β-Adrenergic Supersensitivity of the Transplanted Human Heart Is Presynaptic in Origin

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An increase in cardiac β-adrenergic sensitivity or β-receptor density or both has been described in several animal species after denervating the heart. The transplanted human heart is also denervated and, therefore, may exhibit supersensitivity to β-adrenergic agonists and an increase in β-adrenergic receptor density. In 16 patients examined 1–3 months after orthotopic cardiac transplantation, β-adrenergic receptor density measured by [125I]iodocyanopindolol binding in endomyocardial biopsy specimens was not significantly different in transplant recipients compared with normal controls (transplant=1,429±199, control=1,728±263 fmol/g wet wt; p=NS). However, when normalized to Lowry protein, the [125I]iodocyanopindolol in β-adrenergic receptor density in biopsy tissue from transplant recipients was significantly lower than in tissue from controls (transplant=58.1±6.2, control=93.5±13.4 fmol/g Lowry protein; p=0.011). Atrial sinus node activity of the denervated donor heart and the innervated atrial cuff of the native recipient heart could be detected on the surface electrocardiogram in six patients. In these six patients, the heart rate response to graded infusions of epinephrine (taken up by the adrenergic nerve terminals) and isoproterenol (not taken up by the adrenergic nerve terminals) was measured. The epinephrine dose-response curve in transplanted donor atria was significantly to the left of the native recipient atrial dose-response curve (p <0.0001). The isoproterenol dose-response curves for native and transplanted atria were not different. We conclude that myocardial β-adrenergic receptors are not increased in human orthotopic cardiac allografts and that there is no evidence for β-receptor–mediated supersensitivity of postsynaptic origin. However, human orthotopic cardiac allografts exhibit adrenergic supersensitivity of the sinus node to agents that are ordinarily removed by the neuronal uptake system. This supersensitivity is, therefore, presynaptic in origin and should increase the response of the transplanted heart to circulating epinephrine or norepinephrine. (Circulation 1989;79:344–349)

We have reported that rabbit and rat heterotopic cardiac allografts analyzed 4 weeks after transplantation have increased β-adrenergic receptor density and increased isoproterenol–stimulated adenylate cyclase activity presumably due to denervation.1 An increase in cardiac β-adrenergic sensitivity or β-receptor density has also been described in several other animal species after denervation of the heart.2–5 Although the heart of human cardiac transplant recipients has been shown to be denervated,6–8 early studies with small numbers of subjects did not demonstrate increased sensitivity to catecholamines.9–11 In contrast, two recent studies have reported increased sensitivity of the transplanted human heart to β-adrenergic stimulation.12,13

Increased sensitivity to sympathomimetic amines may result from changes occurring either before or after the presynaptic nerve ending.14 When supersensitivity results from changes occurring before the synapse, it is classically termed “supersensitivity of presynaptic origin.”15 An example of supersensitivity of presynaptic origin is the adrenergic supersensitivity produced by cocaine’s inhibition of the neuronal uptake mechanism.14,15 When supersensitivity results from changes occurring after the synapse, such as an increase in adrenergic receptor density, it is termed “supersensitivity of postsynaptic origin.”15 The supersensitivity of the transplanted heart may, therefore, be presynaptic or postsynaptic in origin.
In orthotopic transplantation, the posterior walls of the atria and much of the interatrial septum are not excised. Thus, the recipient's native atrium remains innervated and responsive to autonomic influences. The transplanted donor heart is surgically denervated and remains so over time. On the surface electrocardiogram, it is often possible to distinguish "sinus node" activity (P waves) from the remnant of the recipient's native right atrium as well as from the sinus node within the transplanted donor heart. The sinus node activity of the recipient's native atrium, therefore, can serve as an internal innervated control when investigating the denervated donor atrium.

We examined the effect of denervation on β-adrenergic receptor density and catecholamine responsiveness in the intact, orthotopically transplanted human heart. β-Adrenergic receptor density in the transplanted heart was measured in right ventricular endomyocardial biopsy tissue, whereas the rate response of the recipient's native (innervated) and transplanted donor (denervated) sinus nodes to infusions of the β-adrenergic agonists epinephrine and isoproterenol after pretreatment with atropine was used to assess functional activity of innervated and denervated cardiac β-adrenergic receptors. The data indicate that β-adrenergic receptor density and postsynaptic β-adrenergic responsiveness do not increase in human cardiac allografts but that transplanted allografts exhibit supersensitivity of presynaptic origin to a catecholamine that is normally taken up by adrenergic nerve terminals.

Methods

Patients

The study population consisted of 20 orthotopic cardiac allograft recipients transplanted by the Utah Cardiac Transplant Program (UCTP), Salt Lake City, Utah, between March 9, 1985, and April 21, 1987. Patients were clinically stable, had normal cardiac function as evidenced by echocardiogram, and were studied between 25 and 93 days after heart transplantation (mean, 56±5 days). All patients were treated according to a standard immunosuppression protocol that has been described elsewhere. At the time of study, all patients were receiving cyclosporine and azathioprine for immunosuppression, and no patient was receiving corticosteroids in accordance with the UCTP "no-steroid" protocol. All patients gave written, informed consent, which was approved by the Human Subjects Committee of the University of Utah Medical Center.

Endomyocardial Biopsy

Right ventricular endomyocardial biopsy specimens for measuring myocardial β-adrenergic receptor density were obtained from all patients at the time of routine biopsy for rejection surveillance. The procedure for right ventricular endomyocardial biopsy has been previously described. Briefly, a 9F sheath is inserted percutaneously into the right internal jugular vein under local anesthesia. A 50-cm right ventricular biopsy is then inserted through the sheath, and with fluoroscopic guidance, it is positioned against the right intraventricular septum, and specimens are obtained. In this study, three or four specimens were obtained for histologic examination, and three to six specimens were obtained for β-adrenergic receptor analysis.

Of these 20 patients, 16 had no evidence from endomyocardial biopsy for rejection and are included in this study. The remaining four patients had histologic evidence for rejection according to standard criteria and were excluded from further analysis.

Left ventricular function was assessed in cardiac transplant patients by both radionuclide left ventriculography and by echocardiography. Patients with left ventricular ejection fractions of less than 0.50 (radionuclide) were excluded from further analysis. The mean left ventricular ejection fraction measured by radionuclide ventriculography was 0.59±0.02. The mean left ventricular percent fractional shortening measured by echocardiogram was 0.40±0.02.

Myocardial β-adrenergic receptor measurements were also made in nine patients with normal ventricular function (left ventricular ejection fraction >0.50). The mean age of these patients was 43±3 years. Mean hemodynamic variables were left ventricular ejection fraction = 0.58±0.02, cardiac index=3.2±0.3 l/min/m², right atrial pressure = 2±1 mm Hg, and pulmonary artery wedge pressure = 8±1 mm Hg. Seven of these patients underwent endomyocardial biopsy for evaluation of possible anthracycline cardiotoxicity. None had a biopsy score greater than 2.0 on the Billingham scale of anthracycline damage. One patient was evaluated because of an apparent familial cardiomyopathy in his offspring. Another patient had chest pain after an acute viral illness, and acute myocarditis was suspected. The endomyocardial biopsy from these patients was normal.

Endomyocardial β-Adrenergic Receptor Measurement

We have previously described in detail the technique of β-adrenergic receptor density measurement in endomyocardial biopsy specimens. Briefly, biopsy specimens were immediately placed in ice-cold 10 mM Tris buffer, 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) buffer, pH 8.0, and grossly visible fibrous tissue was dissected free. The remaining sample was blotted dry and weighed. Tissue weight ranged from 16.0 to 30.0 mg (mean, 25.0±1.0 mg). Crude membrane preparations were then made by homogenization, extraction of contractile proteins with 0.5 M KCl, and multiple washes of a pellet from centrifugation at 50,000g. The radioligand, [125I]iodocyanopindolol (ICYP) was used for identifying β-adrenergic receptors. Duplicate tubes containing seven increasing concentrations of ICYP...
with and without $10^{-6}$ M (-)propranolol were prepared. The assay was begun with the addition of membrane preparation and incubated for 120 minutes, followed by dilution with buffer and vacuum filtration. Maximum bound ICYP and the ICYP dissociation constant were determined by analysis of saturation binding data with a nonlinear least-squares regression analysis of one form of the Michaelis-Menton equation. Protein measurements were made by Peterson's modification\textsuperscript{22} of Lowry et al's methods.\textsuperscript{23}

**Catecholamine Measurements**

Right atrial blood samples for catecholamine analysis were obtained from all patients. In six patients, a coronary sinus catheter was inserted into the right internal jugular vein with a 7F multipurpose catheter (MA-2, Argon Medical, Athens, Texas), and the catheter position was confirmed by contrast dye injection and blood gas measurement of the coronary sinus sample. Simultaneous coronary sinus and arterial (from a radial or femoral artery catheter) blood samples were obtained. Samples for catecholamine determination were placed into iced EGT A tubes and analyzed by a radioenzymatic method (CAT-a-Kit, Amersham, Chicago, Illinois).\textsuperscript{24}

**Isoproterenol and Epinephrine Infusion Studies**

On the day after endomyocardial biopsy, sinus node activity from the recipient's native and transplanted right atria was easily identified as two distinct P waves on the surface electrocardiogram in six of the 16 evaluable patients. The response of native and transplanted atrial heart rate to isoproterenol and epinephrine was examined in these six patients. Each was placed in a supine position, and an 18-gauge intravenous catheter was inserted into a forearm vein. The rates of the native and transplanted atrial P waves were measured with a 12-lead surface electrocardiogram at a recording speed of 50 mm/min. Arterial blood pressure was measured with a sphygmomanometer cuff placed around the arm yielding the highest blood pressure. After at least 20 minutes with the patient free from outside stimulation, the baseline rate for native and transplanted P waves was recorded. Parasympathetic blockade was initiated with an atropine bolus of 0.04 mg/kg i.v., a dose that has been shown to effectively block parasympathetic influence on heart rate in humans.\textsuperscript{25} Five minutes after atropine administration, native and transplanted atrial rates were measured again (after atropine baseline). The dose-response curves of native and transplanted atria to epinephrine and isoproterenol were then recorded.

The order in which epinephrine and isoproterenol was administered was determined randomly. Four patients first received isoproterenol and two patients first received epinephrine. Each dose was administered as an intravenous bolus during a 10-second interval. Epinephrine and isoproterenol were each administered in the following doses: 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.12, 0.16, and 0.24 $\mu$g/kg. Each drug was first administered at a dose of 0.01 $\mu$g/kg and was followed by the next highest dose every 5 minutes until all doses were administered or until either an adverse effect (e.g., hypertension [diastolic blood pressure $>105$ mm Hg], hypotension [systolic blood pressure $<100$ mm Hg], and ventricular ectopy) or donor heart rate of greater than 150 beats/min was observed. After a 30-minute washout period to allow the heart rate to return to baseline after atropine, the second drug was administered in a similar fashion.

The native and transplant atrial rates were measured at 30 seconds, 1 minute, 2 minutes, 3 minutes, and 4 minutes after each intravenous bolus of drug. The fastest atrial rate was used to determine the dose-response relation. The change in heart rate over baseline was used to define the dose-response relation. Blood pressure was measured after the 1- and 3-minute electrocardiographic recordings.

**Statistical Methods**

The values for $\beta$-adrenergic receptor density for transplant recipients and normal controls were compared by the Student’s $t$ test for unpaired samples. The correlation between $\beta$-adrenergic receptor density and days after transplantation was evaluated with linear regression. The dose-response curves for native and transplant atrial rates for epinephrine and isoproterenol were compared by a previously described method of multiple linear regression.\textsuperscript{26} All values are mean $\pm$ SEM. Differences between groups were considered significant when $p$ was less than 0.05.

**TABLE 1. Myocardial $\beta$-Adrenergic Receptor Density**

<table>
<thead>
<tr>
<th>Transplant patients</th>
<th>Days 35–51</th>
<th>Days 53–93</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_{max}$ (fmol/g Lowry protein)</td>
<td>58.1 $\pm$ 6.2</td>
<td>70.1 $\pm$ 8.8</td>
<td>45.9 $\pm$ 6.87</td>
</tr>
<tr>
<td>$B_{max}$ (fmol/g wet wt)</td>
<td>(23.5–114.7)</td>
<td>(42.0–114.7)</td>
<td>(23.5–77.6)</td>
</tr>
<tr>
<td>$B_{max}$</td>
<td>1,429 $\pm$ 199</td>
<td>1,356 $\pm$ 279</td>
<td>1,503 $\pm$ 302</td>
</tr>
<tr>
<td>$K_d$</td>
<td>13.9 $\pm$ 2.2</td>
<td>13.7 $\pm$ 3.5</td>
<td>14.1 $\pm$ 3.0</td>
</tr>
<tr>
<td>$K_d$</td>
<td>(3.6–35.5)</td>
<td>(3.6–35.5)</td>
<td>(4.6–31.6)</td>
</tr>
</tbody>
</table>

Range of values given in parentheses.

$B_{max}$, myocardial $\beta$-adrenergic receptor density.

*p = 0.011 vs. normal subjects; $t_p = 0.008$ vs. normal subjects.
Results

Myocardial β-Adrenergic Receptor Density

The mean values for β-adrenergic receptor density and ICYP dissociation constant from right ventricular endomyocardial biopsy specimens for the 16 transplant recipients and nine normal controls are given in Table 1. Data for the eight recipients studied earliest after transplantation (days 25–51) and the eight recipients studied latest after transplantation (days 53–93) are also shown in Table 1. When β-adrenergic receptor density was normalized to gram wet weight of tissue, no significant difference existed between transplant recipients and controls. However, with normalization to Lowry protein, which is a method that tends to ignore collagen protein, the β-adrenergic receptor densities of all transplant recipients and recipients studied 53–93 days after transplantation were significantly lower than controls. The β-adrenergic receptor density of recipients studied early after transplantation was not different from controls. Figure 1 is a plot of the transplant recipients’ β-adrenergic receptor density per gram of Lowry protein as a function of the time in days after transplantation, with a significant negative correlation between these two variables (r = 0.541, p = 0.029). There were no significant differences between coronary sinus and systemic arterial catecholamines.

Isoproterenol and Epinephrine Infusion

Atropine administration resulted in a significant increase in the rate of recipients’ native atria (native atrial rate before atropine = 77 ± 6 beats/min, after atropine = 96 ± 7 beats/min; p = 0.019). The transplanted atrial rate did not change with atropine administration (transplanted atrial rate before atropine = 84 ± 8 beats/min, after atropine = 84 ± 9 beats/min; p = NS), which is consistent with denervation. As illustrated in Figure 2, the epinephrine dose-response curve in transplanted atria was significantly to the left of the dose-response curve in native atria (p < 0.0001), indicating an increased sensitivity of the transplanted atrial sinus node to epinephrine. However, as illustrated in Figure 3, the isoproterenol dose-response curves were not different for native and transplanted atria, which is indicative of a lack of increased sensitivity of the donor sinus node to isoproterenol.

The average arterial pressure at baseline (after atropine) was 140 ± 8/88 ± 3 mm Hg. Arterial pressure did not change significantly upon administration of the maximum dose of isoproterenol (average arterial pressure = 165 ± 10/88 ± 5 mm Hg) or maximum dose of epinephrine (average arterial pressure = 167 ± 14/96 ± 6 mm Hg).

Discussion

Our results indicate that β-adrenergic receptor density does not increase in human cardiac tissue after orthotopic transplantation. This absence of up-regulation differs from our earlier findings with rabbit and rat isograft tissue where β-adrenergic receptor density was increased in the heterotopic, nonworking graft. In the present study with human patients, we observed a decrease in β-adrenergic receptor density with an increase in time after transplantation. As a result, the β-receptor density of patients who were studied relatively early after transplantation (days 25–51) was not different from control patients, but the β-receptor density of patients who were studied later after transplantation (days 53–93) was significantly lower than control values. Denniss and coworkers have also reported no difference in β-adrenergic receptor density between transplant recipients and normal subjects.

There are significant differences between the heterotopic transplanted heart of the previously used animal models and the orthotopic heart of human transplant recipients. The workload of the intra-

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**TABLE 2. Transplant Recipient Catecholamines**

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
<th>Dopamine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial</td>
<td>158 ± 22</td>
<td>58 ± 20</td>
<td>72 ± 24</td>
</tr>
<tr>
<td>(n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>170 ± 37</td>
<td>74 ± 18</td>
<td>145 ± 93</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>208 ± 45</td>
<td>54 ± 15</td>
<td>94 ± 72</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Abdominal heterotopic heart of the animal models is decreased because blood is not ejected. In contrast, the workload of the right ventricle of a human orthotopic transplanted heart is either normal or increased because of the recipient’s preexisting pulmonary arterial hypertension. This increase in workload may result in right ventricular enlargement and hypertrophy. We have previously reported that patients with pulmonary hypertension and right ventricular failure develop a chamber-specific decrease of right ventricular β-adrenergic receptor density. Similarly, the decrease in β-adrenergic receptor density over time observed in our transplant patients may have been secondary to an increase in transplanted right ventricular workload and right ventricular hypertrophy. However, echocardiographic measurements of right ventricular dimensions were not obtained in our transplant patients, and a limitation in our study design was that we did not attempt to correlate serial measurement of β-receptor density with changes in right ventricular workload or muscle mass.

A significant increase in native atrial sinus node rate was observed after atropine administration. The transplanted atrial sinus node rate did not change with atropine, confirming parasympathetic denervation. In addition, arterial and coronary sinus norepinephrine blood concentrations were not different, which is consistent with denervation because the innervated, nonfailing heart extracts norepinephrine and epinephrine through cardiac adrenergic neuronal uptake.

The transplanted sinus node exhibited an increased response to epinephrine but not to isoproterenol. Because there is active uptake of epinephrine by myocardial adrenergic nerve terminals, the observed increased sensitivity of the denervated sinus node to epinephrine is most likely a presynaptic phenomenon reflecting the absence of neuronal uptake and the resultant increase in myocardial interstitial concentrations of epinephrine within the transplanted heart. In contrast, there is no uptake of isoproterenol by adrenergic neurons and, therefore, any increase in sensitivity to isoproterenol is a postsynaptic phenomenon, such as may be observed with an increase in the affinity or density of β-adrenergic receptors. We did not observe an increased sensitivity of the donor sinus node to isoproterenol. This absence of supersensitivity of postsynaptic origin is consistent with the absence of up-regulation of β-adrenergic receptors of donor ventricular myocardium.

Our findings apparently conflict with those of Yusuf and coworkers, who reported an increased sensitivity of the denervated transplanted human heart to isoproterenol. In their study, native and transplanted ventricular rate responses to isoproterenol in heterotopic cardiac transplant recipients were compared with ventricular rates of orthotopic cardiac transplant recipients and normal controls. They showed an increased sensitivity to isoproterenol of the transplanted hearts with respect to native hearts or normal controls. However, patients were not pretreated with atropine, and it is possible that the control hearts and native hearts of heterotopic transplant recipients were less sensitive to isoproterenol because of intact baroreceptor reflexes and increased parasympathetic tone. Such effects cannot be excluded by their experimental design. In addition, the native heart of a heterotopic transplant recipient is not normal and may exhibit β-receptor down-regulation because of heart failure. Therefore, in this previous study, the apparent increased sensitivity of the transplanted heart might have resulted from a decreased sensitivity of the recipient’s native heart.

The increased sensitivity of transplanted allografts to catecholamines that are normally taken up by adrenergic nerve terminals has important clinical
implications. Among them is that epinephrine and norepinephrine will be more potent inotropes for the treatment of ventricular dysfunction in transplanted hearts because denervation will increase the sensitivity to these drugs. This supersensitivity will in effect increase the β/α-receptor response ratio of each of these agents. In addition, the exaggerated response to circulating epinephrine also helps explain how the exercise response of cardiac transplant recipients is preserved.

In summary, myocardial β-adrenergic receptors are not increased in human orthotopic cardiac allografts. Human orthotopic cardiac allografts exhibit supersensitivity of the sinus node of presynaptic origin consistent with denervation, but they do not exhibit supersensitivity of postsynaptic origin.

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