaggerates the excitatory effects of cyclosporine.

Neuroendocrine Summary

Neuroendocrine hyperactivity in heart transplant recipients may be ascribed to cyclosporine, antihypertensive medications, or skeletal muscle deconditioning. However, cardiac denervation offers an alternative and attractive explanation for the hypersecretion of NE, AVP, and PRA during exercise. Virtually all of the neuroendocrine hyperactivity in our heart transplant patients could be explained by ablation of atrial stretch receptor afferents. Hypersecretion of NE, AVP, and PRA is certainly plausible, given the ablation of afferent neural connections between the transplanted heart and the brain of heart transplant recipients. In animal models, limited reinnervation by the sympathetic nervous system usually occurs within the first year after autotopic heart transplantation.58,59 In contrast, physiological evidence for vagal reinnervation after heart transplantation in humans is less clear.5,53,60,61

Mechanoreceptors in the recipient atria may also be unable to contribute to the tonic inhibition of AVP, PRA, and sympathetic traffic during exercise. Although the native atria are left intact and the sensory afferents are not intentionally severed, they may be diminished in number or sensitivity or permanently ablated. Impaired firing patterns of atrial mechanoreceptors are observed during volume-overloaded CHF.62 Also, sympathetic hyperactivity increases coronary vasoconstriction and induces myocardial tissue damage in certain types of CHF.63 Finally, the coronary artery blood source is permanently diverted to the donor organ during transplant surgery, and the recipient atrial remnant must rely on collateral circulation.14 There was no evidence of physiological reinnervation in any of our patients during a postural shift from a supine to an erect seated position, and the tachycardic response to exercise was blunted in all heart transplant recipients. The present study, however, was not designed to isolate cardiac stretch receptor reflexes. Exclusive tests of the afferent limb of cardiac reflex arcs are difficult to implement in humans but warrant further investigation.

Clinical Implications

The clinical implications of these findings are speculative but offer rationale for the early introduction of therapy designed to buffer neuroendocrine hyperactivity (e.g., β- or α/β-adrenergic blockade and demand pacemaker). Although circulating catecholamines provide chronotropic and inotropic support for the dener-
vated heart during exercise, extreme sympathetic arousal increases peripheral vascular resistance and the work of the heart. Plasma NE levels above 1.8 ng/ml behave as a circulating a-agonist vasoconstrictor.\textsuperscript{64} Mean plasma NE in our heart transplant recipients at peak exercise during the GXT was 3.9 ng/ml (ninefold to 10-fold greater than rest) and possibly imposed limits on exercise capacity by increasing afterload and opposing further increases in cardiac output through arterial baroreflex.\textsuperscript{65}

Although the heart transplant patients in the present study were receiving ACE inhibitors (20 mg enalapril b.i.d. and 60 mg captopril b.i.d.), both baseline and exercise levels of plasma Ang II and aldosterone were normal (Figure 1). These data indicate that pharmacological suppression of the RAA system is incomplete in heart transplant recipients receiving ACE inhibition at doses that are clinically effective in other hypertensive populations. The PRA, Ang II, and aldosterone data, together with the observation of markedly increased AVP, suggest that neuroendocrine mechanisms alter the ability of heart transplant recipients to regulate fluid volume even in the presence of normal glomerular function and cardiac hemodynamics.\textsuperscript{8,30} Consequently, neuroendocrine-mediated volume expansion may contribute to the incidence and severity of hypertension in heart transplant patients.\textsuperscript{1,2,66}

Summary

Heart transplant recipients demonstrated exaggerated neuroendocrine responses during exercise performed at the same relative intensity compared with innervated matched controls. We interpret these data as a possible indication of ablation of cardiac mechanoreceptor afferents and unopposed neuroendocrine stimulation in heart transplant patients. Furthermore, chronic neuroendocrine hyperactivity is likely in ambulatory heart transplant recipients. Although cyclosporine nephrotoxicity is implicated in the development of transplant hypertension,\textsuperscript{1,2} our data suggest that chronic neuroendocrine hyperactivity, which alters renal volume regulation,\textsuperscript{30,66} also contributes to the incidence and severity of hypertension in heart transplant recipients.

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