Age-Related Changes in β-Adrenergic Neuroeffector Systems in the Human Heart

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Background Aging decreases cardiac β-adrenergic responsiveness in model systems and in humans in vivo. The purpose of this study was to comprehensively evaluate the age-related changes in the β-receptor-G protein-adenyl cyclase complex in nonfailing human hearts.

Methods and Results Twenty-six nonfailing explanted human hearts aged 1 to 71 years were obtained from organ donors and subjected to pharmacological investigation of β-adrenergic neuroeffector systems. The population was subdivided into the 13 youngest and 13 oldest subjects, total β-receptor density assessed by maximum [125I]ICYP binding (Bmax) was reduced in older hearts by 37% in left ventricles and 31% in right ventricles (both P<.05), and the downregulation was confined to the β1 subtype (r=.78 left ventricle β1 density versus donor age). Older donor hearts exhibited a 2- to 4-fold rightward shift of ICYP-isoproterenol (ISO) competition curves and demonstrated 43% fewer receptors in a high-affinity agonist binding state (P<.05). Older hearts exhibited decreased adenylyl cyclase stimulation by ISO, by forskolin (β1-agonist), and by the G protein-sensitive probes Gpp(NH)p and NaF. In contrast, there was no change in response to manganese, a specific activator of the adenylyl cyclase catalytic unit. Toxin-catalyzed ADP ribosylation in membranes prepared from older versus younger hearts revealed a 29% to 30% reduction (P<.05) with chologer toxin (G1) but no difference with pertussis toxin (G2). The systolic contractile response of isolated right ventricular trabeculae to ISO was decreased by 46%, with a 10-fold increase in ISO Emax in older relative to younger donor hearts.

Conclusions There is a profound decrease in cardiac β-adrenergic responsiveness with aging. This occurs by multiple mechanisms including downregulation and decreased agonist binding of β1-receptors, uncoupling of β-adrenoceptors, and abnormal G protein-mediated signal transduction. (Circulation. 1994;90:1225-1238.)

Key Words • aging • receptors, adrenergic, beta • proteins • myocardium

A consistent across-species effect of age on cardiac function is the diminished response of atrial and myocardial tissues to catecholamines.1-5 Although age-related decreases in myocardial β-adrenergic receptor responsiveness have been described in several animal systems and in humans, the basis for β-adrenergic subsensitivity of aging myocardium has not been completely elucidated. This is particularly true for the human heart, where species uniqueness of adrenergic neuroeffector mechanisms may preclude direct transfer of information obtained in laboratory animal models.6,7

Previous studies in rat models have failed to reveal consistent age-related changes in cardiac β-adrenergic receptor density or agonist affinity,6-8 but have revealed a decrease in maximum adenylyl cyclase stimulation in response to the β-agonist isoproterenol9-11 and the G protein/adenyl cyclase agonists Gpp(NH)p, sodium fluoride, and forskolin.12-14 These results suggest an age-related decrease in the functional activity of the stimulatory G protein (G1) or the adenylyl cyclase catalytic unit. The available human data have been obtained from biochemical studies of peripheral lymphocytes and in vivo isoproterenol challenges. Most of the lymphocyte studies have not reported a reduction in total β-receptor density with aging,15-18 although other investigations found a decrease in high-affinity agonist binding sites19 and decreased adenylyl cyclase production in response to isoproterenol,20,21 salbutamol,22 prostaglandin E1,23 and sodium fluoride.24

There are several limitations to these studies. Rat myocardium is predominantly an α1-adrenergic system (α1: β ratio >2:1) and contains virtually no β1-receptors6,7,22; the nonfailing human heart is a β-adrenergic system (α1: β ratio <1:5) that contains 20% to 25% β1-receptors,7 which are functionally coupled to heart rate and inotropic activity.26,27 Although it has been postulated that peripheral lymphocyte β-receptors may reflect alterations in β-receptor density and overall β-adrenergic responsiveness of less-accessible target tissues like the heart or the lungs,28,29 human lymphocytes do not contain β1-receptors and are therefore not a reliable surrogate system for the heart.30 Lymphocyte β-adrenergic receptors are not innervated, and this leads to a lack of concordance between behavior of myocardial and lymphocyte β-receptors in disease conditions manifested by increased adrenergic neurotransmitter activity30-32 or during β1-receptor specific therapeutic interventions.30,32,33
The purpose of this study was to comprehensively evaluate age-related changes in β-adrenergic neuroeffector systems in the explanted nonfailing human heart. We postulated that increased adrenergic neurotransmitter activity accompanying aging would produce changes in the myocardial β-adrenergic neuroeffector system that are similar to the effects of heart failure, including downregulation of β₁-adrenergic receptors and alterations of G protein function.

Methods

Tissue Procurement

Nonfailing human hearts were obtained from the Stanford, Utah, and Colorado Cardiac Transplant Programs via the Northern California, Intermountain, and Colorado organ procurement agencies. Twenty-six nonfailing hearts were obtained from organ donors excluded from heart donation because of age, body size, or AB blood type incompatibilities. In all cases, hearts were refrigerated (at least two) attempts were made to place these hearts for organ donation using the United Network for Organ Sharing (UNOS). Additionally, previously transplanted and presumably denuerated nonfailing hearts were harvested from nine patients undergoing retransplantation for graft atherosclerosis. In all cases, written consent for organ donation for research purposes was obtained from the subject or from a family member.

None of the organ donors had a known cardiac history, and all hearts were procured locally. In all cases, an echocardiogram obtained as part of the organ donation process revealed normal left ventricular function (defined as a shortening fraction >25%). The donors had been maintained on respirators for 0.5 to 3 days, and they had not been given β-adrenergic agonists other than dopamine. None of the previously transplanted patients received dopamine, other β-agonists or β-blocking agents before retransplantation. The explanted hearts were rapidly removed and immediately immersed in ice-cold, oxygenated physiological salt solution. The time between removal and homogenization was less than 30 minutes in all cases.

Biochemical Studies

Receptor Binding

Aliquots (5 to 10 g) of left and right ventricular free wall were dissected free of epicardial fat and endocardial tissue and placed in 10 mmol/L Tris, 1 mmol/L EGTA buffer, pH 8.0. The tissue was finely minced with scissors and homogenized with a Polytron tissue homogenizer (Brinkmann Instruments) using three consecutive bursts. A crude membrane fraction was made by extracting the contractile proteins in 0.5 mol/L KCl and repeatedly washing a 50 000 g pellet. Membranes were stored at −70°C in buffer of 250 mmol/L sucrose, 50 mmol/L Tris, 1 mmol/L EGTA, pH 7.5, at protein concentration of 5 to 10 mg/mL. Relation to the initial homogenate recovery of β-adrenergic receptors was 90.4±7.8% (n=5) in this crude membrane preparation. Membrane yield was assessed by measuring the Mn²⁺ response of adenyl cyclase in the extracted membrane preparations.

For receptor assays, the total population of β-adrenergic receptors was radiolabeled with [³²P]ICYP as previously described. Briefly, seven increasing concentrations of ICYP between 3.125 and 150 pmol/L in the presence or absence of 1 μmol/L (-)propranolol were used to construct the specific binding curve. The assays were performed within 2 months of tissue procurement in a buffer of 150 mmol/L NaCl, 20 mmol/L Tris, 1 mmol/L ascorbate, pH 7.5, at 30°C. Maximum binding (Bmax) and dissociation constant (Kd) were determined by nonlinear least-squares fit of the specific binding curve as previously described. The percentages of β₁-receptors and β₂-receptors were determined by computer modeling of ICYP binding data to mathematical models which fit the data of [³²P]ICYP to nonlabeled ICYP competition curves using either 50 or 100 pmol/L ICYP to maintain the radioligand concentration at 3 to 5 × Kd.

The ability of isoprenaline to displace ICYP from human myocardial β₁- and β₂-receptors was assessed by ICYP competition curves using 50 or 100 pmol/L ICYP to maintain the radioligand concentration at 3 to 5 × Kd. Twenty point curves between (−)isoprenaline concentrations of 10 pmol/L and 1 mmol/L were measured under steady-state conditions. Competition curve slope, IC₅₀, percentage of receptors in a high (Kₐ) or low (Kₘ) affinity state, and Kₐ and Kₘ were determined by computer modeling.

Adenyl Cyclase Stimulation

A gently prepared particulate fraction that yields maximal hormone-stimulated adenyl cyclase activity was prepared as previously described. Relation to the initial homogenate recovery of β-adrenergic receptors was 63.1±7.5% (n=5) in this membrane preparation. Two gram aliquots of left and right ventricular myocardium were removed and homogenized in sucrose/Tris buffer. The 1085 g pellet was washed twice and resuspended to a final concentration of 8 to 12 mg/mL and frozen at −80°C until used.

Adenyl cyclase assays were performed as previously described. Myocardial membranes (0.05 to 0.125 mg per tube) were suspended in 100 mmol/L Tris buffer, pH 7.3, at 30°C and exposed to the agonist drug of choice. The standard reaction mixture included 0.1 mmol/L MgCl₂, with 0.5 mmol/L MgCl₂ in excess, 10−⁵ mol/L ATP, 10 mmol/L phosphocreatine, 1 mmol/L eAMP, and 1.75 U creatine kinase per incubation tube. A second assay condition consisted of the above mixture minus ATP plus 10⁻³ mol/L (−)propranolol [Gpp(NH)p (guanyl imidodiphosphate) condition]. A third assay was used to assess Mn²⁺ stimulation and consisted of the Gpp(NH)p condition minus MgCl₂ (Mn²⁺ condition). Recovery was determined by trace labeling with H-eAMP, and the reaction was started by trace labeling with [³²P]-e-AMP. Newly formed [³²P]-e-AMP was recovered by the technique of Salomon.

Column recovery routinely was 70% to 80%, with assay blanks <0.003% of added e-AMP.

Toxin-Catalyzed ADP Ribosylation

G protein substrates of pertussis toxin were radiolabeled using pertussis toxin–catalyzed incorporation of [³²P]ADP-ribose as previously described. In brief, 50 μmol/L of the particulate fraction described for the adenyl cyclase assay was incubated for 90 minutes at 30°C in 100 μL of 100 mmol/L Tris-Cl (pH 8.0) containing 6 mmol/L MgCl₂, 2 mmol/L GTP, 10 mmol/L thymidine, 2.5 mmol/L ATP, 10 mmol/L isoniazid, 10 μmol/L [³²P]NAD (10 Ci/mmol), and activated pertussis toxin. Pertussis toxin was activated by dialyzing with 10 mmol/L Tris-Cl (pH 8.0) for 1 hour at 4°C and then incubating in 100 mmol/L dithiothreitol (DTT) for 30 minutes at room temperature. The reaction was stopped by centrifugation at 15 000 g for 5 minutes and washing the pellet with 50 mmol/L of ice-cold Tris-Cl (pH 8.0) containing 5% (wt/vol) sucrose, 6 mmol/L MgCl₂, 1 mmol/L EDTA, and 1 mmol/L DTT.

G protein substrates of cholera toxin were radiolabeled by incubating 100 μmol/L of membranes for 90 minutes at 30°C in 100 mmol/L KPO₄ buffer (pH 7.0) containing 2 mmol/L GTP, 2.5 mmol/L ATP, 20 mmol/L thymidine, 20 mmol/L arginine, 10 μmol α-protein, 10 μmol/L [³²P]NAD (20 Ci/mmol), and 5 μg activated cholera toxin. Cholera toxin was activated by incubating in 100 mmol/L DTT for 30 minutes at 30°C. The reaction was stopped as in the pertussis toxin–catalyzed reactions, and the membrane pellets were resuspended in 50 μL of a solution containing 62.5 mmol/L Tris-Cl (pH 6.8), 2% SDS, 10% glycerol, and 5 μg mercaptoethanol. Proteins were electrophoretically separated on a 7.5% SDS-polyacrylamide gel. Gels were dried on cellophane and imaged by exposure to betaxolol or ICYP-CGP 207012A competition curves using either 50 or 100 pmol/L ICYP to maintain the radioligand concentration at 3 to 5 × Kd.
Kodak XAR-5 film with an intensifying screen at −70°C for 24 to 72 hours.

The signal intensity of the 45-kD and 52-kD appropriate bands on the autoradiograms was analyzed using a computer-assisted, two-dimensional densitometer (Loaies). Autoradiographic measurements were corrected for total protein in each lane by using the Coomassie blue-stained proteins on the same gels. Four randomly selected Coomassie blue-stained protein bands were quantified using two-dimensional transmission densitometry as previously described. The levels of the ADP-ribosylated proteins in membranes from younger versus older hearts were then calculated as a percentage of the mean of the younger. Because each sample was assayed multiple times in separate experiments and the values for each of these measurements were averaged, the mean of the younger controls may not equal 100%.

Immunochromic Measurement of Gα

The α-subunit of Gα, the “stimulatory” G protein, was measured in the above particulate fraction by Western blotting. Briefly, 1 mL of the frozen (−70°C) human left ventricular cardiac membrane preparation was thawed by dilution with 1 mL of HME buffer (10 mmol/L HEPES, pH 7.2, 5 mmol/L MgCl2, 1 mmol/L EDTA) containing 40 μL of a protease inhibitor cocktail (aprotinin and leupeptin, each 10 μg/mL) plus benzamidine and bacitracin (each 100 μg/mL). The thawed suspension was centrifuged in a motorized Potter-Elvehjem homogenizer in 2 mL of a storage buffer (20 mmol/L Tris, pH 7.4, 0.1% Triton X-100, 5 mmol/L β-mercaptoethanol) and filtered through four layers of sterile gauze prewet with the storage buffer and then stored in 100-μL aliquots at −70°C. The protein concentration was determined by Lowry43 versus a BSA standard curve.

For subsequent Western analysis, an aliquot was thawed and diluted 1:1 in 2× Laemmli loading buffer (4% SDS, 0.1 mol/L Tris, pH 6.8, 20% glycerol, 2% β-mercaptoethanol, bromophenol blue), and aliquots of 10 or 30 μg of membrane protein (3 to 16 μL) were loaded onto a 10% polyacrylamide minigel (7×8 cm). Electrophoresis was at a constant current of 30 mA was continued until the bromophenol blue tracking dye just ran off the end of the resolving gel. Resolved proteins were transferred to nylon membranes (Ran-Free, Schleicher & Schuell) by electroblotting (Hoefer Scientific) for 2 hours at a constant current of 200 mA. Membranes were blocked overnight in 5% nonfat dry milk dissolved in a phosphate buffered saline containing 0.1% Tween 20 (PBS-T) at room temperature.

Blots were removed from the blocking solution and washed in PBS-T (1×5 minutes+4×10 minutes). Primary antibody binding was accomplished using a rabbit anti-Gα (Calbiochem) antibody at a 1:1000 dilution in PBS-T. Blots were incubated for 2 hours at room temperature with constant agitation, then washed as before in PBS-T. Blots were then incubated in a 1:2000 dilution of the goat-anti-rabbit/horse-radish peroxidase–conjugated secondary antibody (Jackson Immunoresearch Laboratories, Inc) for 1.5 hours at room temperature with constant agitation and then thoroughly washed (1×5 minutes+6×10 minutes).

Detection of Gα was accomplished by an enhanced chemiluminescent method (ECL, Amersham) and the results recorded by exposure of film (X-Omat AR, Kodak). The 45-kD splice form of human recombinant Gα was obtained from Thomas W. Gettys (Medical College of South Carolina). The particulate left ventricular membrane fraction was loaded at a protein concentration within the linear range of quantitative densitometry for both the 52-kD and 45-kD splice forms.

Gα protein levels between the two groups were compared after the film was analyzed by densitometry. Samples from younger and older left ventricles were run on the same gel. The average densitometry value for the younger preparations run on each gel was then taken as the normalized control value for that blot, and each individual younger and older sample was calculated as a percentage of that control value.

Tissue Catecholamines and Neuropeptide Y

Aliquots of left and right ventricular free wall suitable for tissue catecholamine and neuropeptide Y (NPY) measurements were immediately frozen at −80°C. For catecholamine measurements, 100 to 200 mg of tissue was homogenized within 2 weeks of procurement in 0.4N HCL containing 5 mmol/L glutathione using 1 mL/100 mg tissue. Homogenization was performed by three consecutive bursts with a Polytron tissue homogenizer and then 20 passes through a motor-driven mortar and pestle. This material was centrifuged at 1500×g 15 minutes, and the supernatant was removed and frozen at −70°C until used. For NPY measurements, 25- to 75-mg aliquots of tissue were thawed, minced, and boiled in 0.01N HCl for 10 minutes and homogenized.

Tissue norepinephrine, dopamine, and epinephrine levels were measured radioenzymatically using kits obtained from Amersham (Cat-a-Kit). Results were expressed as nanograms of catecholamine per gram of original tissue wet weight. NPY tissue levels were measured by radioimmunoassay (RIA). One half of the homogenized aliquot was used for protein measurement; the other half was used for RIA. The lyophilized tissue samples were reconstituted in 250 mL of PBS plus Triton buffer. After centrifugation, the supernatant (200 μL) was separated into plastic tubes to which specific antiserum (100 μL at 1:160 000 dilution) was added. The assay tubes together with a series of standard NPY dilutions were then incubated for 96 hours at 4°C. After incubation, dextran-coated charcoal was added to each tube, and the resultant mixture was centrifuged for 30 minutes at 4°C. The amount of NPY in each tube, expressed as picograms per tube, was calculated from the bound-to-reference ratio. The results were expressed as picogram per milligram of homogenate protein.

Protein and Creatine Kinase Measurements

The protein concentration for calculation of β-receptor density and NPY were determined by the Peterson modification of the Lowry method. The protein concentration for calculation of adenyl cyclase was measured by the method of Lowry.

Soluble creatine kinase activity was measured in the supernatant of the 1085g centrifugation used to process a particulate fraction of adenyl cyclase assays by a spectrophotometric technique.

Contractile Response of Isolated Right Ventricular Trabeculae

The contractile response of isolated human cardiac preparations was assessed as previously described. Briefly, trabeculae of uniform size (1 to 2×6 to 8 mm) were mounted in an eight-chamber muscle bath containing Tyrode's buffer bubbled with 95% O2/5% CO2. After equilibration, tension was applied to achieve maximal contraction (about 1 g), and the trabeculae were field stimulated by a 5-millisecond pulse at 10% above threshold. After a 2-hour equilibration period, full dose-response curves to isoproterenol were performed using 0.3 or 0.5 log unit dose increments between 10 nmol/L and 10 μmol/L. After completion of the isoproterenol dose-response curve and washout of isoproterenol, the maximal response to calcium was measured by administering calcium chloride at a final concentration of 2.5 mmol/L, 5 mmol/L, and then 10 mmol/L. Tension was recorded as the stimulated tension minus baseline tension, and the maximum response was taken as the greatest amount of net tension produced at any point in the dose-response curve. The concentration of isoproterenol that produced 50% of the maximum developed tension (EC50), the maximum muscular tension and percentage of response at each dose of isoproterenol (percentage of maximum re-
TABLE 1. Clinical Characteristics of Nonfailing Organ Donors, Retransplant Patients, and Donors Used for the Previous Transplant in Retransplant Patients

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th>Older</th>
<th>Retransplanted Recipients</th>
<th>Retransplant Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=13)</td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>Age, y</td>
<td>20±2.9</td>
<td>50.4±2.8</td>
<td>49.7±5.0</td>
<td>28.8±3.1</td>
</tr>
<tr>
<td></td>
<td>(1-35)</td>
<td>(37-71)</td>
<td>(24-66)</td>
<td>(19-46)</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>8/5</td>
<td>8/5</td>
<td>1/8</td>
<td>5/4</td>
</tr>
<tr>
<td>Number of patients given dopamine, %</td>
<td>7(54)</td>
<td>7(54)</td>
<td>0</td>
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</tr>
<tr>
<td>Dose of dopamine, µg/kg per minute</td>
<td>6.5±3.0</td>
<td>5.0±2.1</td>
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<td>...</td>
</tr>
<tr>
<td></td>
<td>(0-40)</td>
<td>(0-25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of dopamine, h</td>
<td>5.0±2.3</td>
<td>4.1±2.0</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>(0-25)</td>
<td>(0-24)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SEM. Values in parentheses are range or number of observations.

Drugs and Chemicals

All drugs and chemicals were obtained from commercially available sources using biochemical or molecular biology grade material.

Statistical Analysis

The data analysis was performed with use of SYSTAT/SYGRAPH Software (Systat Inc). The different groups were compared by unpaired t test and linear regression analysis. For the muscle bath data, the maximum contractile response was computed for each trabecula and analyzed as described above. For muscle bath and adenyl cyclase data, the EC50s were determined by nonlinear computer modeling with use of the GRAPHPAD/INPLOT 4.1 software (Graphpad Software). The ICYP-isoproterenol competition curves were analyzed by a nonlinear least-squares curve fitting procedure. All probability values of ≤5% were considered statistically significant. All values are expressed as mean±SEM.

Results

The clinical characteristics of the donor population are presented in Table 1. The study population was divided into younger and older groups, based on the median age of the overall study population (37 years). Thirteen donors were less than 37 years of age (younger group), and 13 donors were 37 years of age or greater (older group). There were eight female and five male subjects in the younger donor group and eight female and five male subjects in the older donor group. Seven younger donors and seven older donors received dopamine before organ donation. The dose and duration of dopamine exposure did not differ in the two groups (Table 1).

The clinical characteristics of the eight male subjects and one female subject who comprised the cardiac retransplant population are also illustrated in Table 1. The mean age of the recipients at the time of the second transplantation was 49.7±5.0 years, and the mean age of the donors used for the previous transplant was 26.6±3.3 years. The age of the previous donor plus the duration of the transplant (24.6±4.2 months) gave the age of the heart removed from the retransplant patients, 28.8±3.1 years (range, 19 to 46 years). The mean ejection fraction of the previously transplanted hearts was 55±4%, measured by radionuclide angiography less than 2 months before retransplantation.

β-Adrenergic Receptor Density in Innervated (Donor) and Denervated (Previously Transplanted) Human Hearts

Total β-adrenergic receptor density and β1 and β2 subpopulation characteristics are illustrated in Fig 1. The younger group had higher total β-receptor (βtot) and β1 subpopulation densities compared with the older group. The β1tot was 108±8.3 fmol/mg in younger versus 67.8±3.3 fmol/mg in older left ventricles and 113.9±8.6 fmol/mg (younger) versus 79.1±7.7 fmol/mg (older) in right ventricles (both P<.05). Similarly, the β1-receptor density was 84.2±6.2 fmol/mg (younger) versus 48.7±2.3 fmol/mg (older) donors for left ventricles and 94.1±8.4 fmol/mg (younger) versus 62±6.4 fmol/mg (older) for right ventricles. The relation between β1-receptor density and donor age is illustrated in Fig 2, where a significant inverse correlation (r = −.78, P<.0001) is evident.

In contrast, there were no age-related changes in β2-receptor density in the donor ventricles (Fig 1). The β2-receptor density was 23.7±2.8 fmol/mg for younger and 18.6±2.0 fmol/mg for older left ventricles and 17.6±3.5 (younger) versus 17.6±2.5 fmol/mg (older) for the right ventricles. No significant differences in β-receptor subpop-

![Graph](image-url)
ulations between the left and right ventricles were observed, although there was a trend for higher \( \beta_1 \)-adrenergic receptor density in right ventricle preparations consistent with our previous observations.\(^{27}\) Finally, we did not observe any significant changes in antagonist affinity (ICYP K\(_h\)) between the two groups in either ventricle (13.2±2.3 versus 14.5±3.7 pmol/L for younger and older groups for left ventricle and 12.6±2.6 versus 15.8±3.7 pmol/L for right ventricle). Membrane yield, assessed by Mn\(^{2+}\) stimulation of adenyl cyclase in membranes prepared from older and younger ventricles, was not different between the two groups (left ventricle, 114±25 pmol/L cAMP/min per milligram [younger] versus 114±29 [older]; right ventricle, 117±21 [younger] versus 131±23 [older]).

The previously transplanted hearts had a marked decrease in myocardial norepinephrine and dopamine content, confirming denervation of at least a majority of adrenergic neurons. In previously transplanted left ventricles, the myocardial norepinephrine content was 63±21 ng/g wet wt (\( P < .05 \) versus all donor left ventricles shown in Table 2), and the dopamine content was 20±9 ng/g wet wt (\( P < .05 \) versus donor left ventricles).

\( \beta_1 \)-adrenergic receptor data from previously transplanted, denervated hearts are illustrated in Figs 3 and 4. The mean \( \beta_1 \)-receptor density for these nine denervated hearts was 65.9±8.4 fmol/mg. There was no correlation between \( \beta_1 \)-receptor density and recipient age (Fig 3) or age of the heart on explantation (Fig 4). This contrasts with the significant inverse correlation observed in Fig 2 for the innervated, nonfailing hearts.

125\(^{I}\)ICYP-Isoproterenol Competition Curves

The results of 125\(^{I}\)ICYP-isoproterenol competition curves are presented in Table 3 and Fig 5. Computer modeling of these curves for both left and right ventricles exhibited shallow slope factors ("pseudo-Hill" coefficient <1, \( P < .05 \) versus unity), consistent with more than one population of binding sites. Only one ICYP-isoproterenol competition curve from the younger group modeled significantly better for a one-site fit in the left ventricle. However, four older ventricles exhibited a one-site fit, with the binding sites falling in a low-affinity class (K\(_s\) >50 nmol/L). Accordingly, in left ventricular membranes, the younger group exhibited a significantly (\( P < .05 \)) higher percentage of receptors in a high-affinity state (%K\(_h\)=40±7% [younger] versus 21±4% [older]) and a lower percentage of receptors in a low-affinity state (%K\(_l\)=59±6% [younger] versus 76±4.2% [older]). Similarly, in younger donor right ventricle preparations, there was a trend toward a higher percentage of K\(_{r}\) (34±6% [younger] versus 18±5% [older], \( P = .06 \)) and lower percentage of K\(_{r}\) (70±4.7% [younger] versus 77±5.4% [older], \( P = .20 \)). The K\(_{r}\) value was similar in the younger and older ventricles. However, there was a trend for a higher K\(_{r}\) value in older ventricles that was more apparent in the right ventricle (410±189 nmol/L [younger] versus 1110±426 nmol/L [older], \( P = .10 \)).

Age-related changes in the percentages of K\(_{r}\) and K\(_{r}\) for left ventricle membrane preparations are illustrated in Fig 5. There was a significant inverse relation between the percentage of K\(_{r}\) and donor age (\( y = -0.76x + 59.3; \)

![Fig 2. Scatterplot: Left ventricular \( \beta_1 \)-adrenergic receptor density in relation to age for nonfailing donor hearts.](image)

![Fig 3. Scatterplot: Left ventricular \( \beta_1 \)-adrenergic receptor density in relation to recipient age in nine previously transplanted hearts.](image)

**Table 2. Myocardial Catecholamines, Neuropeptide Y, and Soluble Creatine Kinase Activity**

<table>
<thead>
<tr>
<th></th>
<th>Younger (n)</th>
<th></th>
<th></th>
<th>Older (n)</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
<td>LV+RV</td>
<td>LV</td>
<td>RV</td>
<td>LV+RV</td>
</tr>
<tr>
<td>Norepinephrine, ( \mu )g/g wet wt</td>
<td>728±211 (10)</td>
<td>1237±260 (10)</td>
<td>982±173</td>
<td>752±150 (10)</td>
<td>706±129 (10)</td>
<td>729±97</td>
</tr>
<tr>
<td>Dopamine, ( \mu )g/g wet wt</td>
<td>363±105 (10)</td>
<td>527±163 (10)</td>
<td>445±96</td>
<td>418±120 (11)</td>
<td>520±130 (12)</td>
<td>470±88</td>
</tr>
<tr>
<td>Epinephrine, ( \mu )g/g wet wt</td>
<td>86±39 (10)</td>
<td>118±58 (10)</td>
<td>102±34</td>
<td>112±36 (12)</td>
<td>88±17 (12)</td>
<td>100±20</td>
</tr>
<tr>
<td>Dopamine/norepinephrine ratio</td>
<td>0.96±0.38 (10)</td>
<td>0.62±0.24 (10)</td>
<td>0.79±0.22</td>
<td>0.88±0.24 (11)</td>
<td>0.93±0.21 (10)</td>
<td>0.90±0.16</td>
</tr>
<tr>
<td>Neuropeptide Y, pg/mg protein</td>
<td>73±13 (6)</td>
<td>65±12 (6)</td>
<td>69±9</td>
<td>83±13 (6)</td>
<td>110±22 (6)</td>
<td>97±13*</td>
</tr>
<tr>
<td>Creatine kinase, IU/g wet wt</td>
<td>944±75 (12)</td>
<td>994±102 (11)</td>
<td>968±61</td>
<td>1178±85 (11)</td>
<td>960±70 (12)</td>
<td>1069±60</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; RV, right ventricle.

Data are for left and right ventricles combined; *\( P = .08 \) younger vs older donors. LV+RV is the LV and RV data grouped together.
There was a positive correlation between the percentage of $K_i$ and donor age ($y=0.73x+40; r=0.66, P<.0001$).

**Adenylyl Cyclase Data**

Basal activity and net maximum adenylyl cyclase stimulation in response to various agonists are presented in Table 4. There was a trend toward lower basal adenylyl cyclase activity in older right ventricle preparations, which reached statistical significance when the left and the right ventricles were combined for analysis. Under standard assay conditions, older left ventricles exhibited significantly lower maximum adenylyl cyclase stimulation in response to the nonselective $\beta$-agonist isoproterenol, the selective $\beta_1$-agonist zinterol, and the adenylyl cyclase catalytic activator forskolin. Isoproterenol- and zinterol-mediated adenylyl cyclase maximal activities were respectively 33% and 48% lower in older versus younger left ventricles. There were similar respective decreases of 24% and 37% in isoproterenol- and zinterol-mediated adenylyl cyclase maximum stimulation in older right ventricle preparations, which were of borderline statistical significance. When the left and right ventricles were pooled for analysis, older ventricles exhibited respective 30% and 43% decreases in isoproterenol- and zinterol-mediated adenylyl cyclase maximum stimulation (both $P<.05$ versus younger ventricles). The significant inverse relations between isoproterenol- and zinterol-mediated adenylyl cyclase maximum stimulation and donor age are illustrated in Fig 6.

In contrast to reduced maximal stimulation of adenylyl cyclase to either isoproterenol or zinterol, increasing age had no effect on $EC_{50}$ values ($P=NS$ by regression analysis; isoproterenol $EC_{50}=1.6 \pm 1.0 \times 10^{-6}$ mol/L left ventricle [younger] versus $6.0 \pm 2.6 \times 10^{-7}$ mol/L left ventricle [older], 2.1 $\pm 1.5 \times 10^{-6}$ mol/L right ventricle [younger] versus 1.7 $\pm 0.6 \times 10^{-6}$ right ventricle [older]; zinterol also $P=NS$ between younger and older groups in left and right ventricles).

Because adenylyl cyclase stimulation was assessed in a lower-yield particulate fraction that was prepared differently than the high-yield membrane fraction used to measure $\beta$-adrenergic receptors, we measured $\beta$-adrenergic receptors in the cyclase preparations of a subset of 17 hearts. As for the high-yield membrane fraction, total $\beta$-receptor density was decreased in the nine older versus eight younger left ventricles (respectively 66.4 $\pm 3.1$ versus 88.4 $\pm 9.4$ fmol/mg, $P<.05$). As for the higher-yield membrane preparation, downregulation of the total pool of $\beta$-adrenergic receptors was confined to loss of $\beta_1$-receptors ($\beta_1$, 48.9 $\pm 3.1$ versus 70.2 $\pm 4.7$ fmol/mg, $P<.05$; $\beta_2$, 17.5 $\pm 1.6$ versus 17.6 $\pm 2.0$, $P=NS$).

The older ventricles exhibited significantly less adenylyl cyclase stimulation in response to forskolin, an adenylyl cyclase catalytic activator whose activity may be influenced by G proteins ($G_i$ and $G_o$) (39). These age-related differences were more apparent for left ventricle membrane preparations, where a significant inverse relation was observed between adenylyl cyclase maximum stimulation by forskolin and donor age ($r=-.56, P=.006$).

The adenylyl cyclase data obtained under Gpp(NH)p and Mn$^{2+}$ assay conditions are also presented in Table 4. For the Gpp(NH)p condition, basal adenylyl cyclase activity was significantly less in older versus younger left ventricle preparations. However, no age-related significant differences were observed in adenylyl cyclase basal activity for right ventricle preparations ($8.7 \pm 1.4$ pmol/L cAMP/min per milligram [younger] versus $6.7 \pm 0.9$ [older], $P=.23$). Older donor hearts exhibited significantly less maximum adenylyl cyclase stimulation in response to NaF. These differences reached statistical significance for left ventricle preparations or when the left and right ventricles were combined for analysis. Moreover, regression analysis of adenylyl cyclase stimulation in response to NaF yielded a significant inverse correlation coefficient for both left and right ventricles ($r=-.64$ [left ventricle], $r=-.48$ [right ventricle]; both $P<.05$). A significant inverse linear relationship was observed between Gpp(NH)p-mediated adenylyl cyclase stimulation and donor age for both the left ($r=-.61, P<.001$) and right ventricles ($r=-.55, P=.012$).
When the catalytic unit of adenyl cyclase was pharmacologically probed by MnCl₂ (Mn²⁺ condition), there were no age-related differences in adenyl cyclase maximum stimulation for either right or left ventricle or when both chambers were combined for analysis (Table 4).

**Toxin-Catalyzed ADP Ribosylation**

Table 5 gives data for toxin-catalyzed ADP ribosylation in particulate fractions prepared from left ventricles of a subset of younger (≤37 years) and older (>37 years) hearts. As previously reported  in particulate fractions of human left ventricle, cholera toxin–catalyzed ADP ribosylation yields two labeled bands at 45 kD and 52 kD. For cholera toxin, there was a 29.4% decrease (P=.02) in the 45-kD band and a 29.8% decrease in the 52-kD band (P=.004) in younger versus older left ventricles. Pertussis toxin–catalyzed ADP ribosylation yielded labeling of a major band at 40 kD, and the intensity did not differ in younger versus older hearts.

**Measurement of G,Gα by Immunoblots**

Because functional data from adenyl cyclase assays and toxin-catalyzed ADP ribosylation experiments indicated decreased activity of G,Gα, we measured the amount of G,Gα protein by Western blotting. Fig 7 (upper) is an immunoblot that demonstrates labeling by the G,Gα antibody of a predominant 52-kD and a smaller amount of the 45-kD protein in left ventricular particulate fractions. The upper panel of Fig 7 includes a recombinant standard that contains only the 45-kD splice form

### Table 4. Adenyl Cyclase Data as Net Maximum Stimulation (Agonist Stimulation Minus Basal Activity, pmol/L cAMP/min per milligram) Under Standard, Gpp(NH)₃p, and Mn²⁺ Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Younger Donors (n)</th>
<th>Older Donors (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
</tr>
<tr>
<td>Standard condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal activity</td>
<td>12.2±0.8</td>
<td>12.7±1.0</td>
</tr>
<tr>
<td>(12) (10) (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>40.4±3.9</td>
<td>36.4±5.4</td>
</tr>
<tr>
<td>(12) (10) (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinterol</td>
<td>14.2±2.4</td>
<td>11.6±1.9</td>
</tr>
<tr>
<td>(10) (10) (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forskolin</td>
<td>308±19</td>
<td>262±14</td>
</tr>
<tr>
<td>(12) (10) (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gpp(NH)₃p condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gpp(NH)₃, 10⁻⁴ mol/L</td>
<td>20.5±3.4</td>
<td>16.6±2.5</td>
</tr>
<tr>
<td>(9) (8) (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaF, 10⁻² mol/L</td>
<td>66.5±8.1</td>
<td>57.4±15.7</td>
</tr>
<tr>
<td>(10) (8) (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn²⁺ condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MnCl₂, 10⁻² mol/L</td>
<td>42.9±10</td>
<td>51.4±7</td>
</tr>
<tr>
<td>(5) (6) (11) (10) (8) (18)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LV indicates left ventricle; RV, right ventricle.

*P<.05, †P<.10 younger vs older donors for respective chamber. LV+RV is the LV and RV data grouped together. See "Methods" for details of assay conditions.
as well as human red blood cell membranes containing the 45-kD protein. Fig 7 (lower) indicates that both the 52-kD and 45-kD labeling can be competed away by preincubation with the specific peptide sequence to which the antibody was raised.45 Table 5 gives quantitative data for younger and older preparations from gels loaded with protein amounts (10 \( \mu \)g for the 52-kD and 30 \( \mu \)g for the 45-kD proteins), which gave signals within the linear range of detection. As can be observed, there is no decrease in the amount of \( \mathrm{G}_{\alpha} \) in older preparations as measured by immunoblotting; in fact, there is a tendency for the older left ventricles to have a greater amount of labeling of both the 52-kD and 45-kD bands.

**Myocardial Catecholamines, Neuropeptide Y, and Creatine Kinase**

Table 2 gives tissue catecholamines, NPY, and soluble creatine kinase data. The younger and older groups had similar myocardial levels of norepinephrine, dopamine, and epinephrine. The older hearts exhibited a trend toward higher levels of NPY. This trend was restricted to right ventricle preparations (65±12 pg/mg protein [younger], \( n=6 \), versus 111±22 [older], \( n=6; P=.09 \)). Younger and older hearts exhibited similar levels of creatine kinase and similar dopamine to norepinephrine ratios.

**Contractile Response of Isolated Right Ventricular Trabeculae**

Fig 8 illustrates the maximum muscle contraction of isolated right ventricular trabeculae in response to isoproterenol and calcium. An average of 2.5 trabeculae per heart was examined. The maximum tension response to isoproterenol was 23.8±3.3 millinewtons (mN) (n=28 trabeculae) in the younger group versus 12.9±2.0 mN (n=32) in the older group (\( P<.05 \)). In response to calcium, trabeculae for the younger donors developed 11.7±2.2 mN in maximum tension versus 8.4±1.3 mN for the older hearts (\( P=.23 \)). The ratio of the response of isoproterenol divided by the response to calcium (Iso/Ca\(^{2+} \) ratio) was 2.09±0.19 in younger versus 1.27±0.20 in older hearts (\( P=.01 \)).

By regression analysis, the maximum response to isoproterenol was inversely related to age (\( r=-.42, P<.05 \)), as was the Iso/Ca\(^{2+} \) ratio (\( r=-.54, P<.05 \)). In contrast, the response to Ca\(^{2+} \) was not related to age (\( r=.14, P=\text{NS} \)). Complete dose-response curves to isoproterenol for the younger and older hearts are presented in Figs 9 and 10. The older group exhibited a rightward shift of the isoproterenol dose-response curve, with a 10-fold increase in EC\(_{50} \) (46±10 nmol/L [younger] versus 460±149 nmol/L [older], \( P=.009 \)). This resulted in significantly less maximum developed tension at each concentration of isoproterenol in the older versus younger hearts (Fig 10).

**Discussion**

In this study, we describe age-related changes in the \( \beta \)-adrenergic neuroeffector systems in explanted human hearts. In innervated explanted hearts, there was a gradual decline in \( \beta \)-adrenergic receptor density with age, which was specific for the \( \beta_1 \)-receptor subpopulation. There was no change in \( \beta_2 \)-adrenergic receptor density with age. This age-related change in \( \beta_2 \)-adrenergic receptor density was observed only in innervated ventricles and was not observed in previously transplanted, denervated hearts. Older innervated hearts

![Graph](image_url)

**Fig 6. Scatterplots: Net maximum adenyl cyclase (AC) stimulation by isoproterenol (left) and zinterol (right) for left ventricular myocardial preparations in relation to donor age.**

**Table 5. Toxin-Catalyzed ADP Ribosylation in a Particulate Fraction Prepared From Younger (<37 years) Vs Older (>37 years) Left Ventricles**

<table>
<thead>
<tr>
<th>Group</th>
<th>Pertussis Toxin-Catalyzed ADP Ribosylation (n=8 Younger, 7 Older)</th>
<th>Cholera Toxin-Catalyzed ADP Ribosylation (n=7 Younger, 6 Older)</th>
<th>( \mathrm{G}_{\alpha} ) Ab Labeling (n=8 Younger, 8 Older)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52 kD</td>
<td>45 kD</td>
<td>52 kD</td>
</tr>
<tr>
<td>Younger</td>
<td>101.7±8.2</td>
<td>104.7±8.5</td>
<td>100.0±4.3</td>
</tr>
<tr>
<td>Older</td>
<td>104.0±14.3</td>
<td>71.4±3.8*</td>
<td>73.9±7.6*</td>
</tr>
</tbody>
</table>

ADP ribosylation data are the % incorporation of \(^{32P}\)ADP ribose compared with younger controls; immunoblot data are \( \mathrm{G}_{\alpha} \) antibody labeling of older vs younger controls.

*\( P<.05 \) vs younger; †\( P<.10 \) vs younger.
also exhibited a significantly lower percentage of β-receptors in a high-affinity agonist binding state, indicating reduced agonist affinity in the aging human heart.

Under standard assay conditions, we observed a significant inverse correlation between age and maximum adenyl cyclase stimulation in response to the nonselective β-agonist isoproterenol and the selective β2-agonist zinterol. We also measured a significant age-related decrease in adenyl cyclase stimulation in response to forskolin, which activates adenyl cyclase via a G protein-sensitive effect on the adenyl cyclase catalytic unit. These changes were more apparent and reached statistical significance only in the left ventricle or on combined analysis of left and right ventricles. When adenyl cyclase was stimulated through G proteins by the nonhydrolyzable guanine nucleotide (Gpp[NH]p) or by NaF, there was also a significant age-related decrease in maximum adenyl cyclase activity. In contrast, there was no age-related change in maximum adenyl cyclase stimulation by Mn2+, an adenyl cyclase catalytic unit activator whose action is not influenced by G proteins.

In older explanted hearts, we observed a marked decrease in the maximum tension response to isoproterenol as well as a reduced isoproterenol/Ca2+ ratio, similar to what has recently been reported in isolated human ventricular myocytes. Additionally, the EC50 value for isoproterenol was markedly increased (by 10-fold) with age. Despite these changes in the β-receptor pathway, younger and older hearts had similar myocardial levels of the adrenergic neurotransmitters norepinephrine and NPY and similar levels of dopamine and epinephrine. Younger and older ventricles also exhibited similar levels of creatine kinase and no significant difference in the tension response to calcium, unlike what has been reported in isolated human ven-
tricular myocytes. Thus, indices of adrenergic innervation and the amount of functioning myocardium were not different in younger versus older ventricles.

Since the majority (>70%) of the β-adrenergic receptors in both younger and older hearts were of the β₁ subtype, β₁- and β₂-receptors are similarly coupled to inotropic response, and isoproterenol is a nonselective β agonist, stimulation of muscle contraction by isoproterenol is a β₁-selective response. Because there are few or no spare β-receptors in the human heart, a decrease in isoproterenol maximum stimulation of contraction should be due to the reduced β₁-receptor density. The decrease in maximal tension response to isoproterenol in older hearts (by 46%) was in fact in good agreement with the decrease in β₁-receptor density (by 42%). In contrast, β₂-receptors are more tightly coupled (by 4- to 5-fold) than β₁-receptors to adenylyl cyclase stimulation. Thus, stimulation of adenylyl cyclase by both the nonselective β₁-agonist isoproterenol and the selective β₂-agonist zinterol is β₂-selective. Since in older hearts β₁-receptor density was unchanged and isoproterenol or zinterol maximum stimulation of adenylyl cyclase was decreased by respective values of 30% and 43%, β₂-receptors were mildly uncoupled from pharmacological response. Again, since there are no spare β₂-receptors in the human heart, a reduction in maximum isoproterenol stimulation of adenylyl cyclase should be a direct reflection of the degree of uncoupling when there is no receptor downregulation.

Agonist affinity was assessed from the %Kᵦ, computed from the tension-response curve in the muscle bath system (primarily β₁ response), the %Kᵦ of ICYP-isoproterenol competition curves performed in myocardial membranes (where β₁-receptors are more dense than β₂-receptors), and the EC₅₀ of adenylyl cyclase dose-response curves (primarily β₂ response). Older hearts exhibited a marked rightward shift in the isoproterenol tension response, a reduction in %Kᵦ deduced from ICYP-isoproterenol competition curves, and a rightward shift in adenylyl cyclase dose-response curves to either isoproterenol or zinterol. Accordingly, the age-related reduction in agonist affinity appears to be confined to the β₁-receptor subtype.

There have been mixed reports in model systems of decreases in β-adrenergic receptor density with aging, and a consensus has not emerged in either animal or human leukocyte work. Human lymphocytes do not contain β₁-adrenergic receptors, and it is perhaps not surprising that previous studies in this system failed to report consistent age-related differences in β₁-adrenergic density. A rightward shift in isoproterenol competition curves or a lesser shift by guanine nucleotides has not been reported in a pure or predominantly β₁ system. However, Amerini and coworkers described a significant increase in isoproterenol EC₅₀ in aged rabbit ventricular strips. Thus, the age-related decrease in β₁-receptor agonist affinity deduced from muscle contraction dose-response curves and isoproterenol competition curves is in agreement with previous animal data.

Age-related β₂-receptor subsensitivity has been previously reported in human lymphocytes. In the current study, β₂-receptor uncoupling was documented by decreased maximal isoproterenol- and zinterol-mediated adenylyl cyclase stimulation in older ventricles whose β₂-receptor density was not different from younger controls. However, we failed to observe a significant rightward shift in isoproterenol and zinterol adenylyl cyclase dose-response curves in older hearts. Due to the limited reserve of β₁ or β₂-adrenergic receptors, receptor uncoupling is manifested as decreased maximal responsiveness rather than a right-shifted dose-response curve. Accordingly, our results do not support an age-related decrease in β₂-receptor agonist binding affinity in human heart.

The mild uncoupling of β₂-receptors could be due either to less efficient coupling of receptors to the stimulatory G protein (Gₛ), to enhanced activity of the inhibitory G protein (Gᵢ), or to decreased catalytic...
activity of adenylyl cyclase. An abnormality in $G_i$ is unlikely in aged heart, because in human myocardial preparations increased functional activity of $G_i$ does not typically result in decreased stimulation of adenylyl cyclase by NaF, and $G_i$ activity as assessed by pertussis toxin-catalyzed ADP ribosylation was not different in older versus younger hearts. The decreased responses of older hearts to agents that stimulate adenylyl cyclase via G protein activation (Gpp[NH]p and NaF) or by an activation of the adenylyl cyclase catalytic unit that is influenced by G proteins (forskolin) suggests that the receptor uncoupling and/or the apparent affinity change may be related to altered G protein function and more specifically to decreased functional activity of $G_i$. That the decreased adenylyl cyclase responsiveness to Gpp(NH)p, NaF, and forskolin was due to decreased $G_i$ activity was strongly supported by cholera toxin-catalyzed ADP ribosylation data, where incorporation of $^{[32P]}$ADP-ribose into 45-kD and 52-kD substrates we have previously identified as two splice forms of $G_i$, was decreased by 29% to 30% in preparations from older left ventricles. Moreover, the degree of decrease in cholera toxin-catalyzed ADP ribosylation was similar to the percent reduction in Gpp(NH)p, NaF, or forskolin stimulation in the older group (by respective values of 34%, 37%, and 30%). However, we found no age-related reduction in Mn$^{2+}$ stimulation of adenylyl cyclase in aged hearts. Mn$^{2+}$ is a relatively selective probe for the adenylyl cyclase catalytic unit, and unlike forskolin, its action is not affected by guanine nucleotides. Therefore, our results do not support a decrease in function or amount of the adenylyl cyclase catalytic unit with aging, as has been proposed in human lymphocytes.

The results with G protein-sensitive probes of adenylyl cyclase stimulation and toxin-catalyzed ADP ribosylation indicate an abnormality of $G_i$ in aging heart, which could account for uncoupling of $\beta$-adrenergic receptors, a decrease in agonist affinity of $\beta_i$-receptors, or both. Such an abnormality has recently been reported by Vatner et al in aging nonhuman primate hearts. The decrease in functional activity of $G_i$, suggested by adenylyl cyclase stimulation data and supported by cholera toxin-catalyzed ADP ribosylation appeared to be a posttranslational modification, inasmuch as there was no decrease in the 52-kD or 45-kD $G_i\alpha$ splice form detected on immunoblots of older versus younger preparations. Although we did not perform experiments designed to elucidate the nature of such a modification, recent data indicate that palmitoylation of $G_i\alpha$ can be regulated by $\beta$-agonist exposure, an effect that does not alter the total amount of immunochromatically detectable $G_i\alpha$. Such a modification, i.e., depalmitoylation, could functionally uncouple $G_i\alpha$ by interfering with its ability to attach to the cell surface membrane. Another way in which chronic adrenergic stimulation could produce posttranslational modification in $G_i\alpha$ is through protein kinase A or C-modulated phosphorylation.

The combination of decreased NaF- and forskolin-stimulated adenylyl cyclase activities and lack of change in immunochromatically detectable $G_i$ agrees with recent work in the aging rat heart. This study also demonstrated no change in immunodetectable $G_i$ in aging hearts. Based on decreased forskolin binding, Shu and Scarpate concluded that the adenylyl cyclase catalytic unit (C) was decreased in amount rather than $G_i\alpha$ being functionally altered. However, forskolin binding actually detects the $G_i\alpha$-C complex rather than free C and thus will be sensitive to decreases in either $G_i\alpha$ or C. Shu and Scarpate did not report toxin-catalyzed ADP ribosylation or Mn-stimulated adenylyl cyclase data, so their results are also consistent with a posttranslational modification of $G_i\alpha$.

In contrast to innervated myocardium obtained from organ donors, there was no correlation between $\beta_i$-receptor density and age in previously transplanted hearts that exhibited profound depletion of adrenergic neurotransmitters. Other parameters shown in the current investigation to be related to age such as $\beta_i$-agonist affinity and $\beta_i$-receptor coupling are also not age related in denervated human heart. Although these observations are limited by the truncated age group and the small number of cases in the retransplanted group as well as the presence of graft atherosclerosis in these normally functioning denervated hearts, the data suggest that cardiac innervation may be a necessary condition for age-related downregulation of myocardial $\beta$-adrenergic receptors in the "physiologically" aging human heart. One possible explanation for this is an age-related increase in cardiac adrenergic drive. In support of this hypothesis is the recent report by Ng et al of an age-related increase in skeletal muscle sympathetic nerve activity in both men and women and several reports of increased systemic norepinephrine levels with age. However, due to the avidity of the cardiac neuronal norepinephrine uptake system, myocardial $\beta$-adrenergic receptors respond mostly to cardiac adrenergic drive, and increased cardiac adrenergic drive in aging has not been reported. In the current study, tissue norepinephrine level as an indirect index of cardiac adrenergic activity was different in older versus younger hearts. In model systems, there is no consensus concerning age-related changes in myocardial levels of norepinephrine. Accordingly, increased cardiac adrenergic drive as an explanation for decreased $\beta_i$-receptor density or the other changes in $\beta$-receptors that occur with aging is only speculative.

There are many similarities between the aged and the failing heart. Both aged and failing myocardia exhibit increases in left ventricular mass and decreased diastolic function. Morphologically, myocyte hypertrophy and replacement fibrosis occur in both aging and heart failure animal models. The aging and failing myocardia exhibit decreased functional reserve and defective cardiac energetics. The age-related changes in the behavior of the $\beta$-adrenergic neuroeffector systems reported in the older hearts are quite similar to the changes previously described in heart failure (Table 6) and, as in this clinical syndrome, may have been caused by compensatory cardiac adrenergic activation. Heart failure from dilated cardiomyopathy (idiopathic or ischemic) is characterized by $\beta_i$-adrenergic receptor downregulation and uncoupling of $\beta_i$-adrenergic receptors from adenylyl cyclase as well as abnormalities of $G$ protein function that are manifested by decreases in Gpp(NH)p and forskolin responsiveness. Similarly, aged and failing human hearts are characterized by increased systemic adrenergic drive.
TABLE 6. Comparison of Changes in Failing (Idiopathic Dilated Cardiomyopathy) Vs Aged Human Left Ventricular Myocardium

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Change in Failing LV</th>
<th>Change in Aged LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_1$-Receptor density</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>$\beta_2$-Receptor agonist affinity</td>
<td>NSC</td>
<td>↓</td>
</tr>
<tr>
<td>$\beta_2$-Receptor coupling</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Gpp(NH)p-, forskolin-stimulated adenyl cyclase</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>$G_i$ function</td>
<td>↓</td>
<td>NSC</td>
</tr>
<tr>
<td>NaF-stimulated adenyl cyclase</td>
<td>NSC</td>
<td>↓</td>
</tr>
<tr>
<td>$G_o$ function</td>
<td>NSC</td>
<td>↓</td>
</tr>
<tr>
<td>Tissue norepinephrine</td>
<td>↓</td>
<td>NSC</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; NSC, no significant change.

Despite these similarities, there are also differences between the aged and the failing heart (Table 1). In contrast with previous studies in heart failure,68,86 we did not observe an age-related decrease in myocardial content of norepinephrine and dopamine. Decreased adenyl cyclase stimulation by NaF does not occur in human myocardium failing as the result of ischemic or idiopathic dilated cardiomyopathy,35,37 and $G_o$ function or amount is not decreased in the failing human heart.36,37 In heart failure, the decreased myocardial adenyl cyclase response to Gpp(NH)p and forskolin is explained by a posttranslational increase in the functional activity of $G_i$,36,87 whereas $G_i$ functional activity did not appear to be increased by aging. Finally, the decrease in the agonist affinity of $\beta_2$-receptors described in this study does not occur to the same magnitude in the failing human heart,88 if it occurs at all.96

There are methodological considerations that could have affected the conclusions of this investigation. Although all organ donors had normal systolic function measured echocardiographically, before tissue harvest these had been exposed to high levels of circulating catecholamines, which could have produced acute desensitization phenomena. Also, several hours on a respirator or administration of dopamine could have produced acute changes in the $\beta$-adrenergic neuroeffector systems. These potential limitations could not be avoided because of the nature of the study. However, myocardial $\beta$-receptor downregulation is likely to take several weeks or months to develop and is not present after a 48-hour catecholamine infusion in humans.89,90 In dogs, several weeks of continuous high-dose norepinephrine causes a decrease in mechanical response to isoproterenol but does not cause $\beta$-receptor downregulation.91,92 Finally, younger and older donors were exposed to similar doses and durations of dopamine administration and had similar intervals between brain injury and cardiac transplantation. Therefore, although high levels of catecholamines related to brain injury or dopamine administration could have produced desensitization changes, these effects should have been equally manifest in both younger and older subjects and would have been confined to acute desensitization phenomena.

The age-related alterations in $\beta$-adrenergic signal transduction could have important clinical implications. The process of aging is associated with gradual decline in oxygen consumption during maximum exercise, reduction in exercise-associated increases in cardiac index and heart rate,93,94 and increased left ventricular end-diastolic volume, mimicking the physiology observed during $\beta$-blocker therapy.95 Since older individuals exhibit an even greater degree of adrenergic activation during exercise than younger subjects,96,97 it can be argued that the elderly are even more dependent on adrenergic drive to increase cardiac performance during exercise than are younger individuals. Therefore, similar to heart failure,98 it is likely that much of the decline in peak exercise capacity that occurs with age is related to changes in $\beta$-adrenergic receptor mechanisms described in this study.

In summary, the human heart exhibits an age-related decrease in myocardial $\beta_1$-adrenergic receptors, uncoupling of $\beta_2$-receptors, decreased $\beta_2$-receptor agonist affinity, and decreased $G_o$-mediated activation of adenyl cyclase in vitro. These changes result in decreased contractile response of right ventricular trabeculae to $\beta$-agonist stimulation. The signals and molecular mechanisms that account for these age-related changes will require further investigation.

Acknowledgments

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