Desipramine attenuates loss of cardiac sympathetic neurotransmitters produced by congestive heart failure and NE infusion

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Liang, Chang-seng, Akito Yatani, Yoshihiro Himura, Michihiro Kashiki, and Susanne Y. Stevens. Desipramine attenuates loss of cardiac sympathetic neurotransmitters produced by congestive heart failure and NE infusion. Am J Physiol Heart Circ Physiol 284: H1729–H1736, 2003.—We reported recently that inhibition of neuronal reuptake of norepinephrine (NE) by desipramine prevented the reduction of sympathetic neurotransmitters in the failing right ventricle of right heart failure animals. In this study, we studied whether desipramine also reduced the sympathetic neurotransmitter loss in animals with left heart failure induced by rapid ventricular pacing (225 beats/min) or after chronic NE infusion (0.5 μg·kg⁻¹·min⁻¹). Desipramine was given to the animals for 8 wk beginning with rapid ventricular pacing or NE infusion. Animals receiving no desipramine were studied as controls. We measured myocardial NE content, NE uptake activity, and sympathetic NE, tyrosine hydroxylase, and neuropeptide Y profiles by histofluorescence and immunocytochemical techniques. Effects of desipramine on NE uptake inhibition were evidenced by potentiation of the pressor response to exogenous NE and reduction of myocardial NE uptake activity. Desipramine treatment had no effect in sham or saline control animals but attenuated the reduction of sympathetic neurotransmitter profiles in the left ventricles of animals with rapid cardiac pacing and NE infusion. In contrast, the panneuronal marker protein gene product 9.5 profile was not affected by either rapid pacing or NE infusion, nor was it changed by desipramine treatment in the heart failure animals. The study confirms that excess NE contributes to the reduction of cardiac sympathetic neurotransmitters in heart failure. In addition, it shows that the anatomic integrity of the sympathetic nerves is relatively intact and that the neuronal damaging effect of NE involves the uptake of NE or its metabolites into the sympathetic nerves.

neuronal uptake activity; histofluorescence; tyrosine hydroxylase; neuropeptide Y; protein gene product 9.5

Earlier studies from our laboratory have shown that right heart failure produced by progressive pulmonary constriction and tricuspid valve avulsion is associated with a selective reduction of norepinephrine (NE) reuptake and downregulation of myocardial β-adrenoceptor density in the failing right ventricle (33). The sympathetic nerve terminal profiles, as measured by catecholaminergic histofluorescence, and tyrosine hydroxylase and neuropeptide Y immunocytochemistry are also reduced only in the failing right ventricle (27). These findings suggest that the reductions of myocardial β-adrenoceptors and sympathetic nerve neurotransmitters in congestive heart failure (CHF) are caused by local mechanisms occurring only in the failing myocardium; the correspondent left ventricle is relatively unaffected despite exposure to the same systemic elevation of plasma catecholamines (33). A similar chamber specific reduction of myocardial β-adrenoceptors was reported in humans with right heart failure secondary to primary pulmonary hypertension (7).

More recently, we showed that the chamber-specific reduction of myocardial β-adrenoceptor density was abolished by the administration of desipramine, a NE uptake inhibitor, which reduced NE uptake activity in both the right and left ventricles of right heart failure animals (34). These findings support a hypothesis that β-receptor downregulation is caused by increased interstitial NE (16) in animals because of either reduced reuptake or increased release of NE. Chronic administration of excess NE has also been shown to cause similar cardiac sympathetic nerve terminal dysfunction as that in heart failure (27). In contrast, desipramine prevented the reduction of sympathetic nerve neurotransmitters in the failing right ventricle (34). Likewise, desipramine prevented degenerative changes of sympathetic nerve varicosities in the saphenous vein produced by chronic intravenous infusion of NE (2). The purpose of this study was to determine whether desipramine treatment also attenuated the cardiac sympathetic neurotransmitter loss that occurs after prolonged NE infusion or in pacing-induced cardiomyopathy (27). Cardiac noradrenergic nerve terminal function was determined by measuring myocardial NE uptake activity, NE histofluorescence, and tyrosine hydroxylase and neuropeptide Y immunocytochemistry. Tyrosine hydroxylase is the rate-limiting step in the biosynthesis of NE (31). Neuropeptide Y is a sympathetic neurotransmitter that is coreleased with NE (37).
but is under different controls of biosynthesis and metabolism (23). In addition, to determine whether there was structural loss of the sympathetic nerve fibers, we measured tissue protein gene product (PGP) 9.5, a pan-neuronal marker (24, 28) unrelated to sympathetic neurotransmitters.

METHODS

The present study was approved by the University of Rochester 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.

Surgical Preparation of Animals

Two experimental protocols were included in this study. Adult mongrel dogs weighing 17–30 kg were used. All animals underwent an initial sterile left thoracotomy under general anesthesia with intravenous pentobarbital sodium (25 mg/kg) and mechanical ventilation using a respirator (Harvard Apparatus; South Natick, MA) for placement of a heparin-filled Tygon catheter (1.02 mm inner diameter, Norton; Akron, OH) in the left atrium, main pulmonary artery, and descending aorta. An implantable micromanometer (Konigsberg Instruments; Pasadena, CA) was inserted into the apex of the left ventricle. The catheters and the lead from the micromanometer were exteriorized at the nape of the neck. One week later, the animals were assigned to receive either an implantable pacemaker (protocol 1) or a subcutaneous osmotic minipump (protocol 2).

Protocol 1: CHF. A Medtronic LEGEND SSIR model 8416 or MINIX SSI model 8340 multiprogrammable pulse generator (Minneapolis, MN) modified for rapid cardiac pacing for animal use was placed in a cervical pocket and connected to a bipolar transvenous ventricular pacing lead, which was inserted into an external jugular vein and positioned to the apex of the right ventricle. Dogs were assigned to receive either rapid ventricular pacing at a rate of 225 beats/min (CHF group) or control pacing at a rate of 100 beats/min (sham group). Pacing was maintained throughout the study period as verified by weekly electrocardiographic and hemodynamic recordings. CHF was assessed by the development of tachypnea and ascites and elevated left atrial pressures. The Konigsberg micromanometer was connected to the Gould recorder for measuring left ventricular pressure and the first derivative of left ventricular pressure (dP/dt) using an electronic differentiator. Arterial samples were taken for measuring plasma NE (43). Heart rate was obtained from the electrocardiogram. Cardiac output was measured by injecting indocyanine green (Cardio-Green, Hynson, Westcott & Dunning; Baltimore, MD) into the pulmonary artery and sampling the arterial blood for dye concentrations with a Gilford model 140 cardiac output system (Oberlin, OH). Resting hemodynamic measurements were made in triplicate at least 5 min apart, at least 1 h after insertion of the Millar catheter. Averages of the triplicate measurements were used for statistical analyses. All measurements, including the following biochemical and histoimmunocytochemical studies, were performed by individuals without knowledge of the individual animal’s group assignment.

Experimental Design

Animals were randomly divided into two experimental subgroups under each study protocol, according to desipramine assignment. Desipramine (Merrell Dow; Cincinnati, OH) was administered orally at a dose of 225 mg once daily for 8 consecutive weeks beginning with either the beginning of pacing or pump infusion. At this dose, desipramine produced significant inhibition of tissue NE uptake in the heart and was also well tolerated by the animals (34). In this study, a bolus dose of 0.5 μg/kg NE was given intravenously at weeks 0 and 8 to demonstrate that the pressor response to NE was exaggerated after 8 wk of desipramine treatment. After each NE injection, the animals were monitored continuously until the hemodynamic changes returned to baseline. At week 8 after completion of the hemodynamic studies, animals were euthanized with pentobarbital sodium (>100 mg/kg) at least 20 min after NE administration. After death, the hearts were excised immediately, and the right and left ventricles were separated and weighed. The left ventricular weight included both the septum and left ventricular free wall. Muscle blocks were removed from the left ventricular free wall 3 cm below the atrioventricular groove. Fresh muscle samples were used for measuring myocardial NE uptake activity. The remainder of tissue blocks was used for measuring tissue NE content and sympathetic nerve histofluorescence and immunochemical studies.

Resting Hemodynamic Measurements

Animals were acclimatized to the laboratory and personnel and trained to lie in a lateral decubitus position with minimal restraint on a table by the time of final hemodynamic measurements. For animals with rapid cardiac pacing, the pacemaker was reprogrammed to a subthreshold level at least 2 h before the final hemodynamic measurement. In all animals, the previously implanted intravascular catheters were connected to Spectramed P23XL pressure transducers (Oxnard, CA) and an eight-channel Brush model 480 recorder (Gould; Cleveland, OH) for measuring left atrial and aortic pressures. The Konigsberg micromanometer was connected to the Gould recorder for measuring left ventricular pressure and the first derivative of left ventricular pressure (dP/dt) using an electronic differentiator. Arterial samples were taken for measuring plasma NE (43). Heart rate was obtained from the electrocardiogram. Cardiac output was measured by injecting indocyanine green (Cardio-Green, Hynson, Westcott & Dunning; Baltimore, MD) into the pulmonary artery and sampling the arterial blood for dye concentrations with a Gilford model 140 cardiac output system (Oberlin, OH). Resting hemodynamic measurements were made in triplicate at least 5 min apart, at least 1 h after insertion of the Millar catheter. Averages of the triplicate measurements were used for statistical analyses. All measurements, including the following biochemical and histoimmunocytochemical studies, were performed by individuals without knowledge of the individual animal’s group assignment.

Myocardial NE Uptake Activity

Myocardial NE uptake activity was measured in quadruplicate by incubating fresh tissue slices at 37°C for 15 min in 50 nM L-[3H](N)NE (13.8 Ci/mmol, New England Nuclear) (33). Nonspecific accumulation of radioactivity was determined by parallel incubation of quadruplicate tissue slices at 4°C. Specific 3H uptake activity, defined as the difference in radioactivity between tissue slices incubated in a 3HNE-containing solution at 37°C and those at 4°C, is considered to approximate NE uptake activity (33).

Plasma and Myocardial NE Contents

Plasma and tissue NE were measured radioenzymatically (43) using a Cat-A-Kit assay system (Amersham; Arlington Heights, IL). Tissue samples were minced, suspended in 0.4 N perchloric acid with 5 mmol/l reduced glutathione (pH 7.4), homogenized with a Brinkman Polytron PCU-2 homogenizer
(8-s bursts × 3 at setting 8, Brinkman Instruments; Westbury, NY), and centrifuged at 500 g. 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 performed using a modification (6) of the sucrose-potassium phosphate-glyoxylic acid (SPG) condensation method of de la Torre (15). Tissue blocks from the fresh heart were rapidly frozen on dry ice and stored in liquid nitrogen. Blocks were mounted on a frozen tissue microtome and cut in sections 16 μm thick. Sections were picked up on the glass slides, dipped in SPG solution, dried, and stored at −20°C for 2.5 min, coverslipped, and viewed under epifluorescent illumination using a Nikon fluorescence microscope equipped with filters designed for catecholamine fluorescence visualization. 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-mm² (0.003536 mm³) field; the results of five fields were summed to provide an average for each ventricle.

Immunocytochemistry for tyrosine hydroxylase, neuropeptide Y, and PGP 9.5. Ventricular muscle blocks were fixed for 24 h in 4% paraformaldehyde in 0.15 mol/l phosphate buffer (pH 7.4) at 4°C. The blocks were then transferred to 25% sucrose in 0.15 mol/l phosphate (pH 7.4) and prepared for cryostat sectioning (20°C) for either longitudinal or cross section at a thickness of 16 μm. Sections were stained for tyrosine hydroxylase (1:60,000 dilution, Chemicon; Temecula, CA), neuropeptide Y (1:8,000 dilution, Incstar; Stillwater, MN), or PGP 9.5 (1:20,000 dilution, Accurate Chemical and Scientific; Westbury, NY) in 0.4% Triton X-100 in buffer plus 0.15% normal goat serum for 24 h at 4°C with gentle agitation. On the following day, sections were rinsed and incubated in biotinylated secondary antibody (goat anti-rabbit IgG diluted 1:1,000 in buffer plus 0.15% normal goat serum), followed by avidin-biotin-peroxidase complex (Vector kit; Vector Laboratories, Burlingame, CA; 20 μl reagent A and 20 μl reagent B in 20 ml of 0.15 M phosphate buffer). Sections were rinsed four times, 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. 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) as a photographic montage onto 35-mm slides. The nerve profiles projected onto a graph paper were counted morphometrically (6) in a 0.00885-mm³ field. The results of five fields were averaged for each ventricle.

Statistical Analysis

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 unpaired data for comparison of difference between two group means and by two-way ANOVA and multiple-range tests for determining the significance of difference of the means among the subgroups. A P value < 0.05 was considered statistically significant.

RESULTS

Effects of Desipramine on NE Pressor Response and Myocardial NE Uptake

Figure 1 summarizes the peak pressor responses to intravenous NE before and after desipramine treatment in both cardiac pacing and infusion protocol animals. NE injection caused an abrupt increase of blood pressure, which returned to the basal value within 3 min. It increased mean aortic pressure by 35–40 mmHg in animals at baseline (week 0). The pressor response to NE was potentiated in animals after 8 wk of desipramine treatment. In contrast, the pressor response at week 8 did not differ from the baseline values in control animals receiving no desipramine. Figure 1 also shows that the magnitude of the pressor response to NE was smaller in desipramine-treated CHF and NE animals compared with their respective control sham and vehicle animals.

The efficacy of desipramine on NE inhibition was also evidenced by the reduction of myocardial NE uptake activity (Fig. 2). Figure 2 also shows that compared with the sham-operated and vehicle-infused animals, myocardial NE uptake activity was reduced in the CHF and NE-infused animals without desipramine treatment.

Resting Hemodynamics

Table 1 shows the resting hemodynamics and plasma NE concentration in the pacing protocol ani-
There were no significant differences in body weight among the CHF and sham groups. Left ventricular weight tended to be greater in the CHF animals, but the difference between the CHF and sham animals was not statistically significant. Compared with the sham animals, the CHF animals exhibited an increased heart rate, elevated left atrial pressure, and lower mean aortic pressure, cardiac output, and left ventricular peak first derivative of pressure rise ($dP/dt$). Plasma NE concentration was increased in CHF animals. Desipramine treatment produced no statistically significant changes in any of the hemodynamic parameters in either the sham or CHF animals, nor did desipramine affect plasma NE concentrations in the sham or CHF animals.

Table 2 shows the effects of chronic NE infusion and desipramine treatment in the infusion protocol animals. Table 2 shows that despite the marked elevations in plasma NE concentration, there were no changes in basal hemodynamics and ventricular weight in the NE animals except for a significant decrease in heart rate. Desipramine treatment produced no statistically significant changes in any of the resting hemodynamic parameters and plasma NE concentration in the NE and saline vehicle groups, although the plasma NE concentration and left ventricular $dP/dt$ tended to be greater in the NE animals treated with desipramine.

**Myocardial NE and SPG Histofluorescence**

Figure 3 shows that left ventricular NE content was reduced in CHF animals, regardless of whether it was measured by a radioenzymatic assay or SPG histochemical fluorescence. Desipramine treatment produced a small reduction of myocardial NE in sham animals, but left ventricular NE content increased slightly in desipramine-treated CHF animals compared with the untreated animals. Saline infusion produced no effect on myocardial NE content (vehicle group). In contrast, NE infusion reduced myocardial NE histofluorescence, which was attenuated by desipramine. Desipramine treatment had no effect on left ventricular NE content in the vehicle group.

**Sympathetic Neurotransmitter Immunocytochemistry**

Figure 4 shows that myocardial tyrosine hydroxylase- and neuropeptide Y-immunoreactive profiles were reduced by 50–70% in the CHF animals. Desipramine treatment had no effect on the myocardial tyrosine hydroxylase and neuropeptide immunoreactive profiles of the sham animals but significantly attenuated the reductions of both neurotransmitter profiles that occurred in the left ventricles of CHF animals.

Figure 4 also shows that the tyrosine hydroxylase and neuropeptide Y profiles were significantly reduced in the left ventricles of NE-infused animals. Desipramine treatment affected neither neurotransmitter profiles in the saline-infused animals. In contrast, desipramine reduced the reductions of myocardial tyrosine hydroxylase- and neuropeptide Y-immunostained profiles that occurred in the NE-infused animals.

**PGP 9.5 Immunocytochemistry**

Figure 5 shows the PGP 9.5-immunostained profiles in the left ventricles of the various experimental groups. Figure 5 shows that, unlike the sympathetic

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**Table 1. Resting hemodynamics, heart weights, and plasma [NE] in sham-operated and tachycardia-induced CHF animals**

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
<th>Desipramine</th>
<th>CHF Control</th>
<th>Desipramine</th>
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<td>$n$</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, kg</td>
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<td>23±1</td>
<td>23±1</td>
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<td>95±3*</td>
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<tr>
<td>Left atrial pressure, mmHg</td>
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<td>7±1</td>
<td>25±2*</td>
<td>26±2*</td>
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<td>LV $dP/dt$, mmHg/s</td>
<td>3,073±146</td>
<td>3,032±189</td>
<td>1,489±38*</td>
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<td>Cardiac output, l/min</td>
<td>4.22±0.31</td>
<td>3.90±0.26</td>
<td>2.60±0.17*</td>
<td>2.94±0.29*</td>
</tr>
<tr>
<td>Plasma [NE], ng/ml</td>
<td>0.22±0.06</td>
<td>0.20±0.05</td>
<td>1.32±0.23*</td>
<td>1.20±0.20*</td>
</tr>
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</table>

Values are means ± SE; $n$, no. of animals. [NE], norepinephrine concentration; CHF, congestive heart failure; $dP/dt$, peak first derivative of the left ventricular (LV) pressure rise. *P < 0.05 vs. sham control, as measured by two-way ANOVA and multiple-range comparisons.
neurotransmitters tyrosine hydroxylase and neuropeptide Y, left ventricular PGP 9.5 profiles were affected by neither CHF nor NE infusion. Desipramine treatment did not significantly alter the number of PGP 9.5 profiles in the CHF or sham-operated animals.

**DISCUSSION**

Myocardial NE depletion is a well-known hallmark of CHF (10). This reduction of myocardial NE is caused by a number of factors, including increased release, decreased synthesis, and reduced reuptake of NE (11, 18, 27, 44, 45). Tyrosine hydroxylase is the rate-limiting step in the biochemical synthesis of NE. Early studies have demonstrated that cardiac tyrosine hydroxylase activity is reduced in both human CHF (18) and animals with experimental CHF (44). More recently, we have shown that tyrosine hydroxylase content is diminished in animals with CHF using either immunohistochemistry (27, 29, 33, 35) or Western blot analysis (unpublished data). The reduction of cardiac tyrosine hydroxylase was confirmed in the present study in animals with pacing-induced cardiomyopathy and NE infusion. Thus decreased tyrosine hydroxylase function is likely to be responsible, at least in part, for the reduction of myocardial NE in CHF. A decrease in

![Fig. 3](image-url) Effects of desipramine treatment on myocardial tissue NE content as measured by either a radioenzymatic method (A) or sucrose-potassium phosphate-glyoxylic acid-induced histofluorescence (B) in tachycardia-induced CHF and NE animals. The number of experiments in each group is the same as that shown in Fig. 1. Bars indicate SEs. *P < 0.05 vs. control sham or vehicle animals receiving no desipramine; †P < 0.05 vs. control CHF or NE animals; ‡P < 0.05 vs. desipramine-treated sham or vehicle animals.

![Fig. 4](image-url) Effects of desipramine treatment on myocardial tyrosine hydroxylase-immunostained profiles (A) and neuropeptide Y-immunostained profiles (B) in tachycardia-induced CHF and NE animals. The number of experiments in each group is the same as that shown in Fig. 1. Bars indicate SEs. *P < 0.05 vs. control sham or vehicle animals receiving no desipramine; †P < 0.05 vs. control CHF or NE animals; ‡P < 0.05 vs. desipramine-treated sham or vehicle animals.
Desipramine also increases NE transmitter washout and reduces NE clearance and increased plasma NE (11). Desipramine also increases NE transmitter washout with stimulation of the sympathetic nerves of supply at a high rate (12). Thus the effects of desipramine on plasma NE may vary depending on the experimental conditions and duration of administration. The effects of desipramine are expected to be greater in a heightened sympathetic state when the contribution of NE reuptake for NE spillover to plasma is increased. This may account for the slight, albeit statistically insignificant, increase of plasma NE in the desipramine-treated NE infused animals.

The dose of desipramine chosen for this study was sufficient to produce a 60–70% reduction of myocardial NE uptake (Fig. 2). The exaggerated pressor responses to exogenous NE in the desipramine-treated animals (Fig. 1) are consistent with the known NE uptake inhibitory action of desipramine on peripheral blood vessels. Similar responses have been reported previously (32, 34). The inhibitory action of desipramine on NE uptake may be responsible in part for the small reduction of myocardial NE in sham animals, but this action per se probably does not account for the increase of myocardial NE in desipramine-treated CHF animals. Furthermore, our results indicate that myocardial NE content was not increased by exogenous administration of subhypertensive doses of NE. The bolus dose of NE used to demonstrate the exaggerated pressor response of NE by desipramine produced only a modest transient increase of blood pressure in the animals and was unlikely to be responsible for the increase of myocardial NE in desipramine-treated animals.

Desipramine treatment exerted no significant effects on the resting hemodynamics in sham-operated and vehicle control animals, nor did it change plasma NE concentration in the animals. Earlier studies (20, 21, 47) also showed no effects of desipramine on plasma NE, probably because desipramine has a sympathoinhibitory effect via stimulation of presynaptic α2-receptors (19). However, plasma NE increased after long-term administration of desipramine in depressed subjects because of the decreased clearance of NE with a blockade of NE reuptake into the nerve terminals (49). Prolonged administration of desipramine also has been shown to desensitize the central and peripheral α2-adrenergic receptors to NE, thus leading to decrease of sympathoinhibition and increase of NE release (8, 14). In patients with CHF, desipramine has been shown to decrease NE clearance and increased plasma NE (11). Desipramine also increases NE transmitter washout with stimulation of the sympathetic nerves of supply at a high rate (12). Thus the effects of desipramine on plasma NE may vary depending on the experimental conditions and duration of administration. The effects of desipramine are expected to be greater in a heightened sympathetic state when the contribution of NE reuptake for NE spillover to plasma is increased. This may account for the slight, albeit statistically insignificant, increase of plasma NE in the desipramine-treated NE infused animals.

The neuropeptide Y-containing nerve terminals in the heart are generally considered to represent postganglionic sympathetic nerves. These nerves originate in the stellate and other paravertebral ganglia, where numerous neuropeptide Y-immunoreactive cell bodies have been identified (36, 38). Although part of myocardial neuropeptide Y may be localized in nonadrenergic nerves (13, 26), the peptide generally coexists with NE in adrenergic neurons and is coreleased with NE (25, 37), despite their separate presence in small and large vesicles, respectively (23). In the human heart, neuropeptide Y-containing neurons have been identified in epicardial coronary arteries and myocardium by immunofluorescent staining methods (50). Furthermore, because tissue neuropeptide Y is severely depleted in the heart after surgical (4) or chemical (3, 41) sympathectomy, neuropeptide Y serves as a useful marker of myocardial adrenergic innervation.

Myocardial neuropeptide Y is reduced, in direct proportion to cardiac NE, in failing human myocardium (5, 7, 22). Because a neuronal uptake system has not been identified for neuropeptide Y, the decrease in tissue neuropeptide Y probably reflects increased release of neuropeptide Y, decreased biosynthesis, or both.

PGP 9.5 is a neuron-specific cytoplasmic protein originally detected by two-dimensional polyacrylamide gel electrophoresis in human brain extracts (28). It belongs to a family of ubiquitin COOH-terminal hydroxylases expressed throughout the mammalian central and peripheral nervous systems. This protein is present in all neuronal tissues derived from the neural crest (24), independent of the NE content or sympathetic stimulation. PGP 9.5 is not increased after nerve growth factor-induced differentiation in rat PC12 cells (48). In our present study, the neuronal marker PGP...
9.5 was affected by neither rapid ventricular pacing nor NE infusion. Thus we speculate that the cardiac sympathetic nerves were largely structurally intact in CHF and NE-infused animals and that the changes of neurotransmitters within the nerve endings were caused by functional abnormalities that are potentially reversible when the primary insult is removed. This is supported by a recent study from our laboratory (29) showing rapid normalization of cardiac sympathetic transmitter nerve profiles within 1 wk after discontinuation of rapid cardiac pacing.

Our present study extends our prior identification of a role of NE in sympathetic nerve terminal dysfunction. Because the decrease of noradrenergic neurotransmitters was blocked by desipramine in the CHF and NE infusion studies, we speculate that NE would have to enter the desipramine-sensitive NE transporter site to exert its effects within the sympathetic nerve terminals. Furthermore, because antioxidant vitamins and superoxide dismutase have been shown to protect the sympathetic nerve terminals from NE-induced damage (2, 35, 46), the neurotoxic effects of NE probably are mediated via oxygen free radicals derived from NE metabolism. Because superoxide dismutase, particularly the form conjugated with polyethylene glycol, is a large molecule with poor intracellular distribution (40, 51), one may speculate that NE released from sympathetic stimulation in CHF is first metabolized to oxygen free radicals in the interstitial space of the heart and then exerts the neurotoxic effects after entering the sympathetic nerve endings via the NE reuptake binding site. However, the exact nature of oxygen free radicals derived from NE metabolism outside the cells is not known. In addition, because oxidative stress is often associated with activation of tissue inflammatory mediators, such as tissue necrosis factor-α and various interleukins, which are known to exist in CHF (39), and play a functional role in the initiation of neuropathy (17, 42), the possibility that sympathetic nerve terminal dysfunction is mediated via the proinflammatory cytokines in CHF should be considered as well. Additional studies are needed to determine the exact mechanisms by which NE metabolites affect the NE uptake, tyrosine hydroxylase, and synthesis of NE and neuropeptide Y.

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