Myocardial Dysfunction in Donor Hearts
A Possible Etiology

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Background—Potential cardiac donors show various degrees of myocardial dysfunction, and the most severely affected hearts are unsuitable for transplantation. The cause of this acute heart failure is poorly understood. We investigated whether alterations in calcium-handling proteins, β-adrenoceptor density, or the inhibitory G protein Gia could account for this phenomenon in unused donor hearts (n=4 to 8). We compared these with end-stage failing hearts (n=14 to 16) and nonfailing hearts (n=3 to 12).

Methods and Results—Myocardial samples were obtained from unused donor hearts displaying ejection fractions <30%. Both trabeculae and isolated myocytes responded as poorly as those from the group of failing hearts to increasing stimulation frequency with regard to inotropic function in vitro. Immunodetectable abundance of sarcoplasmic reticulum calcium-ATPase and sodium calcium exchanger were greater (177%; P<0.01) and smaller (29%; P<0.01), respectively, in the unused donor hearts relative to the failing group, which suggests that alterations of these proteins are not a common cause of contractile dysfunction in the 2 groups. Myocytes from the unused donor group were desensitized to isoprenaline to a similar degree as those from the failing heart group. However, β-adrenoceptor density was reduced in the failing (P<0.001) but not in the unused donor heart group (P=0.37) relative to the nonfailing heart group (n=5). Gia activity was increased in samples from unused donor and failing hearts relative to nonfailing hearts (P<0.05).

Conclusions—Increased activity of the inhibitory G protein Gia is a significant contributory factor for impaired contractility in these acutely failing donor hearts. (Circulation. 1999;99:2565-2570.)

Key Words: transplantation • heart failure • proteins • receptors, adrenergic, beta • contractility

The shortage of donor organs considerably limits the availability of heart transplantation and has led to the search for ways to increase the size of the donor pool. Myocardial dysfunction occurs to various degrees in all brain-dead organ donors, and we and others have found this to be so severe in ~20% of cases that it precludes the use of these hearts for transplantation.1 Because much of the early posttransplantation mortality is due to graft failure, the use of such hearts is surrounded by uncertainty and conjecture.

The present study was designed to examine the origin of myocardial dysfunction in potential organ donors. Cellular mechanisms considered important in end-stage heart failure were examined in a group of unused donor hearts with ejection fractions <30%. Characteristically, myocardium from end-stage failing hearts is slow to relax, perhaps owing to reduced levels of expression of the sarcoplasmic reticulum calcium-ATPase (SERCA2).2 Furthermore, end-stage failing myocardium is desensitized to β-agonists because of β-adrenoceptor (β-AR) downregulation coupled with upregulation of the inhibitory G protein Gia.3,4 We have characterized the contractile behavior of cells and trabeculae from unused donor hearts and compared it with that seen in hearts with end-stage failure. Myocardial samples were analyzed for abundance of the key calcium-handling proteins SERCA2, sodium calcium exchanger (NCX), and phospholamban (PLB). Finally, β-AR density was assessed and compared with that seen in both normal and failing myocardial tissue samples, together with the expression of Gia. Our data show that myocardial dysfunction in donor hearts is associated with impaired contractile function in vitro, decreased β-AR sensitivity, and elevated Gia activity.

Methods

Tissue Retrieval and Storage
Myocardium was obtained from Royal Brompton and Harefield Hospital CABG patients with good ventricular function (the nonfailing group, with ejection fractions [EFs] >60%) and those with moderate heart failure (New York Heart Association [NYHA] class...
II or III). Samples from end-stage heart failure (NYHA IV) were taken at the time of transplantation. The cause of all heart failure in these patients was either ischemic heart disease (IHD) or idiopathic dilated cardiomyopathy (DCM). In all cases, ventricular function was assessed angiographically. Ventricular function in potential cardiac donors was assessed by transesophageal echocardiography, with the transgastric short-axis area used as an assessment of fractional shortening and hence EF. Hearts from those individuals with EFs \(< 30\%\) coupled with poor hemodynamic parameters (blood pressure, left and right atrial pressures) were not used for transplantation but were retrieved normally (with ice-cold St Thomas cardioplegia solution) and used for homograft valve preparation with EFs \(> 30\%\).

Samples from unused donor (UD) hearts were transported to the laboratory in ice-cold St Thomas cardioplegia solution. Tissue was divided into 3 portions that were either frozen in liquid N2 and stored \(-80\,^\circ\text{C}\) for later biochemical analysis, used to isolate myocytes as previously described, or used as a source of trabeculae for contraction measurements.

Ethical approval was obtained from the Royal Brompton and Harefield Hospital Ethical Committee, and informed written consent was obtained in all cases.

### Myocardial Dysfunction in Donor Hearts

#### Clinical Details of Unused Donor Hearts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Cause of Brain Death</th>
<th>Sex</th>
<th>EF, %</th>
<th>Time in ITU, h</th>
<th>Drug/Dose, µg/kg (-1)·min (-1)</th>
<th>BP, mm Hg</th>
<th>ECG</th>
<th>Biochemistry</th>
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<tr>
<td>1</td>
<td>13</td>
<td>HI</td>
<td>F</td>
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<td>19</td>
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<td>115/70</td>
<td>ST</td>
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<td>ICH</td>
<td>F</td>
<td>&lt;30</td>
<td>24</td>
<td>ADR 0.12</td>
<td>100/70</td>
<td>ST</td>
<td>WCC 11.2</td>
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<td>90/60</td>
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<td>WCC 12.0</td>
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<td>M</td>
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<td>M</td>
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<td>95/60</td>
<td>ST</td>
<td>WCC 6.7</td>
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</table>

NA indicates noradrenaline; DOP, dopamine; ADR, adrenaline; DOB, dobutamine; AR, aramine (metaraminol); and ITU, intensive care unit. ECG results are shown as either sinus rhythm (SR) or sinus tachycardia (ST). Blood biochemistry and hematology results are white cell count (WCC; \(\times 10^9/\text{L}\)), urea (Ur), creatinine (Cr), potassium (K\(^+\)), and sodium (Na\(^+\)), all in mmol/L.

Cause of death in the majority of cases was intracranial hemorrhage (ICH) or head injury (HI). All patients were taking \(\geq 1\) inotropic agent.

#### In Vitro Assessment of Contractile Function

Myocyte and trabeculae contractile responses were assessed as previously described.\(^6\) Contraction of both isolated myocytes and multicellular preparations was studied at \(32\,^\circ\text{C}\) and either 2 mmol/L Ca\(^{2+}\) (for trabeculae) or 2 to 10 mmol/L Ca\(^{2+}\) (for myocytes). The effect of a range of frequencies on contraction amplitude and postrest behavior was examined after rest intervals of 10, 30, and 180 seconds. For isolated myocytes, a concentration-response curve to isoproterenol was also constructed.

#### Biochemical Analysis

Western blot analysis was performed on samples of myocardium as described previously.\(^7\) \(\beta\)-Adrenoceptors were identified by \([^{125}\text{I}]\)iodocyanopindolol binding as previously described\(^8\) with minor modifications. Briefly, cardiac homogenates were centrifuged for 20 minutes at 50,000g, and the pellet was washed by recentrifugation. After resuspension of the final pellet in binding buffer (154 mmol/L NaCl, 10 mmol/L Tris, pH 7.4), aliquots of the membrane suspension were incubated in a total volume of 250 \(\mu\)L for 90 minutes at \(37\,^\circ\text{C}\). Incubations were terminated by rapid vacuum filtration over Whatman GF/C filters, which were washed with 20 mL of binding buffer. Nonspecific binding was defined by 1 \(\mu\)mol/L CGP 12177. \(\beta\)- and \(\beta\)-adrenoceptors were estimated from competition experiments with the highly \(\beta\)-selective antagonist CGP 20712A.

\(G_m\) activity was assessed by pertussis toxin–induced \([^{32}\text{P}]\)ADP ribosylation and standardized to both the amount of total protein and 3’-nucleotidase activity as a membrane marker. The \([^{32}\text{P}]\)ADP ribosylation of \(G_m\) by pertussis toxin was performed for 14 hours at \(4\,^\circ\text{C}\) in a volume of 40 \(\mu\)L containing 100 mmol/L dithiothreitol, 2 mmol/L ATP, 1 mmol/L GTP, 50 mmol/L \([^{32}\text{P}]\)NAD (800 Ci/mmol), and 20 \(\mu\)g/mL pertussis toxin that had been activated by incubation with 50 mmol/L dithiothreitol for 1 hour at \(20\,^\circ\text{C}\) before the labeling reaction. Samples were subjected to SDS-PAGE (10% w/v acrylamide and 16 cm total gel length). Gels were stained with Coomassie blue and dried before autoradiography was performed. Incorporated \([^{32}\text{P}]\)ADP ribose was quantified by cutting out the bands from dried gels and by scintillation counting.

#### Statistical Analysis

Significance was assessed on grouped data with either Student’s \(t\) test for unpaired data or an ANOVA where indicated. Data from 1 to 3 cells for each patient were pooled before analysis so that \(n\) values refer to patients. Values are expressed as mean \pm SEM.

#### Results

Transesophageal echocardiography was performed on 107 potential heart and heart-lung donors examined by the Royal Brompton and Harefield Hospital transplant team. Of these, 23 organs (21.5%) were not used for transplantation because of EFs \(<30\%\) coupled with poor hemodynamic parameters, including elevated left atrial pressure, arterial blood pressure, right atrial pressure, and pulmonary artery pressure. In all cases, cardiovascular history and cardiovascular risk factors were negative, and there were no noted episodes of cardiac arrest. The clinical details of these patients are shown in the Table.
um exhibits a flat or negative Bowditch response. Figure 1 summarizes the force-frequency relationships seen in trabeculae preparations (Figure 1A) and isolated myocytes (Figure 1B) from the UD group, along with end-stage failing (F; both DCM and IHD) and nonfailing (NF) myocardium. The force-frequency relationship in trabeculae was indistinguishable between the F and UD groups. Similarly, increasing the stimulation frequency failed to increase the contraction amplitude of myocytes from either the UD or F groups (Figure 1B); however, contraction amplitude was significantly increased in myocytes from NF myocardium ($P_{0.001}$; $n=12$ (NF), 14 (F), and 5 (UD)). Analysis of the increase in contraction from 0.1 to 1 Hz for the 3 groups differed significantly by ANOVA ($P_{0.03}$).

Postrest Contraction Amplitude Was Increased in UD Myocardium
Potentiation of the postrest contraction amplitude relative to prerest levels is thought to reflect a gain of calcium in the sarcoplasmic reticulum (SR) during quiescence.$^{10}$ SR function in myocardium of both the UD and F groups was assessed by comparison of the amplitude of the first beat after a rest period. We have previously shown that myocytes isolated from normal myocardium tend to show postrest decay, whereas failing myocardium exhibits postrest potentiation.$^{11}$ Postrest amplitude from the UD group was examined, and the first beats after different rest periods at 4 stimulation frequencies are presented in Figure 2A. A comparison between the postrest amplitude from the UD and F groups is presented in Figure 2B. Amplitude of the first beat after indicated rest period in trabeculae from UD (solid bars) and F (open bars) myocardium. Data are mean±SEM; $n=8$.

Beat Duration Was Increased in UD Myocardium
Myocyte beat duration was analyzed in terms of time to peak contraction (TTP), time to 50% relaxation ($R_{50}$), and time to 90% relaxation ($R_{90}$). A comparison between cells from the UD group (4 patients) and data gathered from the NF (20 patients) and F (61 patients; both IHD and DCM) groups is shown in Figure 3A. TTP was similar in both the NF and UD groups, both being significantly shorter than for cells from the F group. However, relaxation times for the UD group were prolonged. $R_{50}$ values in myocytes from the UD group were even longer than in those from the F group and were significantly different from either the F or NF groups. $R_{90}$ values were also prolonged, dramatically so in some myocytes. This prolongation of relaxation, particularly the second phase, is shown in representative traces in Figure 3B.

$\beta$-AR Sensitivity Was Decreased in UD Myocardium
Responses of isolated myocytes from UD, F, and NF groups to isoproterenol were compared (Figure 4). Myocytes from the UD group showed a similar degree of $\beta$-AR desensitization to that seen in the moderately failing groups investigated previously, corresponding to cells from patients with NYHA class II or III failure.

Key Calcium-Handling Protein Expression in UD Was Significantly Different From End-Stage Failing Myocardium
When Western blot analysis was used, expression of SERCA2 was found to be significantly higher in the UD group than in the F group ($P_{0.01}$) whereas NCX was significantly lower ($P_{0.01}$). PLB levels were also higher in the UD group relative to the F group (60%; $P_{0.05}$; data not
shown). Protein levels are shown in Figure 5, with the F group divided into IHD and DCM. The relationship between the UD and F groups was similar to that previously reported between normal and failing myocardium for these proteins.12,13

**β-AR Number Was Unchanged in UD Myocardium**
Because of the functional β-AR desensitization seen in the UD group, β-AR number was measured in myocardial samples from this group. Measurements were compared with the NF and F (DCM and IHD) groups. β-AR number was not significantly different between the UD and NF groups but was decreased in the F group, in agreement with previous results14 (Figure 6A; P<0.001).

**Discussion**
The major finding of this study is that myocardial dysfunction seen in brain-dead organ donors appears to be unrelated to alterations in calcium-handling protein levels or β-AR receptor density. However, we detected a significant elevation in the level of the inhibitory G protein Giα.

Characteristic changes in the expression of SERCA2, NCX, and PLB have been reported in human heart failure.
For example, SERCA2 mRNA has been found to be reduced in studies of human failing myocardium compared with nonfailing myocardium.\textsuperscript{17-19} However, some studies report a decrease at the protein level, whereas others show no change in expression.\textsuperscript{18,19} A significant correlation has been demonstrated relating the abundance of SERCA2 protein and myocardial function as assessed by the force-frequency relationship.\textsuperscript{13,19} The majority of reports find no change in protein levels of PLB between failing and nonfailing myocardium.\textsuperscript{19,20} Studer et al\textsuperscript{12} found that mRNA and protein levels of NCX were significantly increased in failing myocardium, a result recently confirmed by Flesch et al.\textsuperscript{21} Correspondingly, the level of activity of the NCX is increased in failing compared with nonfailing myocardium.\textsuperscript{22} Because both NCX and SERCA2 act primarily to remove calcium from the cytosol, this increase in NCX activity could theoretically compensate for the reduced SERCA2 activity. It has been observed that epicardial muscle strips from patients in whom NCX is upregulated at the same time that SERCA2 is downregulated have better preserved function than those patients in whom SERCA2 is decreased and NCX unchanged.\textsuperscript{2}

Our data demonstrate that hearts with impaired systolic and diastolic function, as assessed by echocardiography, also demonstrate impaired function in vitro (slow relaxation and poor frequency response). However, the levels of SERCA2 and NCX are significantly different from those seen in end-stage failing myocardium and more closely resemble those seen in normal heart. This is evidence against a link between the alteration in calcium-handling protein levels and loss of the frequency response or impairment of relaxation. Additional support for the possibility of uncoupling the force-frequency relationship from alterations in calcium-handling proteins comes from studies showing an abnormal force-frequency relationship in dilated cardiomyopathy without a decrease in the level of SERCA2 expression but with a reduced SERCA2 Vmax.\textsuperscript{23}

The loss of function of the catecholamine signaling pathway is well known to accompany end-stage heart failure, as seen in the UD group, was suggested by our finding that isolated myocytes were less responsive to the \( \beta \)-agonist isoproterenol. However, \( \beta \)-AR downregulation did not occur, which suggests that it is the signal transduction pathway coupling the receptors to the contractile apparatus that is impaired. Impaired myocardial adenylate cyclase activity from brain-dead organ donors has been reported previously in both adult humans\textsuperscript{24} and pediatric animal models.\textsuperscript{25} In the present study, myocardial samples from the UD group had significantly elevated levels of \( G_m \) activity compared with normal myocardium, with values comparable to those found in end-stage heart failure. It is likely that this accounts for the decrease in \( \beta \)-AR sensitivity.

Increased \( G_m \) activity may also provide a link to explain decreased function in the presence of normal SERCA2 levels. Exposure of rats to isoproterenol causes an increase in \( G_m \),\textsuperscript{26} and treatment of neonatal myocyte cultures with norepinephrine both decreases basal cAMP and increases \( G_m \).\textsuperscript{27} Hence, increasing \( G_m \) may act to reduce the tonic activity of SERCA2 by decreasing basal cAMP. It may be that \( G_m \) upregulation is an acute response in heart failure, followed in the long term by downregulation in \( \beta \)-AR density.

It could also be considered that \( G_m \) plays a more fundamental role in the induction of heart failure. A provisional study\textsuperscript{28} reports that transgenic mice with increased \( G_m \) levels develop a form of dilated cardiomyopathy. Of particular relevance to the present study is the finding that \( G_m \) levels were elevated by 225\% in patients who died of “catecholamine refractory” cardiogenic shock after myocardial infarction.\textsuperscript{3} It may therefore be that a similar pathophysiological mechanism is responsible for both cardiogenic shock and brain-death–induced acute heart failure and that the 2 conditions are, in clinical terms, the same entity.

Conclusions

We have demonstrated that myocardial dysfunction seen in unused donor hearts is associated with significantly elevated levels of the inhibitory \( G \) protein \( G_m \). Although the function of myocardial tissue from these subjects was impaired, protein levels of SERCA2 and NCX and density of \( \beta \)-ARs were distinctly different from failing hearts and resembled those of nonfailing tissue. The increase in \( G_m \) could play a central role not only in \( \beta \)-AR desensitization but also in fundamental changes in muscle contractility. These observations, coupled with our previous finding that inactivation of \( G_m \) with pertussis toxin restores \( \beta \)-AR function in myocytes from human hearts with end-stage failure,\textsuperscript{29} may be valuable in the design of therapeutic strategies to restore myocardial function in these patients and so increase the volume of this valuable resource.

Acknowledgments

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References


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