Cardiac Noradrenergic Nerve Terminal Abnormalities in Dogs With Experimental Congestive Heart Failure

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Background. We have shown previously that norepinephrine (NE) uptake activity is reduced in the failing right ventricle of animals with right heart failure (RHF) produced by tricuspid avulsion and progressive pulmonary constriction. However, it is unknown whether this defect in neuronal NE uptake is related to reduction of noradrenergic nerve terminals or whether these changes also occur in animals with left heart failure (LHF). It is also unknown whether increased NE release in heart failure contributes to the noradrenergic nerve abnormalities.

Methods and Results. We measured myocardial NE content, NE uptake function, and noradrenergic nerve profiles in dogs with either RHF or LHF induced by rapid ventricular pacing. NE uptake activity was measured using \[^3H\]NE, and noradrenergic nerve profiles were visualized by glyoxylic acid (SPG)-induced histofluorescence and tyrosine hydroxylase immunocytochemical staining. To study the effects of excess NE, we exposed normal dogs to 8 weeks of chronic NE infusion using subcutaneous osmotic minipumps. RHF and LHF animals exhibited reduced myocardial contractile function and congestive heart failure, as evidenced by reduced cardiac output and elevated right atrial pressure. However, unlike that in LHF, left atrial pressure was not increased in RHF. The animals also showed an increase in plasma NE and a decrease in cardiac NE. In addition, SPG-induced histofluorescence correlated significantly with NE uptake activity \((r=0.712, P<0.001)\) and tyrosine hydroxylase immunoreactive profiles \((r=0.569, P<0.001)\) in the right ventricles of RHF dogs and in both ventricles of LHF dogs. The numbers of catecholaminergic profiles and tyrosine hydroxylase profiles significantly correlated with cardiac filling pressures. Chronic infusion of NE decreased heart rate in normal dogs but had no effect on either mean aortic pressure or left atrial pressure; like heart failure, it resulted in significant decreases in myocardial NE uptake activity and numbers of SPG-induced catecholaminergic histofluorescence and immunoreactive tyrosine hydroxylase profiles.

Conclusions. Myocardial NE uptake activity was reduced only in the failing ventricles with elevated filling pressure in RHF and LHF. These changes probably were caused by loss of noradrenergic nerve terminals in the failing ventricles, as evidenced by the reductions of catecholaminergic histofluorescence and tyrosine hydroxylase immunostained profiles. Furthermore, since similar reductions of myocardial NE uptake and noradrenergic nerve profiles could be produced by chronic NE infusion in normal dogs, elevated NE levels may play a role in the development of cardiac noradrenergic nerve abnormalities in congestive heart failure. (Circulation. 1993;88:1299-1309.)

**Key Words** • congestive heart failure • sympathetic nerves • catecholamines • tyrosine hydroxylase • norepinephrine

We have shown in dogs with right heart failure (RHF) produced by progressive pulmonary constriction and tricuspid avulsion that myocardial \(\beta\)-adrenoeceptor density is reduced in the right ventricle.1 Since the reduction of myocardial \(\beta\)-adrenoceptors is prevented by the \(\beta\)-receptor-blocking agent nadolol,2 we speculate that the decrease in \(\beta\)-receptor density in RHF is caused by excessive noradrenergic stimulation, a phenomenon known as agonist-induced downregulation.3,4 However, since the decrease in \(\beta\)-adrenoceptors does not occur in the left ventricle that is exposed to the same elevated circulating norepinephrine (NE) as the right ventricle of the RHF animals, some local tissue factors must exist and play an important role in the chamber-specific \(\beta\)-adrenoceptor down-regulation in the failing right ventricle.1 Similar findings were recently reported in humans with RHF secondary to primary pulmonary hypertension,2 confirming the importance of local rather than systemic mechanisms in mediating the \(\beta\)-receptor downregulation.

Studies on adrenergic transmission6,7 have shown that the major mechanism responsible for removal of previously released NE into the interstitium and rapid termination of the effects of noradrenergic impulses in most organs involves active transport systems at the adrenergic nerve terminals that pump NE back to nerve terminals. Agents that inhibit the NE reuptake trans-
port mechanism are known to potentiate the effects of NE. To study the role of NE reuptake mechanism, we found that the ability of ventricular tissue slices incubated with [3H]NE to take up NE was reduced in the right ventricle of RHF dogs. Furthermore, because the reduced NE uptake activity in the right ventricle correlates significantly with the decrease in myocardial β-receptor density, we speculate that the NE uptake mechanism may be causally related to myocardial β-receptor downregulation. However, similar studies have not been performed in left heart failure (LHF). It is also unknown whether this defect in NE uptake is related to a reduction of noradrenergic nerve terminals. Thus, we carried out the present experiments to investigate whether myocardial NE uptake activity is reduced in LHF, using a rapid ventricular pacing model. The LHF model would also allow us to measure the NE uptake activity of the failing heart in vivo using an NE tracer technique developed by Rose et al., which cannot be used to measure right ventricular NE uptake. In addition, experiments were performed to determine whether the changes of myocardial NE uptake activity in both models of RHF and LHF are associated with reduced noradrenergic nerve profiles by measuring catecholaminergic histofluorescence and immunoreactive tyrosine hydroxylase, the rate-limiting enzyme for NE synthesis. Finally, to investigate the role of elevated NE on these changes, we administered NE to normal dogs, using subcutaneous osmotic minipumps. The studies indicate that elevated NE plays an important role in the changes of cardiac noradrenergic nerve terminals that occur in heart failure.

Methods

The present studies were approved by the University of Rochester Committee on Animal Resources and conformed to the guiding principles approved by the Council of the American Physiological Society and the National Institutes of Health Guide on the humane care and use of laboratory animals. Aseptic thoracotomies were performed in animals after intravenous sodium pentobarbital (25 mg/kg) anesthesia and under mechanical ventilation.

Animal Preparations

Right heart failure. Male adult mongrel dogs weighing 22 to 27 kg underwent a two-step surgical preparation to induce RHF using a modified technique of Barger et al. The surgical procedures that entailed staged right and left thoracotomies for anterior tricuspid valve avulsion, placement of a silicone rubber balloon occluder around the pulmonary artery, and insertion of indwelling catheters in the right atrium, main pulmonary artery, left atrium, and aorta, and a Konigsberg micromanometer (Konigsberg Instruments, Pasadena, Calif) in the left ventricle were described previously. Two weeks later, the balloon occluder was inflated progressively to constrict the pulmonary artery at 4- to 7-day intervals to produce RHF. Sham-operated dogs underwent two surgical thoracotomies without tricuspid valve avulsion and balloon occluder placement. Animals were studied 8 weeks after second surgery.

Left heart failure. Adult mongrel dogs weighing 21 to 26 kg underwent left thoracotomy for placement of Tygon catheters into the main pulmonary artery, left atrium, and aorta and a Konigsberg micromanometer into the left ventricle. One week later, a custom-designed implantable Medtronic multiprogrammable pulse generator (Medtronic, Minneapolis, Minn) was placed in a cervical pocket and connected to a bipolar transvenous ventricular pacing lead that was inserted into an external jugular vein and positioned at the right ventricular apex. Dogs were assigned to receive either rapid ventricular pacing at a rate of 225 beats per minute (LHF) or control pacing at a rate of 100 beats per minute. Final hemodynamic studies were performed after 8 weeks of pacing.

Chronic NE infusion. Adult mongrel dogs weighing 21 to 27 kg were anesthetized and instrumented with chronic indwelling catheters in the left atrium, main pulmonary artery, and descending aorta and a Konigsberg transducer into the left ventricle. One week later, an Alzet model 2ML4 osmotic minipump (Alza Corp, Palo Alto, Calif) was implanted subcutaneously at the nape of the neck under local anesthesia with xylcaine. The animals then received either normal saline or NE at a rate of 0.5 μg·kg⁻¹·min⁻¹. A second minipump was implanted 3 to 4 weeks later to maintain constant NE infusion for 8 weeks. Animals were studied at the end of 8 weeks of infusion.

Resting Hemodynamic Measurements

Right heart failure. Dogs were trained to lie quietly in a lateral decubitus position for the hemodynamic studies. The previously implanted catheters were attached to Spectramed P23XL (Spectramed, Inc, Oxnard, Calif) transducers and an 8-channel Brush model 480 recorder (Gould, Inc, Instruments System Division, Cleveland, Ohio) for measuring heart rate and right atrial, left atrial, and aortic pressures. The Konigsberg transducer was connected to the Brush recorder for measuring left ventricular pressure. The rate of rise of left ventricular pressure (dP/dt) was derived using an electronic differentiator. The ratio of left ventricular dP/dt at a developed pressure of 50 mm Hg during isovolumic systole and developed pressure (dP/dt/P), which has been shown to be relatively independent of ventricular afterload, was calculated as an index of left ventricular contractility. A transducer-tipped catheter (Millar Instruments Inc, Houston, Tex) was inserted via an external jugular vein under local xylcaine anesthesia for measuring right ventricular pressure and its peak dP/dt at least 1 hour before the start of hemodynamic measurements. Cardiac output was measured by the indocyanine green (Cardio-Green; Hynson, Westcott, & Dunning, Inc, Baltimore, Md) dye dilution technique, using a Gilford model 140 cardiac output system (Gilford Instrument Laboratories, Inc, Oberlin, Ohio).

Left heart failure. Dogs were prepared for the hemodynamic studies as above for the RHF animals, with the exceptions that no Millar catheter was inserted into the right ventricle and that a developed pressure of 25 mm Hg was used in the calculation for left ventricular dP/dt/P. Furthermore, in order to measure the intrinsic heart rate, the pacemaker was reprogrammed to a subthreshold level at least 2 hours before hemodynamic measurements.

Chronic NE infusion. The procedure for hemodynamic measurements was identical to those described above for the LHF dogs except that a developed pressure of 50 mm Hg was used in the calculation for left ventricular dP/dt/P.
TABLE 1. Resting Hemodynamics in Right and Left Heart Failure Dogs

<table>
<thead>
<tr>
<th></th>
<th>RHF model (n=16)</th>
<th>Control (n=18)</th>
<th>LHF model (n=14)</th>
<th>Control (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>27±1*</td>
<td>23±1</td>
<td>23±2</td>
<td>24±1</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>128±6‡</td>
<td>92±5</td>
<td>140±5‡</td>
<td>105±5</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>103±3*</td>
<td>112±3</td>
<td>96±3†</td>
<td>111±2</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>2.5±0.2‡</td>
<td>4.4±0.2</td>
<td>2.8±0.4*</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>LV peak dP/dt (mm Hg/s)</td>
<td>2104±86‡</td>
<td>3198±101</td>
<td>1436±56‡</td>
<td>2985±224</td>
</tr>
<tr>
<td>LV dP/dt/P (s⁻¹)</td>
<td>33±1‡</td>
<td>41±1</td>
<td>40±1†</td>
<td>47±2</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>13.4±0.8‡</td>
<td>4.8±0.5</td>
<td>9.8±0.9†</td>
<td>3.5±0.9</td>
</tr>
<tr>
<td>Left atrial pressure (mm Hg)</td>
<td>5.8±0.3*</td>
<td>7.7±0.6</td>
<td>30.2±2.1‡</td>
<td>8.0±1.0</td>
</tr>
<tr>
<td>RV peak dP/dt (mm Hg/s)</td>
<td>524±50*</td>
<td>669±35</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RV weight (g)</td>
<td>54±1‡</td>
<td>36±2</td>
<td>44±2*</td>
<td>36±3</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>91±4</td>
<td>99±3</td>
<td>109±4†</td>
<td>95±5</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>1107±99‡</td>
<td>637±24</td>
<td>846±35‡</td>
<td>644±25</td>
</tr>
<tr>
<td>Lung (g)</td>
<td>ND</td>
<td>ND</td>
<td>311±25†</td>
<td>228±11</td>
</tr>
</tbody>
</table>

Values are mean±SE. RHF indicates right heart failure; LHF, left heart failure; bpm, beats per minute; LV, left ventricular; RV, right ventricular; and ND, not done.

*P<.05, †P<.01, ‡P<.001 compared with sham-operated control dogs.

Resting systemic hemodynamic measurements were obtained in triplicate. The averages of the triplicate measurements were used for statistical analyses. Aortic blood was taken for resting NE determinations. In addition, a NE tracer technique was used to measure NE uptake kinetics in LHF and chronic NE infusion studies (see below).

After the hemodynamic studies, the animal was given a lethal dose (>100 mg/kg) of sodium pentobarbital. The heart, lungs, and liver were removed and weighed. The ventricles were separated from the septum and rinsed in an ice-cold oxygenated normal saline. The left ventricular weight included both the septum and left ventricular free wall; the right ventricular weight included only the free wall. Ventricular muscle blocks were removed quickly from ventricular free walls for measuring NE uptake activity, chemical NE content, and noradrenergic nerve profiles.

NE Tracer Study

A modification of the NE tracer technique described by Rose et al. was used to measure NE uptake kinetics in vivo. After the dog was sedated with intravenous morphine (6 mg) and locally anesthetized, angiographic catheters were inserted into the coronary sinus and the ostium of the left coronary artery via an external jugular vein and a carotid artery, respectively. A mixture of [¹⁴C]sucrose, [¹⁴C]sucrose, and [³H]NE was then injected into the coronary artery, and a timed, continuous sampling of coronary sinus blood (19 mL/min) was begun and continued during the ensuing 60 seconds. The activity for each of the three tracers in the coronary sinus samples was divided by the total activity injected to arrive at a relative extraction fraction. The extraction fraction was then plotted against time for each tracer. Using the computerized algorithms developed by Rose et al. and modified for use on an IBM PC using Turbo Pascal, the rate constants for the bidirectional diffusion of sucrose across the capillary membrane, the bidirectional diffusion of NE across the capillary membrane, and the unidirectional transport of NE into the adrenergic nerves from the interstitium (Kₐ) were calculated.

TABLE 2. Plasma and Myocardial Norepinephrine Contents in Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>RHF model (n=16)</th>
<th>Control (n=18)</th>
<th>LHF model (n=14)</th>
<th>Control (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma NE (pg/mL)</td>
<td>798±190*</td>
<td>198±18</td>
<td>1322±235*</td>
<td>220±57</td>
</tr>
<tr>
<td>Myocardial NE (ng/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>77±17*</td>
<td>1341±100</td>
<td>360±68*</td>
<td>1265±152</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>507±105*</td>
<td>1476±102</td>
<td>333±74*</td>
<td>1348±117</td>
</tr>
</tbody>
</table>

Values are mean±SE. NE indicates norepinephrine; RHF, right heart failure; and LHF, left heart failure.

*P<.001 compared with sham-operated control dogs.

Fig 1. Bar graphs showing the decreases in myocardial norepinephrine (NE) uptake activity measured in fresh ventricular slices in dogs (clear columns) with either right heart failure (RHF) or left heart failure (LHF). Bars indicate SE. *P<.001 compared with the sham-operated control dogs (striped columns).
NE Uptake Activity

Myocardial NE uptake activity was measured in quadruplicate by incubating fresh tissue slices at 37°C for 15 minutes in 50 nmol/L 1-[3H(N)]NE (13.8 Ci/mmol; New England Nuclear).\textsuperscript{8} Specific \(^3\)H-uptake activity, defined as the difference in radioactivity between tissue slices incubated in a \(^{3}\)H-NE-containing solution at 37°C and those at 4°C, is taken to approximate NE uptake activity.

Plasma and Myocardial NE Contents

Plasma and tissue NE were measured radioenzymatically,\textsuperscript{15} using Cat-A-Kit assay system (Amersham Corp, Arlington Heights, Ill). Tissue samples were minced and suspended in a 0.4N perchloric acid with 5 mmol/L reduced glutathione (pH 7.4), homogenized with a Brinkmann Polytron PCU-2 homogenizer (8-second bursts times 3 at setting 8; Brinkmann Instruments, Inc, Westbury, NY), and centrifuged at 500g. The supernatant was taken for the radioenzymatic assay.

Anatomic Studies of Ventricular Sympathetic Nerves

Glyoxylic acid--induced histofluorescence for catecholamines. Histofluorescence specific for catecholamines was done using a modification\textsuperscript{16} of the sucrose-potassium phosphate-glyoxylic acid (SPG) condensation method of de la Torre.\textsuperscript{17} 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 either longitudinal or cross section at thickness of 16 μm. Sections were picked up on glass slides, dipped in SPG solution, dried, heated under oil at 95°C for 2.5 minutes, coverslipped, and viewed under ultraviolet light using a Nikon fluorescence microscope equipped with epi-illumination accessories. All sections were photographed at the same magnification (×50) using 35-mm slide film. The number of stained catecholamine profiles were counted in a 0.221-mm\(^2\) (0.003536 mm\(^3\)) field; the results of five fields were summed to provide an average for each ventricle.

Immunochemistry for tyrosine hydroxylase. Ventricular muscle blocks were fixed for 24 hours 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 (pH 7.4) for an additional 24 hours 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 cut at 40 μm, and placed in 0.15 mol/L phosphate buffer. For the following procedure, the buffer was 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 minutes in 10% normal goat serum. The primary anti-tyrosine hydroxylase antibody (Eugene, Ore) was diluted (1:60 000) in 0.4% Triton X-100 in buffer plus 0.15% normal goat serum. Sections were incubated in the primary antibody for 24 hours at 4°C with gentle agitation. On the following day, sections were rinsed 10 times, each for 10 minutes in buffer, and then incubated in the biotinylated secondary antibody (goat anti-rabbit IgG diluted 1:1000 in buffer plus 0.15% normal goat serum) for 2 hours. Sections were subsequently rinsed in buffer six times each for 5 minutes and treated to remove endogenous peroxidase activity by incubating in 5% methanol, 1.5% hydrogen peroxide in phosphate buffer for 30 minutes. Sections were then incubated in avidin-biotin-peroxidase complex (Vector kit; Vector Laboratories, Inc, Burlingame, Calif; 40 μL of reagent A and 40 μL of reagent B in 20 mL of 0.15 mol/L phosphate buffer) for 2 hours. Sections were rinsed four times, 5 minutes each, in 0.1 mol/L sodium acetate with 10 mmol/L imidazole (pH 7.0) and then developed in acetate-imidazole buffer containing 0.1 mol/L nickel (II) sulfate, 0.03% dianisobenzidine, and 0.008% hydrogen peroxide for 5 minutes. All sections were then rinsed three times, 5 minutes each, in 0.15 mol/L phosphate buffer. Sections were finally mounted on gelatin-coated slides, dried, dehydrated through a series of ethanol, cleared in xylene, and coverslipped in Permount. Sections were viewed and photographed at the same magnification (×50) onto 35-mm slides. The number of tyrosine hydroxylase staining profiles were counted in a 0.00885-mm\(^3\) field; the results of five fields were averaged for each ventricle.

Statistical Analysis

Results are expressed as mean±SE. The experimental data were analyzed by Student’s \(t\) test for determining significance of differences between the RHF and sham-operated control dogs, between the LHF and control paced animals, and between NE-infused and saline-infused animals. The degree of relatedness between two variables was determined using Pearson’s correlation coefficient. A value of \(P<.05\) was considered statistically significant.

Results

Resting Hemodynamics in Heart Failure Animals

Table 1 shows the resting hemodynamics in the RHF and LHF studies. Dogs with RHF showed prominent ascites and heavier body weights than sham-operated control dogs. The LHF dogs also showed ascites, but the amount was variable and less than that in RHF dogs. There was no significant difference in body weight between the LHF and the control paced animals. Nevertheless, both RHF and LHF dogs exhibited a higher heart rate and lower mean aortic pressure, cardiac output, and left ventricular peak dP/dt and dP/dt/P compared with their respective control animals. As expected, there was an increase in right atrial pressure and reduction of right ventricular dP/dt in RHF dogs, whereas LHF was associated with an increased left atrial pressure. Left atrial pressure was lower in RHF than the sham-operated dogs. Unlike the selective increase in right ventricular weight in RHF, both right and left ventricles showed a slight increase in weight in LHF. LHF dogs also showed an increase in lung weight, whereas the liver weight was increased in both RHF and LHF dogs.
Fig 3. Facing page. Right ventricular tyrosine hydroxylase immunoreactive nerve profiles (brown) in a right heart failure (RHF, 387 profiles per field) and a sham-operated control dog (control, 1,074 profiles per field). Field, 0.00885 mm²; horizontal bar, 50 μm.

NE Tracer Study in LHF Animals

The rate constant for the bidirectional diffusion of sucrose across the capillaries did not differ between the LHF (0.056±0.009 s⁻¹, n=7) and control animals (0.056±0.011 s⁻¹, n=9). However, the rate constant for the unidirectional uptake of NE (Kn) was markedly reduced in the LHF dogs (0.246±0.019 s⁻¹) compared with control dogs (0.826±0.133 s⁻¹, P<.001). In contrast, the rate constant for the bidirectional diffusion of NE across the capillaries did not differ between the two groups (0.127±0.019 vs 0.152±0.045 s⁻¹).

NE Uptake Activity in Isolated Ventricular Slices

NE uptake activity was reduced in the right ventricular slices of RHF dogs compared with sham-operated dogs but did not differ significantly in the left ventricle between the two groups of animals (Fig 1). However, unlike the chamber-specific reduction of NE uptake activity in RHF dogs, NE uptake activity was reduced in both right and left ventricles of the LHF dogs. NE uptake activity did not differ significantly in LHF between the two ventricles. In addition, the in vitro measurements of left ventricular NE uptake activity correlated significantly with the in vivo rate constant for NE uptake (r=.871, P<.001).

Plasma and Myocardial NE Levels in Heart Failure

Congestive heart failure was associated with increased plasma NE and reduced myocardial NE in both RHF and LHF models (Table 2). The magnitude of NE reduction was significantly greater in the right ventricle than the left ventricle in RHF dogs. In contrast, NE content was reduced to a similar extent in the right and left ventricles in LHF dogs compared with control animals.

SPG Histofluorescence and Tyrosine Hydroxylase Immunostained Profiles

Right heart failure. Representative micrographs depicting the numbers of noradrenergic nerve profiles identified both by SPG-induced histofluorescence and tyrosine hydroxylase immunocytochemistry are shown for the right ventricles of RHF and sham-operated animals in Figs 2 and 3. The group results are presented in Table 3. The noradrenergic nerve profiles measured by both methods were reduced in both ventricles of RHF dogs compared with sham-operated dogs. As with cardiac NE content, a greater reduction in SPG histofluorescence profiles occurred in the right ventricle than the left ventricle in RHF dogs. However, unlike SPG histofluorescence, tyrosine hydroxylase stained nerve profiles did not decrease significantly in the left ventricles of RHF dogs.

Left heart failure. Tissue SPG histofluorescence profiles was reduced by 60% and 56% in the left and right ventricles of LHF dogs compared with control paced dogs (Table 3). Similar reductions also occurred in tyrosine hydroxylase immunostained profiles. The number of histofluorescence profiles significantly correlated with Kn (r=.773, P<.001).

Correlation Coefficients Between Cardiac NE, NE Uptake, and Tyrosine Hydroxylase Immunoreactive Profiles

To determine whether the reduced cardiac NE could be accounted for by reduced neuronal NE reuptake or impaired tyrosine hydroxylase function, we plotted myocardial SPG histofluorescence profiles against either NE uptake activity or tyrosine hydroxylase immunostained profiles. Fig 4 shows that SPG histofluorescence profiles correlated significantly with both NE uptake activity and tyrosine hydroxylase immunoreactive profiles in the ventricles with elevated filling pressures of RHF and LHF dogs. However, in the left ventricles of RHF dogs, SPG histofluorescence profiles correlated with neither NE uptake activity nor tyrosine hydroxylase immunostained profiles (Fig 5).

Correlation Coefficients Between Cardiac NE, Tyrosine Hydroxylase, and Cardiac Filling Pressure

Fig 6 shows a close correlation between the number of catecholaminergic nerve profiles and cardiac filling pressure in the RHF and LHF animals. Strong correlations also existed between tyrosine hydroxylase profiles and left atrial pressure (r=-.847, P<.0001) and between tyrosine hydroxylase profiles and right atrial pressure (r=-.827, P<.0001).

Effects of Chronic NE Infusion

Plasma NE was markedly elevated in dogs receiving NE infusion. At the final hemodynamic study, plasma NE was 4.59±0.49 ng/mL, which was 15 times that in the saline-infused animals (0.30±0.04 ng/mL, P<.0001). Table 4 shows that the two groups did not differ in body

| Table 3. Myocardial Noradrenergic Nerve Profiles in Heart Failure |
|-------------------------------------------------|--------------|--------------|--------------|--------------|
| Profile (number/field) | RHF model | Control | LHF model | Control |
| SPG histofluorescence | | | | |
| Right ventricle | 42±12† (16) | 452±25 (18) | 183±26* (14) | 453±24 (11) |
| Left ventricle | 335±21† (16) | 470±17 (18) | 195±36* (14) | 440±26 (11) |
| Tyrosine hydroxylase | | | | |
| Right ventricle | 255±43† (14) | 1002±47 (13) | 481±72* (11) | 808±45 (9) |
| Left ventricle | 802±59 (14) | 974±43 (13) | 524±106* (9) | 820±46 (8) |

Values are mean±SE. The number of measurements is indicated by the number within parentheses. RHF indicates right heart failure and LHF, left heart failure.

*P<.01, †P<.001 compared with appropriate sham-operated control animals.
weight, nor did they differ in mean aortic pressure, left atrial pressure, left ventricular peak \(\frac{dP}{dt}\), left ventricular \(\frac{dP}{dt}/P\), or cardiac output. There was, however, a significant decrease in heart rate in the NE-infused animals.

Table 5 shows that chronic NE infusion reduced the tissue SPG histochemistry profiles in the left and right ventricles by 40%. These changes in NE-infused animals were associated with reduced tyrosine hydroxylase immunoreactive profiles and myocardial NE uptake activity. Kn was also lower in the NE-infused animals (0.289±0.054 s\(^{-1}\)) than the saline-infused animals (0.956±0.203 s\(^{-1}\), \(P<.01\)). The chemical NE levels, however, did not differ significantly in the left ventricle between the NE (1154±182 ng/g, \(n=8\)) and saline (1565±122 ng/g, \(n=7\), \(t=1.81, P = .094\)) groups.

**Discussion**

Congestive heart failure was produced in both models of experimental heart failure. This was evidenced by the low cardiac output, elevated right atrial pressure, ascites, and hepatomegaly. However, unlike RHF, in which left atrial pressure is reduced, rapid ventricular pacing produces a state of biventricular heart failure as manifested by elevated right and left atrial pressures and an increased lung weight. The present studies extend our prior findings in RHF that cardiac NE content and NE uptake activity are reduced in animals with LHF. A significant correlation existed between the NE uptake activity measured in vitro using the left ventricular tissue slices and the rate constant for the unidirectional NE uptake obtained in vivo from the NE tracer technique.

Our present studies further show that the noradrenergic nerve profiles as demonstrated by SPG-induced catecholaminergic histochemistry and tyrosine hydroxylase immunocytochemistry are reduced in the failing ventricles. In addition, NE uptake-1 (mazindol-binding) site density\(^6\) and neuropeptide \(\text{Y}^{18}\) are reduced in the failing myocardium. The NE uptake-1 site is a plasma membrane marker, whereas neuropeptide \(\text{Y}\) and NE are contained in different intraneuronal storage vesicles but are released together after sympathetic stimulation.\(^19\) Since the NE uptake-1 site and neuropeptide \(\text{Y}\) are structurally and functionally distinct from the NE storage vesicles and tyrosine hydroxylase profiles, the noradrenergic nerve abnormalities in congestive heart failure cannot be explained by a defect in the NE synthesis, storage, or release processes alone. These changes most likely are caused by loss of adrenergic nerve terminals in the failing ventricle. However, the increase in cardiac sympathetic activity in heart failure\(^20\) could also contribute to the reduction of cardiac NE storage, particularly if the NE reuptake mechanism is impaired. Sympathetic denervation has been shown by electron microscopy in atrial myocardium obtained from humans with congestive heart failure.\(^21\) Furthermore, because tyrosine hydroxylase activity was not reduced in the stellate ganglia of the LHF dogs (unpublished data), the neuronal loss probably is caused by a local factor within the failing myocardium rather than by a descending degenerative process from the nerve bodies.

Although its filling pressure was not elevated, the left ventricle showed reduced contractile function in RHF. This was accompanied by reduced cardiac chemical NE content and SPG-induced histochemistry profiles; these changes, however, were much smaller than those in the failing right heart of the same animals. Furthermore, cardiac NE store showed a greater proportional reduction (66%) than SPG-induced histochemistry...
profiles (29%) in the left ventricles of RHF dogs. This is probably related to the higher NE content in the left ventricles as a concentration-dependent quenching of the SPG-induced fluorescence occurs when the tissue NE exceeds 40% of the normal NE concentration. Unlike the right ventricle, the left ventricle showed no significant decreases in tyrosine hydroxylase immunoreactive nerve profiles or NE uptake activity in RHF dogs. The decrease in left ventricular NE in RHF could have been caused by an increased release and washout of NE from the cardiac tissue.

Results of our present study further suggest that while neuronal NE content is reduced, tyrosine hydroxylase immunoreactive profiles did not change significantly in the left ventricles of RHF dogs, suggesting that the noradrenergic nerves are relatively intact in the left ventricle. The decrease in SPG-induced catecholaminergic histofluorescence probably was due to the concentration of NE in some nerves being below the threshold of sensitivity for the SPG technique. Nevertheless, the SPG method is sufficiently sensitive to demonstrate nerves that are partially depleted of NE, as evidenced by the greater reduction of tissue chemical NE content than the nerve histofluorescence profiles. Moreover, since tyrosine hydroxylase immunostained profiles were not reduced in depressed left ventricles without elevated filling pressures in RHF, the increase in cardiac filling pressure may play an important role, either directly or indirectly, in causing the loss of noradrenergic nerve terminals in heart failure. This is further supported by our pilot studies in dogs without tricuspid avulsion that NE uptake activity did not decrease after progressive pulmonary constriction, which produced right ventricular hypertrophy without elevated right atrial pressure.

Prior studies have shown that cardiac neurotransmitter activity is altered in heart failure. Decrease in cardiac NE content has been amply demonstrated in humans and animals with congestive heart failure. However, changes in cardiac NE synthesis in heart failure have been limited to determinations of certain synthetic enzyme activities. Cardiac tyrosine hydroxylase activity decreased in human congestive heart failure. Similarly, tyrosine hydroxylase activity was reduced in the right ventricles of dogs with tricuspid avulsion and pulmonary occlusion for 2 to 8 weeks. The left ventricle showed a relatively small reduction of tyrosine hydroxylase activity during this period. However, tyrosine hydroxylase activity could be reduced to a much lower level in the left ventricle in dogs with RHF for more than 2 years of duration. On the other hand, dopamine β-hydroxylase activity was reduced in the cardiomyopathic Syrian hamster. We know of no published studies that applied the immunohistochemical technique to investigate cardiac innervation in our animal models of congestive heart failure. Nevertheless, a recent study by Wharton et al., using an immunohistochemical technique similar to ours did show a moderate number of tyrosine hydroxylase immunostained nerve fibers in the ventricular muscles in cardiac transplant recipient hearts. However, because neither the degree of heart failure was documented nor normal control hearts were included in that study, it cannot be stated whether the density of the tyrosine hydroxylase immunostained nerve fibers are reduced in the patients with end-stage cardiomyopathies.

As stated previously, an increase in cardiac sympathetic discharges and release of NE into the coronary sinus have been used to explain myocardial NE depletion in heart failure. However, Spann et al., using an in vivo labeling technique, found the absolute levels of specific activity and the rates of disappearance of radiolabeled NE were identical in the left ventricles of normal guinea pigs and animals with heart failure produced by aortic constriction. They concluded that

---

**Table 4.** Resting Hemodynamics in Norepinephrine-Infused Dogs and Saline-Infused Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NE infusion (n=9)</th>
<th>Saline infusion (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>24.4±0.8</td>
<td>22.6±0.8</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>83±4*</td>
<td>105±5</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>121±4</td>
<td>115±3</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>4.01±0.49</td>
<td>4.73±0.31</td>
</tr>
<tr>
<td>LV peak dp/dt (mm Hg/s)</td>
<td>3805±238</td>
<td>3358±130</td>
</tr>
<tr>
<td>LV dp/dt/P (s⁻¹)</td>
<td>45±1</td>
<td>44±1</td>
</tr>
<tr>
<td>Left atrial pressure (mm Hg)</td>
<td>8.3±0.9</td>
<td>8.5±0.5</td>
</tr>
<tr>
<td>RV weight (g)</td>
<td>38±2</td>
<td>37±2</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>118±4</td>
<td>108±5</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>696±46</td>
<td>614±23</td>
</tr>
</tbody>
</table>

Values are mean±SE. The number of experiments in each group is given in parentheses under the heading. NE indicates norepinephrine and bpm, beats per minute.

*P<.001 compared with saline-infused dogs.

---

**Table 5.** Myocardial Noradrenergic Nerve Profiles in Norepinephrine-Infused Dogs and Saline-Infused Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NE infusion</th>
<th>Saline infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPG histofluorescence (profiles/field)</td>
<td>Right ventricle 249±33* (9)</td>
<td>431±19 (10)</td>
</tr>
<tr>
<td></td>
<td>Left ventricle 261±20* (9)</td>
<td>430±26 (10)</td>
</tr>
<tr>
<td>Tyrosine hydroxylase (profiles/field)</td>
<td>Right ventricle 487±36* (9)</td>
<td>890±52 (7)</td>
</tr>
<tr>
<td></td>
<td>Left ventricle 514±35* (9)</td>
<td>871±44 (7)</td>
</tr>
<tr>
<td>NE uptake activity (fmol/mg/15 min)</td>
<td>Right ventricle 76±11† (9)</td>
<td>138±8 (10)</td>
</tr>
<tr>
<td></td>
<td>Left ventricle 80±16† (9)</td>
<td>132±7 (10)</td>
</tr>
</tbody>
</table>

Values are mean±SE. NE indicates norepinephrine. The numbers of experiments are given in parentheses.

*P<.001, †P<.01 compared with saline-infused dogs.
NE release was not increased in the failing heart. Other studies\textsuperscript{11,12,25} have shown that cardiac NE release is reduced in patients with heart failure.

The discrepancies in the above studies may be related to differences in animal models, duration and degree of heart failure, or methods of measurement. Close examination of the state of the noradrenergic nerves in the failing heart may help to resolve these discrepancies among various conditions. We have observed similar findings in two very different models of heart failure suggesting that localized abnormalities of the noradrenergic nerve terminals may have a pathophysiological role in the failing myocardium. These findings also suggest that the changes in noradrenergic nerve profiles cannot be explained by surgical implantation of pulmonary artery occluders or insertion of pacemakers. One possible scenario that seems to explain many of the findings discussed above may also help account for some of the discrepancies in the literature. With the induction of heart failure, cardiac noradrenergic activity increases reflexly to compensate for decreased cardiac output and arterial pressure. The increased cardiac filling pressure may enhance local NE release and predispose the failing ventricle to develop reduced neuronal NE uptake via an unidentified local mechanism. The increased neuronal release and reduced reuptake of NE will then lead to a higher interstitial NE concentration and lower tissue NE content. An increased interstitial NE concentration was recently demonstrated in the pacing-induced failing left ventricle.\textsuperscript{13} If NE depletion is severe enough, SPG-induced histofluorescence will not be sensitive enough to visualize nerves. Under normal conditions, one would expect tyrosine hydroxylase activity to increase to keep up with increased demands for NE. However, in the present study, tyrosine hydroxylase stained profiles were actually decreased in the failing ventricle. These results suggest that the nerves themselves are damaged. As nerves become more damaged, particularly as they lose the capacity for NE synthesis, NE release would actually decrease. Finally, over a long time course, there may be repair or regrowth of nerves, further confusing the picture of damage. From the current study, the implications are that some process is damaging sympathetic nerves to the point at which they are not able to produce enough tyrosine hydroxylase to be visualized in the failing heart with elevated filling pressure.

In the present study, we have provided direct evidence that chronic NE infusion could lead to cardiac noradrenergic nerve abnormalities similar to those found in the failing myocardium. These findings suggest that excess NE plays a primary, pivotal role in the pathogenesis of cardiac noradrenergic nerve damage and that the elevated cardiac filling pressure is not essential for development of noradrenergic nerve abnormalities. However, these changes of noradrenergic nerve terminals occur at much higher plasma NE levels in the NE-infused animals than the LHF dogs. The increased wall tension imposed by the elevated cardiac filling pressure in congestive heart failure may be injurious and contributes to the noradrenergic nerve abnormalities by either causing local NE release or predisposing the cardiac noradrenergic nerves to the neurotoxic effect of NE in the heart.

Unlike that in congestive heart failure, the central sympathetic nervous system may be inhibited in NE-infused dogs. The decrease in heart rate in the NE-infused dogs, which also has been reported previously,\textsuperscript{36,37} may be a manifestation of the centrally mediated cardiac noradrenergic nerve activity inhibition. However, inhibition of noradrenergic nerve activity would not explain the concomitant reductions of NE uptake activity, SPG catecholaminergic profiles, and tyrosine hydroxylase immunostained profiles observed in our NE-infused dogs.

Noradrenergic nerve damage has been shown to occur in saphenous veins after local infusions of NE.\textsuperscript{38} The changes are similar to those produced by 6-hydroxydopamine.\textsuperscript{39,40} Since chemical sympathomectomy is prevented by desipramine or superoxide dismutase, the nerve damage produced by NE has be speculated to be caused by oxygen free radicals derived from NE with an action involving the neuronal uptake mechanism.\textsuperscript{38}

Additional studies are warranted to determine the exact mechanisms by which excess NE causes cardiac denervation and whether desipramine or superoxide dismutase can be used to prevent cardiac denervation in heart failure.

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Cardiac noradrenergic nerve terminal abnormalities in dogs with experimental congestive heart failure.

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