Differential pre- and postsynaptic effects of desipramine on cardiac sympathetic nerve terminals in RHF

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Liang, Chang-seng, Yoshihiro Himura, Michihiro Kashiki, and Suzanne Y. Stevens. Differential pre- and postsynaptic effects of desipramine on cardiac sympathetic nerve terminals in RHF. Am J Physiol Heart Circ Physiol 283: H1863–H1872, 2002—Right heart failure (RHF) is characterized by chamber-specific reductions of myocardial norepinephrine (NE) reuptake, β-receptor density, and profiles of cardiac sympathetic nerve ending neurotransmitters. To study the functional linkage between NE uptake and the pre- and postsynaptic changes, we administered desipramine (225 mg/day), a NE uptake inhibitor, to dogs with RHF produced by tricuspid avulsion and progressive pulmonary constriction or sham-operated dogs for 6 wk. Animals receiving no desipramine were studied as controls. We measured myocardial NE uptake activity using [3H]NE, β-receptor density by [125I]iodocyanopindolol, inotropic responses to duchovamine, and noradrenergic terminal neurotransmitter profiles by glyoxylic acid-induced histofluorescence for catecholamines, and immunocytochemical staining for tyrosine hydroxylase and neuropeptide Y. Desipramine decreased myocardial NE uptake activity and had no effect on the resting hemodynamics in both RHF and sham animals but decreased myocardial β-adrenoceptor density and β-adrenergic inotropic responses in both ventricles of the RHF animals. However, desipramine treatment prevented the reduction of sympathetic neurotransmitter profiles in the failing heart. Our results indicate that NE uptake inhibition facilitates the reduction of myocardial β-adrenoceptor density and β-adrenergic subresponsivity in RHF, probably by increasing interstitial NE concentrations, but protects the cardiac noradrenergic nerve endings from damage, probably via blockade of NE-derived neurotoxic metabolites into the nerve endings.

Concentric heart failure; neuronal norepinephrine uptake; tyrosine hydroxylase; neuropeptide Y

Myocardial β-adrenoceptors are reduced in number in the failing right ventricles of both animals subjected to tricuspid avulsion and pulmonary artery constriction (19) and patients with primary pulmonary hypertension (8). The correspondent left ventricle showed no changes in myocardial β-adrenoceptor density despite exposure to the same elevation of circulating norepinephrine (NE) as the right ventricle. Other studies (2, 56) have also shown that myocardial β-adrenoceptor downregulation occurs only in the ventricles with elevated filling pressures, such as in selective left heart failure produced by aortic regurgitation. In contrast, when biventricular heart failure is produced by doxorubicin (56) or rapid ventricular pacing (15), myocardial β-adrenoceptor density is reduced in both ventricles. These findings suggest myocardial β-receptor changes are caused by local rather than systemic mechanisms in heart failure. Furthermore, because myocardial β-receptor density correlates inversely with cardiac interstitial NE concentration (15), we speculate that β-receptor downregulation occurs in the failing ventricle where interstitial NE is increased by either an increase in NE release, a decrease in tissue clearance of NE, or both.

Increased cardiac NE spillover in heart failure has been well established (18, 38). In addition, work from our laboratories (21, 25, 31) has shown that myocardial NE uptake activity and NE uptake-1 carrier density are reduced in heart failure and correlate significantly with myocardial β-receptor density. Because neuronal NE uptake is the major mechanism for NE clearance from the interstitial space, a decrease in neuronal NE uptake is expected to increase interstitial NE and lead to agonist-induced β-adrenoceptor downregulation. The functional importance of the NE reuptake mechanism in the sympathetic nerve terminals has been demonstrated by Anzai et al. (2) with the use of 6-hydroxydopamine to produce chemical sympathectomy that facilitated the reduction of myocardial β-receptors in both ventricles of animals after aortic regurgitation and abolished the chamber-specific alterations in β-adrenergic signaling in the left heart failure. However, the investigators provided no direct measurements of myocardial NE uptake activity, nor did they consider that an early release of NE after chemical destruction of sympathetic nerves by 6-hydroxydopamine (54) could increase interstitial NE and complicate the interpretation of the results in heart failure. Thus we carried out the present study in the right heart failure...
(RHF) animals with the use of desipramine, an antidepressant agent well known for its neuronal NE reuptake inhibitory action on NE uptake-1 carrier site without causing sympathetic denervation (29, 50). We speculate that desipramine would increase interstitial NE and potentiate the intensity and duration of the physiological action of NE in both the right and left ventricles, and thus would, like 6-hydroxydopamine, abolish the chamber-specific downregulation of the β-adrenoceptors in the RHF animals.

We measured myocardial β-receptor density and NE uptake activity in the present study. Furthermore, because desipramine has been shown to reduce NE-induced sympathetic denervation in the present study, we also studied the functional integrity of cardiac sympathetic nerves by measuring sympathetic contents of NE, its rate-limiting enzyme tyrosine hydroxylase (41), and neuropeptide Y, a neurotransmitter that is coreleased with NE after nerve stimulation, but, unlike NE, is not taken back into the sympathetic nerve endings by a reuptake mechanism (22).

METHODS

Surgical Preparation of the Animals

Healthy adult mongrel dogs (19.6–29.5 kg) were anesthetized with pentobarbital sodium (25 mg/kg iv) and ventilated with room air by a respirator (Harvard Apparatus; South Natick, MA). The animals underwent a modified two-staged sterile surgical procedure (24, 31). During the first operation, a right atriotomy was performed via right thoracotomy to rupture the anterior chordae tendineae of the tricuspid valves and to insert a Tygon catheter (1.02 mm ID; Norton Plastics and Synthetics Division; Akron, OH) in the right atrium. Two weeks later, a left thoracotomy was performed for placement of a silicone rubber hydraulic occluder (Jones; Silver Spring, MD) around the main pulmonary artery, an implantable micrometeron (Konigsberg Instruments; Pasadena, CA) in the left ventricle, and Tygon catheters in the pulmonary artery, left atrium, and descending thoracic aorta. RHF was produced by progressive inflation of the pulmonary artery occluder, beginning 2 wk after the second thoracotomy. Balloon inflation was adjusted at 4- to 7-day intervals for 3 wk to produce a steady increase in right atrial pressure to 12–14 mmHg; no further adjustments were made thereafter. Final hemodynamic studies were made 8 wk after the second thoracotomy.

A separate group of dogs (sham) underwent two surgical procedures identical to those described above, except neither tricuspid valve avulsion nor pulmonary artery constriction was included.

The study was approved by the University Committee on Animal Resources and conformed to the American Physiological Society’s “Guiding Principles in the Care and Use of Animals” and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Protocol

RHF and sham-operated animals were randomized to receive oral desipramine (Merrell Dow; Cincinnati, OH), 225 mg once a day. Animals receiving no desipramine were studied as controls. Thus the animals were divided into four groups: 1) RHF animals receiving no desipramine (RHF control), 2) RHF animals receiving desipramine (RHF desipramine), 3) sham-operated animals receiving no desipramine (sham control), and 4) sham-operated animals receiving desipramine (sham desipramine).

Desipramine was administered daily for six consecutive weeks beginning 2 wk after the second thoracotomy in both the RHF and sham-operated animals. The doses chosen for the study inhibited tissue NE uptake in the heart and potentiate the pressor response to exogenous NE in pilot studies. To verify the inhibition of NE uptake by desipramine, NE (0.5 μg/kg) was administered intravenously before initiation of desipramine treatment (week 2) and at the end of final hemodynamic studies (week 8) and the blood pressure increments before and after desipramine treatment were compared (Fig. 1). NE pressor response was also studied in the control animals receiving no desipramine at week 2 and week 8 after the second thoracotomy, which corresponded in time to the beginning and end of the desipramine treatment.

Animals were monitored weekly for heart rate, blood pressure, and body weight. They were acclimatized to the laboratory environment and trained to lie with minimal restraint on a table. Final hemodynamic studies were carried out in the conscious animals to study the resting hemodynamics and myocardial β-adrenergic sensitivity to dobutamine 8 wk after the second thoracotomy. Arterial blood was taken before β-agonist administrations to determine the resting plasma NE (44). The animals were then euthanized with large doses (>100 mg/kg) of intravenous pentobarbital 2 days later. The hearts were excised, and the right and left ventricles were separated and weighed. Tissue blocks were removed from the ventricular free walls 3 cm below the atrioventricular groove for measuring myocardial NE uptake activity, chemical NE content, and morphometric analysis of the sympathetic nerve endings with the use of NE histochemistry, tyrosine hydroxylase, and neuropeptide Y immunocytochemistry. A crude muscle membrane fraction was prepared immediately by homogenization and differential centrifugation, and pellets were stored in −70°C for measurement of β-adrenoceptor density. Tissue protein was determined in triplicate (36) with bovine serum albumin used as a standard.
as standard. The persons performing myocardial tissue NE, β-adrenergic receptor density, and sympathetic terminal neurotransmitter profile assays were blinded to the treatment assignments of the animals.

Resting Systemic Hemodynamics

The previously implanted intravascular catheters were connected to pressure transducers (model P23Db, Statham Instruments; Oxnard, CA) and a recorder (Brush model 480, Gould Instrument System Division; Cleveland, OH) for measuring blood pressures. The Konigsberg micromanometer was attached to the Brush recorder for measuring left ventricular pressure and its first derivative (dP/dt) using an electronic differentiator. The left ventricular dP/dt at 50 mmHg of developed pressure that occurred during the isovolumic contraction was divided by 50 mmHg of developed pressure. This dP/dt ratio (dP/dt/P) is an index of left ventricular contractility independent of ventricular afterload (13). A transducer-tipped catheter (Millar; Houston, TX) was inserted under local xylcaine anesthesia via an external jugular vein into the right ventricle for measuring right ventricular pressure and dP/dt. Cardiac output was measured by injecting indocyanine green (Cardio-Green, Hynson Westcott and Dunning; Baltimore, MD) into the pulmonary artery and sampling the arterial blood for dye concentrations with a cardiac output system (model 140; Gilford Instrument Laboratories; Oberlin, OH). The animals were allowed to rest for at least 1 h after placement of the Millar catheter before the resting hemodynamic measurements were taken in triplicate at 5-min intervals. Averages of the triplicates were used for statistical analysis.

Plasma and Myocardial NE Contents

Plasma and tissue NE were measured radioenzymatically (44) with the use of Cat-A-Kit assay system (Amersham; Arlington Heights, IL). Fresh heart samples were minced and suspended in a 0.4 N perchloric acid with 5 mmol/l reduced glutathione (pH 7.4), homogenized with a homogenizer (8-s bursts × 3 at setting 8; Polytron PCU-2, Brinkman Instruments; Westbury, NY), and centrifuged at 500 g. The supernatant was taken for the assay.

Myocardial β-Adrenergic Sensitivity

Animals were administered increasing doses of dobutamine (4, 8, and 16 μg·kg⁻¹·min⁻¹) at a rate of 0.988 ml/min. The infusion was continued for 10 min at each dose level. Heart rate, mean aortic pressure, right ventricular dP/dt and left ventricular dP/dt reached a new steady state within 5 min of each infusion; the steady increases of right and left ventricular dP/dt obtained at 8–10 min of infusion were averaged and used for assessing the cardiac inotropic response to dobutamine.

Myocardial NE Uptake Activity

Myocardial NE uptake activity was measured quadruplicately by incubating fresh tissue slices at 37°C for 15 min in 50 nM l-[7-3H]NE (15 Ci/mmol; New England Nuclear; Boston, MA) (31). Nonspecific accumulation of radioactivity was determined by parallel incubation of quadruplicate tissue slices at 4°C. Specific °H uptake activity, defined as the difference in radioactivity between tissue slices incubated in a °HNE-containing solution at 37°C and those at 4°C, is considered to approximate NE uptake activity (31).

Myocardial β-Adrenergceptor Binding Assay

Myocardial β-receptor density was measured by specific binding of [°I]iodocyanopindolol (2,200 Ci/mmol; New England Nuclear), as previously described (32). The maximum number of receptor-binding sites was calculated using the AccuFit saturation two sites program (Lunden Software; Chagrin Falls, OH).

Anatomic Studies of Ventricular Sympathetic Nerves

Glyoxylic acid-induced histofluorescence for catecholamines. Histofluorescence specific for catecholamines was performed using a modification (6) of the sucrose-potassium phosphate-glyoxylic acid (SPG) condensation method of de la Torre (14). Tissue blocks from fresh heart were rapidly frozen on dry ice and stored in liquid nitrogen. Blocks were mounted on a cryostat (−20°C) for longitudinal section at thickness of 16 μm. Sections were picked up on the glass slides, dipped in SPG solution, dried heated under oil at 95°C for 2.5 min, coverslipped, and viewed under epifluorescence illumination using a Nikon fluorescence microscope equipped with filters designed for catecholamine fluorescence visualization. All sections were photographed at the same magnification ×50 with 35-mm slide film. Slides were projected onto a grid, 8 × 8 squares per inch, and the number of lines of intersection, in which the nerve profiles projected, was counted in a 0.221 mm² (0.003536 mm³) field. At least five fields were chosen from each ventricle, and the averaged number of profiles in that ventricle was used for statistics.

Immunocytochemistry for tyrosine hydroxylase and neuropeptide Y. Ventricular muscle blocks were fixed for 24 h in 4% paraformaldehyde in 0.15 mol/l phosphate buffer (pH 7.4) at 4°C. Blocks were transferred to 25% sucrose in 0.15 mol/l phosphate buffer (pH 7.4) for an additional 24 h at 4°C and then frozen on dry ice and stored at −80°C. Frozen tissue blocks were mounted on the chuck of a sliding microtome, sections were cut at 40 μm, and placed in 0.15 mol/l phosphate buffer. For the following procedure, the buffer was composed of 0.15 mol/l phosphate and all steps were carried out at room temperature using gentle agitation, unless otherwise indicated. Before incubation with the primary antibody, sections were rinsed thoroughly in buffer and preincubated for 30 min in 10% normal goat serum. The primary anti-tyrosine hydroxylase (Chemicon; Temecula, CA) and anti-neuropeptide Y (Incstar; Stillwater, MN) antibodies were diluted (1:60,000 for tyrosine hydroxylase and 1:8,000 for neuropeptide Y) in 0.4% Triton X-100 in buffer plus 0.15% normal goat serum. Sections were incubated in the primary antibody for 24 h at 4°C with gentle agitation. On the following day, sections were rinsed 10 times each for 10 min in buffer and then incubated in the biotinylated secondary antibody (goat anti-rabbit IgG diluted 1:1,000 in buffer plus 0.15% normal goat serum) for 2 h. The sections were subsequently rinsed in buffer six times each for 5 min and treated to remove endogenous peroxidase activity by being incubated in 5% methanol and 1.5% hydrogen peroxide in phosphate buffer for 30 min. Sections were then incubated in avidin-biotin-peroxidase complex (Vector kit; Vector Laboratories, Burlingame, CA; 20 μl of reagent A and 20 μl of reagent B in 20 ml of 0.15 M phosphate buffer) for 2 h. Sections were then rinsed four times for 5 min each in 0.1 M sodium acetate with 10 mM imidazole (pH 7.0) and then developed in acetate-imidazole buffer containing 0.1 mol/l nickel (II) sulfate, 0.03% diaminobenzidine, and 0.008% hydrogen peroxide for 5 min. All sections were then rinsed three times for 5 min each in 0.15 M phosphate buffer. Sections were finally mounted on gelatin coated slides, dried, dehydrated through a series of ethanol, cleared in xylene, and
coverslipped in Permount. For quantification of the immunostained nerve fiber density, the slides were viewed and photographed at the same magnification (×50) onto 35-mm slides. The number of neurotransmitter profiles was counted in a 0.00885 mm³ field. Results of five fields were averaged for each ventricle.

Statistical Analyses

All results were expressed as means ± SE. The data were analyzed with a RS/1 Research System (Bolt, Beranek, and Newman Software Products; Cambridge, MA). The experimental data were analyzed by Student’s t-test for comparison of difference between two group means. Two-way analysis of variance was used to study the effects of RHF (vs. sham) and desipramine (vs. control), as well as interaction between RHF and desipramine. A multiple-range test was used to determine the statistical significance of differences among the groups. A P value <0.05 was considered statistically significant.

RESULTS

NE Uptake Inhibition by Desipramine

Figure 1 shows the pressor responses to NE injection at weeks 2 and 8 in the four experimental groups. In control sham and RHF animals without desipramine treatment, the magnitude of blood pressure rise after NE was the same at treatment, the magnitude of blood pressure rise after NE was already markedly reduced in the RHF control animals, the further reduction of NE uptake activity by desipramine in the RHF animals did not reach statistical significance compared with the control RHF animals.

Clinical Manifestations and Resting Hemodynamic Parameters

Dogs developed clinical evidence of ascites after tricuspid avulsion and progressive pulmonary constriction. The RHF animals also exhibited heavier body weight, and higher heart rate, right atrial pressure, right ventricular systolic pressure, and plasma NE concentration than the sham-operated animals (Table 1). Mean aortic pressure, left atrial pressure, and cardiac output were lower in the RHF animals compared with the sham animals. In addition, left ventricular peak dP/dt and dP/dt/P, and right ventricular peak dP/dt were lower in the RHF than sham-operated dogs. Desipramine treatment affected none of the resting hemodynamic parameters, except for a tendency of increases in mean aortic pressure and left atrial pressure in RHF dogs.

Right ventricular weight was increased in RHF, but the difference in left ventricular weight did not differ significantly between the RHF and sham-operated dogs. Desipramine treatment had no effect on the cardiac weights in either the sham or RHF animals.

Myocardial Inotropic Responses to Dobutamine and β-Adrenoceptor Density

Dobutamine infusion increased right and left ventricular dP/dt in a dose-dependent manner in both the RHF and sham animals (Fig. 3). Heart rate and mean blood pressure did not change significantly during the infusions. To compare the magnitude of inotropic effects of dobutamine among the four experimental groups, we calculated the net increases of right and left ventricular dP/dt produced by the highest dose of dobutamine (Table 2). The table shows that dobutamine produced a much smaller increases of right and left ventricular dP/dt in RHF animals compared with the sham animals. Desipramine treatment had no effect on the inotropic responses in the sham animals but caused a further reduction in the inotropic response in both right and left ventricles in RHF animals.

Figure 4 shows the characteristic chamber-specific reduction of myocardial β-adrenoceptor density in the failing right ventricle of RHF animals. Myocardial β-adrenoceptor density did not differ significantly in the left ventricle between the RHF and sham animals. Desipramine treatment produced no significant changes in myocardial β-adrenoceptor density in sham dogs, but it caused a significant reduction of myocardial β-adrenoceptor density in both the right and left ventricles of RHF animals.
Table 1. Resting hemodynamics and heart weights in sham-operated and RHF animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham Control</th>
<th>Desipramine</th>
<th>RHF Control</th>
<th>Desipramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>23.5 ± 0.5</td>
<td>22.3 ± 0.6</td>
<td>26.2 ± 0.7*</td>
<td>26.7 ± 0.7*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>92 ± 3</td>
<td>100 ± 3</td>
<td>135 ± 4*</td>
<td>122 ± 6*</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>114 ± 3</td>
<td>115 ± 4</td>
<td>104 ± 3*</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>4.45 ± 0.25</td>
<td>4.49 ± 0.30</td>
<td>2.47 ± 0.13*</td>
<td>2.86 ± 0.15*</td>
</tr>
<tr>
<td>Left atrial pressure, mmHg</td>
<td>8.2 ± 0.5</td>
<td>6.8 ± 0.8</td>
<td>5.5 ± 0.3*</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>4.8 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>14.2 ± 0.9*</td>
<td>13.2 ± 0.9*</td>
</tr>
<tr>
<td>RV systolic pressure, mmHg</td>
<td>34 ± 2</td>
<td>33 ± 1</td>
<td>45 ± 3*</td>
<td>48 ± 2*</td>
</tr>
<tr>
<td>LV dP/dt, mmHg/s</td>
<td>3,305 ± 129</td>
<td>2,961 ± 109</td>
<td>2,174 ± 88*</td>
<td>2,398 ± 96*</td>
</tr>
<tr>
<td>LV dP/dt/P, mmHg/s</td>
<td>41.3 ± 1.1</td>
<td>42.3 ± 0.9</td>
<td>33.7 ± 0.8*</td>
<td>35.6 ± 0.8*</td>
</tr>
<tr>
<td>RV dP/dt, mmHg/s</td>
<td>699 ± 40</td>
<td>652 ± 25</td>
<td>517 ± 41*</td>
<td>528 ± 29*</td>
</tr>
<tr>
<td>Plasma (NE), pg/ml</td>
<td>364 ± 44</td>
<td>331 ± 44</td>
<td>915 ± 118*</td>
<td>1,015 ± 89*</td>
</tr>
<tr>
<td>RV wt, g</td>
<td>39 ± 1</td>
<td>36 ± 3</td>
<td>55 ± 2*</td>
<td>51 ± 2*</td>
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<tr>
<td>LV wt, g</td>
<td>100 ± 3</td>
<td>104 ± 5</td>
<td>90 ± 3</td>
<td>100 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. RHF, right heart failure; LV, left ventricle; RV, right ventricle; NE, norepinephrine; dP/dt, LV pressure and its first derivative; dP/dt/P, ratio of dP/dt at developed pressure. *P < 0.05, vs. sham control, as measured by two-way analysis of variance and multiple-range comparisons.

Myocardial Tissue NE Content and SPG Histoﬂuorescence

We measured NE stores in myocardial tissue using the radioenzymatic method and in the noradrenergic nerve terminals using SPG histoﬂuorescence (Fig. 5). Desipramine treatment produced no effects on either cardiac chemical NE content or myocardial SPG histoﬂuorescence in sham animals. Myocardial NE content was reduced in both ventricles of RHF animals, but the magnitude of NE reduction was much greater in the right ventricle than the left ventricle. Desipramine treatment had no effect on NE content of the left ventricle of RHF animals but increased NE content in the right ventricle compared with RHF control animals.

Desipramine treatment in the RHF animals signiﬁcantly attenuated the reduction in SPG proﬁles in the left ventricle. The reduction of SPG histoﬂuorescence proﬁles was also smaller in the right ventricle of desipramine-treated RHF animals, but the difference between the control and desipramine-treated RHF animals did not reach statistical signiﬁcance.

Immunocytochemistry for Tyrosine Hydroxylase and Neuropeptide Y

Sympathetic nerve terminal proﬁles visualized by the immunochemical staining of tyrosine hydroxylase and neuropeptide Y are shown in Fig. 6. The ﬁgure shows that the sympathetic nerve proﬁles were not affected by desipramine in either ventricle of the sham animals. Figure 6 also shows that the tyrosine hydroxylase- and neuropeptide Y-immunostained proﬁles were reduced in the right ventricle of RHF animals. However, unlike NE content, neither tyrosine hydroxylase- nor neuropeptide Y-stained nerve proﬁles decreased signiﬁcantly in the left ventricle of the RHF

Table 2. Peak increases of right and left ventricular dP/dt in response to dobutamine

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ΔRVdP/dt, mmHg/s</th>
<th>ΔLVdP/dt, mmHg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>13</td>
<td>713 ± 70</td>
<td>3,074 ± 416</td>
</tr>
<tr>
<td>Sham desipramine</td>
<td>11</td>
<td>667 ± 73</td>
<td>2,391 ± 163</td>
</tr>
<tr>
<td>RHF control</td>
<td>15</td>
<td>532 ± 38*</td>
<td>1,671 ± 140*</td>
</tr>
<tr>
<td>RHF desipramine</td>
<td>15</td>
<td>395 ± 31*†‡</td>
<td>1,203 ± 97*†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. *P < 0.05 vs. sham control; †P < 0.05 vs. RHF control; ‡P < 0.05 vs. sham desipramine, as measured by two-way analysis of variance and multiple-range comparisons.

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animals. Desipramine treatment in RHF animals attenuated the reductions in the tyrosine hydroxylase- and neuropeptide Y-immunostained profiles that occurred in the right ventricle.

DISCUSSION

The RHF model produced by tricuspid avulsion and progressive pulmonary constriction, first reported by Barger et al. (5), has been extensively studied in our laboratory and by others. As expected, the animals in our study exhibited weight gain, ascites, increased right atrial pressure, increased right ventricular systolic pressure as well as right ventricular hypertrophy. The animals also showed decreased cardiac output, a slight reduction in aortic pressure, and decreased right ventricular dP/dt. Left ventricular dP/dt and dP/dt/P were also decreased, but there was no increase in left atrial pressure. The findings are consistent with what we have reported previously (19, 25, 31, 32). Myocardial contractility of the right ventricle is clearly depressed in the RHF animals, as demonstrated in isolated papillary muscle contraction studies (32, 53). However, the assessment of left ventricular contractile function in the RHF animals may be less reliable because the left ventricular dP/dt and peak contractile element velocity can be affected by changes in left ventricular geometry (27) as the right ventricular pressure and volume increase in these animals.

NE is depleted in the failing heart (10). Earlier studies (9, 46) in RHF animals have shown that the decrease in NE store in the failing myocardium is related to increased release, decreased synthesis, and ineffective storage of NE. The decrease in tyrosine hydroxylase activity is thought to be the main mechanism for the decreased NE synthesis (46, 49). The decrease of cardiac tyrosine hydroxylase-immunostained profiles in our present study is consistent with this mechanism. In addition, our present study suggests that the decreased NE uptake is a major local factor for the NE depletion in the failing right ventricle. Similarly, increased cardiac spillover of NE in human heart failure is associated with both increased neuronal release of NE and reduced NE reuptake into the noradrenergic nerve endings (16, 48). Decrease in NE uptake has been demonstrated in human heart failure with the use of a NE tracer technique (47) in vivo and in vitro measurement of NE uptake carrier density (7, 21).

The efficacy of desipramine as a NE uptake inhibitor was demonstrated in our present experiments by the exaggerated pressor response to intravenous NE and diminished NE uptake activities in isolated ventricular tissue preparations. The exaggerated pressor responses are consistent with results of an earlier study of desipramine (30). Our present study also showed the pressor response to NE was attenuated in RHF animals compared with the sham animals. The diminished pressor response in RHF probably was caused by decreased responsiveness of vascular α-adrenergic receptors (20).

As stated previously, desipramine treatment reduced the NE uptake activity of the ventricular myo-
animals. The also reduced by desipramine treatment in the RHF right and left ventricular dP/dt/\text{RHF} \text{ventricles of RHF animals. The inotropic responses of the } \beta\text{-adrenoceptor number in both the right and left ventricles of sham-operated animals, but decreased myocardial sympathetic nervous system activity in the RHF animals. Our results are consistent with the abolition by 6-hydroxydopamine of chamber-specific } \beta\text{-adrenergic downregulation in left heart failure.}

The close interaction between NE and NE uptake activity has been illustrated in a short-term (1 wk) infusion of NE study in rabbits, in which NE infusion, when given alone fails to induce myocardial } \beta\text{-receptor subsensitivity, is capable of causing myocardial } \beta\text{-receptor downregulation and } \beta\text{-adrenergic subsensitivity in animals treated with 6-hydroxydopamine that } \text{reduces NE uptake activity (42). In addition, when NE was given over 8 wk, the animals showed both reduction of myocardial NE uptake and myocardial } \beta\text{-receptor downregulation (17). This close association between myocardial NE uptake activity and } \beta\text{-receptor density suggests that these two phenomena are closely and functionally linked.}

Increased circulatory plasma NE suggests generalized sympathetic nervous system overactivity in RHF. However, the degree of sympathetic stimulation to the right and left ventricles in RHF may vary. Azevedo et al. (3) reported that cardiac NE spillover is reduced by the lowering of cardiac filling pressure in heart failure patients. Thus we speculate that the left ventricle in the RHF animals, which has a normal or low filling pressure, is relatively protected because the amount of NE released in the left ventricle is lower than the right ventricle, which has a much higher filling pressure. This enhanced release of NE in the failing right heart may help explain the chamber-specific reduction of NE uptake activity and myocardial } \beta\text{-receptor density in the right ventricle of RHF animals.}

The results of our present study have further shown that desipramine ameliorates the cardiac sympathetic nerve terminal abnormalities that occur in the failing right ventricle of RHF animals. The RHF animals showed reduced tissue NE and catecholaminergic histofluorescence. The reduction is much greater in the failing right ventricle compared with the left ventricle of the RHF animals, probably because of the greater release of NE and reduced NE uptake activity in the failing heart. The reduction of immunostained tyrosine hydroxylase profiles in the failing myocardium suggests that the reduced synthesis of NE also plays a role to the depletion of cardiac NE. Neuropeptide Y is generally considered to coexist with NE in adrenergic neurons and coreleased with NE after nerve stimulation (37). However, because no presynaptic reuptake mechanism is involved in the elimination of neuropeptide Y, the decrease in tissue neuropeptide Y reflects increased release of neuropeptide Y, decreased biosynthesis, or both.

Myocardial NE uptake activity and tyrosine hydroxylase- and neuropeptide Y-immunoreactive profiles were normal in the left ventricles of RHF animals, despite the reduced neuronal NE content and impaired dP/dt and dP/dt/P. This may imply that the functional structures of sympathetic nerve terminals are intact in the nonfailing left ventricle and that the sympathetic nerve terminal damage is not the cause of impaired cardiac contractility. Cardiac NE content correlates...
poorly with contractile parameters in heart failure patients (45).

We (26) recently studied the temporal relationship between changes of myocardial NE uptake activity and myocardial β-adrenoceptor function during the development and recovery of congestive heart failure in pacing-induced cardiomyopathy. The results suggest that abnormal myocardial NE uptake mechanism may play an important pathophysiological role in heart failure. The pathophysiological importance of the cardiac sympathetic nerve terminal NE uptake function is further supported by a recent study (55) of carvedilol in rats with dilated cardiomyopathy, in which the beneficial effects of carvedilol on cardiac function and myocardial fibrosis are associated with reduction of cardiac adrenergic neuronal damage as measured by [125I]-metaiodobenzylguanidine (MIBG). Cardiac MIBG imaging has been used to assess cardiac sympathetic nerve terminal function in humans (52). These studies have shown that impaired cardiac adrenergic innervation as assessed by MIBG imaging is a valuable prognostic indicator, independent of left ventricular ejection fraction and circulating plasma NE, for increased mortality and morbidity in patients with chronic congestive heart failure (39, 43). MIBG imaging also has been used to select patients who are likely to respond favorably to β-blocker therapy (11, 23, 40) or to evaluate the patient’s prognosis on chronic β-blocker therapy (35). The findings suggest that studies of myocardial adrenergic nerve function are not only physiologically important but also clinically relevant.

Our present study provides no direct evidence for a mechanism responsible for the noradrenergic nerve damage in heart failure. However, we have shown that the cardiac neuronal damage induced by NE (33) and present in heart failure (51) can be attenuated by antioxidant vitamins or superoxide dismutase. Desipramine and superoxide dismutase also have been shown to protect the peripheral sympathetic nerve terminals from neurotoxic oxidative metabolic products of NE (1). These findings suggest that the noradrenergic nerve terminal changes in the failing heart probably are caused by oxygen-free radicals derived from NE metabolism, which exert the neurotoxic effects after entering the nerve endings via the desipramine-sensitive NE transporter site. Backs et al. (4) reported that the decrease of cardiac NE uptake activity and neuronal NE transporter protein was a posttranscriptional phenomenon because NE transporter mRNA did not change significantly in the left stellate ganglion in heart failure. Cyclized NE orthoquinone is one of the oxidized metabolites of NE produced by the Mn3+-pyrophosphate complex. This reaction is coupled with formation of NE hydroquinone, which is unstable and capable of generating reactive oxygen species via continuous oxidation of NADH (34). Studies (28) have also shown that catecholquinones inactivate tyrosine hydroxylase in the tissue, suggesting a posttranslational mechanism for the decrease of tyrosine hydroxylase and neuronal damage. However, the precise mechanisms by which the NE metabolites exert the effects on NE transporter protein and NE and neuromodulatory effects on NE transporter site. Backs et al. (4) reported that the decrease of cardiac NE uptake activity and neuronal NE transporter protein was a posttranscriptional downregulation.

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