Decreased Adrenergic Neuronal Uptake Activity in Experimental Right Heart Failure

A Chamber-specific Contributor to Beta-Adrenoceptor Downregulation

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Abstract

The reduction of myocardial beta-adrenoceptor density in congestive heart failure has been thought to be caused by agonist-induced homologous desensitization. However, recent evidence suggests that excessive adrenergic stimulation may not produce myocardial beta-receptor downregulation unless there is an additional defect in the local norepinephrine (NE) uptake mechanism. To investigate the association between beta-adrenoceptor regulation and NE uptake activity, we carried out studies in 30 dogs with right heart failure (RHF) produced by tricuspid avulsion and progressive pulmonary artery constriction and 23 sham-operated control dogs. We determined NE uptake activity by measuring accumulation of [^{3}H]NE in tissue slices, NE uptake-1 carrier density by [^{3}H]mazindol binding and beta-adrenoceptor density by [^{3}H]dihydroalprenolol binding. Compared with sham-operated dogs, RHF dogs showed a 26% decrease in beta-adrenoceptor density, a 51% reduction in NE uptake activity, and a 57% decrease in NE uptake-1 carrier density in their right ventricles. In addition, right ventricle beta-receptor density correlated significantly with NE uptake activity and NE uptake-1 carrier density. In contrast, neither NE uptake activity nor beta-receptor density in the left ventricle and renal cortex was affected by RHF. Thus, the failing myocardium is associated with an organ- and chamber-specific subnormal neuronal NE uptake. This chamber-specific loss of NE uptake-1 carrier could effectively reduce local NE clearance, and represent a local factor that predisposes the failing ventricle to beta-adrenoceptor downregulation.

Introduction

Human congestive heart failure is associated with an increase in circulating norepinephrine (1–4), increase in myocardial release of catecholamines (5), beta-adrenergic subsensitivity (2, 6, 7), and a reduced number of myocardial beta-adrenergic receptors (6, 7). Since the number of cell surface beta-receptors decreases in a variety of in vitro cell preparations after beta-agonist exposure (8–10), the reduction in myocardial beta-receptor number in heart failure has been thought to be caused by agonist-induced downregulation, known also as homologous desensitization (8). However, chronic intravenous administration of NE in intact dogs has failed to decrease myocardial beta-receptor number (11, 12). Nevertheless, these findings do not refute the role of homologous desensitization since plasma NE concentration is not necessarily similar to the neurotransmitter concentration at the synaptic cleft. The synaptic NE surrounding beta-receptors is influenced not only by the circulating NE, but also by the amount of neuronally released NE, local metabolizing enzymes, and adequacy of NE uptake activity at the presynaptic nerve endings. Because neuronal reuptake is the major mechanism in terminating the pharmacological action of catecholamines, we speculate that unless the NE uptake mechanism is defective, NE infusion may not increase synaptic NE concentration sufficiently high to cause beta-receptor downregulation. Indeed, unlike the response in intact normal dogs, NE infusion causes a decrease in myocardial beta-receptor density in dogs after cardiac denervation (13), a condition that has been shown to reduce NE uptake (14).

Increasing evidence has accumulated that myocardial NE uptake activity is depressed in failing myocardium (15–17). In this study we sought to determine whether the reduction of myocardial beta-adrenoceptor density in heart failure is associated with alterations in NE uptake activity. Studies were performed in dogs with experimental right heart failure (RHF)[1] produced by tricuspid avulsion and progressive pulmonary artery constriction. As in patients with heart failure caused by primary pulmonary hypertension (18), such animals exhibit a chamber-specific reduction of myocardial beta-adrenoceptors in the failing right ventricle, while the left ventricular beta-receptor number remains normal (19). Since both the right and left ventricles are exposed to the same levels of circulating NE, this animal preparation offers a unique opportunity to determine whether a local factor in the right ventricle accounts for the chamber-specific beta-receptor downregulation. We measured beta-adrenoceptor density, NE uptake activity, and NE uptake-1 carrier density in both the right and left ventricles. The left kidney was studied as an extracardiac reference. Results of the study indicate that the beta-adrenoceptor downregulation in RHF is associated with a chamber- and organ-specific reduction in NE uptake-1 carrier density and decrease in NE uptake activity.

Presented in part at the 1987 Fall meetings of the American Society for Pharmacology and Experimental Therapeutics at Honolulu, 18 August 1987, and at the Annual Joint Scientific Sessions of the American Federation for Clinical Research, American Society for Clinical Investigation and American Association of Physicians at Washington, DC, 30 April 1988.

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Received for publication 15 June 1988 and in revised form 1 May 1989.

J. Clin. Invest.

0021-9738/89/10/1267/09 $2.00
Volume 84, October 1989, 1267–1275

1. *Abbreviations used in this paper:* B_{max}, maximum binding site; DHA, dihydroalprenolol; RHF, right heart failure.

Methods

Surgical preparation. RHF was produced in 30 adult mongrel dogs weighing 19–32 kg using a modified technique (19, 20) of Barger and co-workers (21). Animals were anesthetized with sodium pentobarbital (25 mg/kg i.v.) and ventilated with room air using a respirator (Harvard Apparatus Co., Inc., S. Natick, MA). A sterile right thoracotomy was performed through the fifth intercostal space. After the pericardial sac was opened to expose the heart, a purse string suture was placed at the base of the right atrium. With venous return transiently occluded, an incision was made within the purse string, and an index finger inserted into the right ventricle to rupture the chordae tendineae of the anterior tricuspid leaflet. A heparin-filled Tygon catheter (1.02 mm internal diameter; Norton Co., Plastics and Synthetics Division, Akron, OH) was then inserted into the right atrium, and the atrium was closed by tightening the purse string suture. 2 wk later, a left thoracotomy was performed via the fifth intercostal space. A Silastic Jones hydraulic balloon occluder (R. E. Jones, Silver Springs, MD) was placed around the main pulmonary artery. A micromanometer (Konigsberg Instruments, Inc., Pasadena, CA) was inserted into the left ventricular cavity through a stab wound at the apex. Tygon catheters were inserted into the aorta, the main pulmonary artery distal to the occluder, and the left atrium. The catheters were exteriorized to the back of the neck and the chest was closed. The animals received antibiotics for 1 wk after each surgery.

Beginning 2 wk after the second surgery, the pulmonary artery occluder was progressively inflated, once or twice a week, to increase right atrial pressure to 13–17 mmHg. A steady elevation in right atrial pressure and prominent ascites were attained in 2–4 wk. Animals were studied 5–19 wk after the second surgery.

Sham operation was performed in 23 dogs. It differed from the aforementioned two-staged procedure only in that neither tricuspid avulsion nor pulmonary artery constriction was performed.

The study was 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.

Systemic hemodynamic measurements. By the time of study, animals had been acclimatized to the laboratory environment and trained to lie quietly on an examination table. The chronically implanted Tygon catheters were connected to pressure transducers (P23Db; Statham Instruments, Inc., Oxnard, CA) and a multichannel Brush model 480 recorder (Gould, Inc., Instrument Systems Division, Cleveland, OH), for measuring right atrial, left atrial, and aortic pressures. The Konigsberg micromanometer was connected to the Brush recorder for measuring the first derivative of left ventricular pressure (dP/dt). The ratio of left ventricular dP/dt at a developed pressure of 50 mmHg to the developed pressure ($dP/dt/P$), which has been shown to be relatively independent of the ventricular afterload (22), was used as an index of left ventricular contractility. Right ventricular pressure and its peak rate of pressure rise were measured by a catheter (Millar Instruments, Inc., Houston, TX) inserted via an external jugular vein under local anesthesia. Heart rate was obtained from the electrocardiogram. Cardiac output was measured by injecting indocyanine green (Cardio-Green; Hynson, Westcott & Dunning, Inc., Baltimore, MD) into the pulmonary artery and sampling the arterial blood for dye concentrations using a cardiac output system (model 140; Gilford Instrument Laboratories, Inc., Oberlin, OH). Resting systemic hemodynamic measurements were obtained in triplicate at least 1 h after insertion of the Millar catheter. In addition, aortic blood was sampled for plasma catecholamine determinations (see below).

Animals were sacrificed by a lethal dose of sodium pentobarbital. Tissue blocks were removed from the right and left ventricular free walls 3 cm below the atrioventricular groove and from the renal cortex near the lateral margin of the left kidney. Fresh tissue samples were used for measuring NE uptake activity, while other specimens were stored immediately in liquid nitrogen for subsequent measurements of tissue norepinephrine, beta-receptor number, and NE uptake-1 carrier density.

Analysis of plasma and myocardial catecholamine concentrations. Plasma and tissue catecholamines were determined radioenzymatically (23), using the commercially available Cat-A-Kit reagents (Amersham Corp., Arlington Heights, IL). Tissue samples were minced and suspended in a 0.4 N perchloric acid with 5 mM reduced glutathione solution (pH 7.4), homogenized with a Polytron PCU-2 homogenizer (8-s bursts × 3 at setting 8; Brinkmann Instruments, Inc., Westbury, NY), and centrifuged at 500 *g*. The supernatant was taken for catecholamine assay.

Measurement of [^3H]NE uptake activity. The procedure for [^{3}H]NE uptake studies was similar to that previously described by Sharma and Banerjee (24). Tissue slices, 0.5 mm thick and weighing ~ 30 mg, were prepared from blocks of fresh myocardium or renal cortex using a Stadie-Riggs tissue microtome (Thomas Scientific, Swedesboro, NJ). Tissue was kept in an oxygenated modified Krebs solution. The composition of the modified Krebs solution was (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 0.54, $NaHCO_3$ 25, NaH_2PO_4 1, and dextrose 11. Also included in the Krebs solution were EDTA 20 mg/liter, ascorbic acid 200 mg/liter, and nialamide 3.5 mg/liter.

NE uptake activity was measured in triplicate by incubating tissue slices at 37°C for 15 min in three baths of modified Krebs solution containing 12.5, 25, and 50 nM D,L-[7-^{3}H(N)]NE hydrochloride (12.8 Ci/mmol; New England Nuclear, Boston, MA). Nonspecific accumulation of radioactivity (blanks) was determined by parallel incubation of triplicate tissue slices at 4°C. After incubation, tissue slices were rinsed three times with an ice-cold modified Krebs solution, blotted on filter paper, and placed in a test tube containing ice-cold 4% TCA. After 21 h of acid extraction at 4°C, an aliquot was taken for counting ^{3}H-radioactivity by liquid scintillation spectrometry (Packard Instrument Co., Inc., Downers Grove, IL).

Tyramine-induced release of NE from heart slices. Because tissue accumulation of [^{3}H]NE is influenced not only by NE uptake activity but also by the rate of NE release, studies were also performed to determine the rate of release of [^{3}H]NE from heart slices with and without tyramine. Tissue slices were preincubated in duplicate for 30 min at 37°C in a fresh oxygenated modified Krebs solution which contained 50 nM [^{3}H]NE and 100 μM pargyline, a monoamine oxidase inhibitor. The tissue slice was then rinsed three times with a [^{3}H]NE-free Krebs solution, blotted on filter paper, and placed in 4 ml of a modified Krebs solution containing 100 μM pargyline. 10 min later, with the tissue freely suspended, 50 μl of a tyramine solution was added into the incubation medium to achieve a final concentration of 100 μM. Beginning with the addition of tyramine, 50-μl aliquots of the incubation medium were taken for counting ^{3}H-radioactivity at 5-min intervals for 25 min. Finally, 200 μl of a 75% TCA solution was added to extract tissue NE; an aliquot of the incubation solution was taken for calculating the total amount of [^{3}H]NE present in the tissue. Spontaneous release of ^{3}H was determined by parallel incubation of two other tissue slices, using deionized water instead of tyramine. The difference in ^{3}H release between the tissue slices with and without tyramine was taken to indicate the tyramine-induced release of NE.

Preparation of the ventricular and renal particulate fractions. A block of ventricular free wall was trimmed free of fat, large vessels, and 1 mm of its inner and outer surfaces. It was minced and homogenized in an ice-cold 50 mM Tris HCl buffer (buffer I, pH 7.7 at 25°C), using a PCU-2 homogenizer (8-s burst × 5 at setting 8). The homogenate was diluted with additional Tris buffer and centrifuged at 500 *g* for 15 min at 4°C. The supernatant were spun again at 40,000 *g* for 15 min at 4°C. The resulting pellets were resuspended and washed with a second buffer solution containing 50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, pH 7.4 at 25°C (buffer II), and filtered through a 53-μm nylon mesh. This filtrate containing crude membrane fractions was then diluted with buffer II to yield a protein concentration of ~ 0.35 mg/ml for radioligand binding studies. Protein concentration was determined in triplicate by the method of Lowry et al. (25), using bovine serum albumin as a standard.

The renal cortex was trimmed free of capsular membranes and large vessels, minced with scissors, and homogenized in ice-cold buffer I using a Polytron homogenizer (8-s burst × 3 at setting 6). The homogenate was centrifuged at 500 *g* for 15 min at 4°C. The supernatant was spun again at 40,000 *g* for 15 min at 4°C. The resultant pellet was resuspended in buffer I and washed. The final pellet was resuspended in buffer I, filtered through a 53-μm nylon mesh, and used immediately for radioligand binding studies.

Radioligand binding studies. NE uptake-1 carrier density was quantitated by the [^{3}H]mazindol binding assay described by Javitch et al. (26), with minor modifications. Approximately 70 μg of membrane protein was incubated in triplicate at 37°C for 20 min with varying concentrations (3–45 nM) of [^{3}H]mazindol (16.8 Ci/mmol; New England Nuclear) in the presence of either 0.3 μM desipramine (for estimation of nonspecific binding) or vehicle (total binding). Specific binding, defined as the difference between the total and nonspecific binding, accounted for 60–70% of the total binding at 5 nM of [^{3}H]-mazindol.

Beta-adrenoceptor density was quantitated using [^{3}H]dihydroalprenolol (DHA, 95 Ci/mmol; New England Nuclear) as the radioligand (27). Approximately 70 μg of membrane protein was incubated with appropriate concentrations of [^{3}H]DHA (0.5–12 nM), in the presence of either 1 μM L-alprenolol or vehicle, at 37°C for 20 min in a final volume of 0.25 ml. The reaction was terminated by the addition of 5 ml ice-cold buffer II and filtered immediately through Whatman GF/C filters on a Brandel cell harvester (Biomedical Research and Development Laboratories, Inc., Gaithersberg, MD), followed by three additional washes with the same buffer. Specific binding was defined as the difference between binding of the radioligand in the absence and presence of L-alprenolol.

The number of maximum binding sites (B_{max}) and the dissociation constant (K_d) for both radioligand binding studies were calculated using Scatchard analysis (28) with the EBDA software program developed by McPherson (29) (Elsevier Science Publisher, Cambridge, UK).

Data analysis. Results are expressed as mean±SE. The statistical significance of differences between the RHF and sham-operated dogs was determined using analysis of variance for determining equality of variance and *t* test for unpaired data. The degree of relatedness between two variables was determined using Pearson's correlation coefficient and coefficient of determination. The data were analyzed using RS/1 data analysis system (BBN Software Products Corp., Cambridge, MA). Values were considered statistically significant if $P < 0.05$.

Results

Resting systemic hemodynamics. Animals with RHF showed prominent ascites and a heavier body weight than sham-operated controls (Table I). The RHF animals also had a significantly greater right atrial pressure, faster heart rate, lower mean aortic pressure, lower cardiac output, and reduced left atrial pressure, right ventricular d*P*/d*t* and left ventricular d*P*/d*t* and d*P*/d*t*/*P*. In addition, right ventricular free wall weight was greater in the RHF group (50.3±1.9 g) compared with the sham group (39.9±1.3 g, $t = 4.56$, d.f. = 51, $P < 0.001$).

Table I. Hemodynamic Characteristics

	Sham (23)	RHF (30)
Body weight (*kg*)	23.6±0.6	25.7±0.6*
Right atrial pressure (*mmHg*)	3.6±0.2	13.6±0.6‡
Heart rate (*bpm*)	97±3	147±3‡
Mean aortic pressure (*mmHg*)	108±2	95±2‡
Cardiac output (*liters/min*)	3.67±0.11	2.70±0.08‡
Left atrial pressure (*mmHg*)	6.5±0.3	3.8±0.3‡
RV *dP/dt* (*mmHg/s*)	666±22	517±25§
LV *dP/dt* (*mmHg/s*)	3084±99	2196±54‡
LV *dP/dt/P* (s^{-1})	48.2±0.8	38.2±0.7‡

Values are mean±SE. The number of experiments is given in parentheses. RV, right ventricular. LV, left ventricular. * $P = 0.016$, ‡ $P < 0.0001$, and § $P < 0.001$ compared with the sham group.

Table II. Plasma and Tissue Catecholamine Contents

	Sham	RHF
Plasma NE (*ng/ml*)	0.17±0.01 (18)	0.45±0.06* (30)
Plasma epinephrine (*ng/ml*)	0.11±0.01 (18)	0.17±0.02‡ (30)
Tissue NE (*μg/g*)		
Right ventricle	1.22±0.08 (20)	0.07±0.02§ (28)
Left ventricle	1.45±0.11 (20)	0.29±0.05§ (28)
Renal cortex	0.72±0.11 (9)	0.56±0.06 (14)

Values are mean±SE. The number of experiments is given in parentheses. * $P < 0.001$, ‡ $P = 0.003$, and § $P < 0.0001$ compared with the sham group.

Plasma and tissue catecholamine levels. Arterial plasma NE and epinephrine concentrations were elevated in RHF (Table II). In contrast, myocardial NE content was markedly reduced. Furthermore, although NE was reduced in both ventricles, NE depletion was significantly greater in the failing right ventricle than in the left ventricle. In contrast, renal NE content did not differ between the RHF and sham-operated dogs.

Beta-adrenoceptor density. Table III shows that compared with sham-operated dogs, RHF dogs had a 26% decrease in right ventricular beta-adrenoceptor density. In contrast, neither left ventricular nor renal beta-adrenoceptor density differed between the two groups. Nor did the two groups differ in their dissociation constants of the beta-adrenoceptors in the right ventricle and kidneys. However, the dissociation constant of the left ventricular beta-adrenoceptor was slightly greater in RHF dogs than sham-operated dogs.

NE uptake activity and binding sites. Specific ^{3}H uptake activity was measured to approximate NE uptake activity. Fig. 1 shows that the amount of ^{3}H taken up by the myocardial tissue was in direct proportion to the concentrations of [^{3}H]NE present in the incubation medium. Compared with sham-operated dogs, RHF dogs exhibited an attenuated NE uptake

Table III. Binding Characteristics of [^{3}H]DHA to Myocardial and Renal Crude Membrane Preparations

	Sham	RHF
B_{max} (*fmol/mg protein*)		
Right ventricle	107±5 (20)	79±6* (26)
Left ventricle	116±6 (21)	108±5 (27)
Renal cortex	51±6 (6)	55±3 (6)
K_d (*nM*)		
Right ventricle	2.4±0.2	2.7±0.2
Left ventricle	2.3±0.1	3.0±0.2*
Renal cortex	2.3±0.5	1.9±0.4

Values are mean±SE. The number of experiments is given in parentheses. * $P < 0.001$ compared with the sham group.

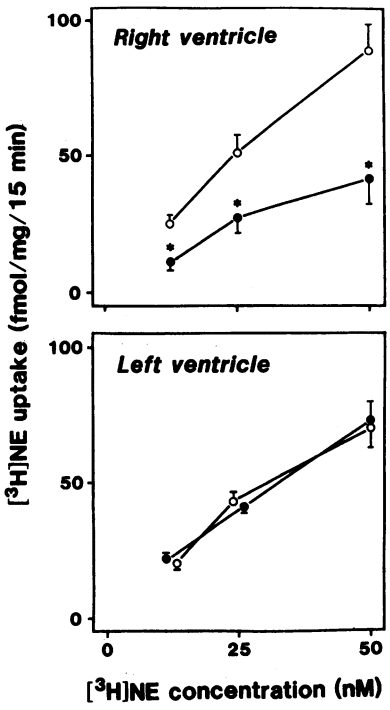

Figure 1. Specific ^3H uptake activity (representing tissue [^3H]NE uptake), at three different concentrations of [^3H]NE, using fresh tissue slices taken from the right and left ventricular free walls of sham-operated (*open circles*) and RHF (*closed circles*) dogs. Bars indicate SE. Asterisks denote values that differ significantly from the sham group at $P < 0.05$. Nonspecific ^3H uptake did not differ significantly between the two groups. The values of nonspecific ^3H uptake (fmol/mg per 15 min, mean±SE) at each of the three incubation concentrations were: left ventricles of the sham animals (5.4±0.6, 10.6±0.9, 19.3±1.5); left ventricles of the RHF animals (4.9±0.3, 11.2±0.9, 20.6±1.2); right ventricles of the sham animals (5.5±0.5, 10.1±0.8, 18.4±1.3); right ventricles of the RHF animals (5.8±0.4, 10.0±0.5, 19.5±1.3).

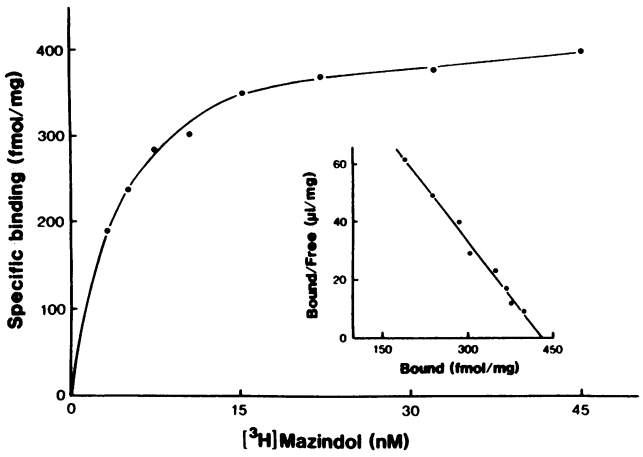

Figure 2. A characteristic [^3H]mazindol radioligand binding study for measuring NE uptake-1 carrier site density. A Scatchard plot is shown in inset.

activity in the right ventricle for the three concentrations of [^3H]NE tested. However, the two groups did not differ in left ventricular NE uptake. Table IV summarizes the NE uptake activity in the right and left ventricles and the renal cortex, at the highest [^3H]NE concentration tested. The table shows that NE uptake was reduced in the right ventricle of the RHF dogs, but neither left ventricular nor renal NE uptake differed significantly between the RHF and sham-operated dogs.

A representative Scatchard plot of specific binding of [^3H]-mazindol is shown in Fig. 2. Specific [^3H]mazindol binding to myocardial membrane was saturable and reversible. The single component, linear Scatchard relationship indicates a single class of [^3H]mazindol binding sites (*inset*). A near-unity Hill coefficient in the Hill plot indicates that there was no cooperativity among binding of the [^3H]mazindol molecules to the cardiac membrane. This property of mazindol binding was not altered by RHF.

Results of [^3H]mazindol binding experiments are summarized in Table V. RHF animals showed a smaller number of maximum mazindol binding sites (B_{max}) in the right ventricle than sham-operated dogs, but mazindol B_{max} did not differ in the left ventricle between the two groups. Neither was the dissociation constant for either ventricle affected by RHF.

Tyramine-induced NE release. Fig. 3 shows the cumulative relative release of ^3H over a 25-min period from the right and left ventricular muscle slices in the presence and absence of tyramine. Neither the spontaneous release nor tyramine-induced release of ^3H differed significantly in the right or left ventricle between the RHF and sham animals.

Correlation of beta-adrenoceptor density with NE uptake and plasma NE. Table VI lists correlation coefficients and coefficients of determination for correlations between beta-adrenoceptor density and biochemical measurements in the failing right ventricle. Beta-receptor density correlated significantly with both NE uptake activity (Fig. 4) and NE uptake-1 carrier density. NE uptake activity also correlated significantly with NE uptake-1 carrier density (Fig. 5). In addition, there was an inverse correlation between beta-receptor density and NE content of the failing hearts. In contrast, right ventricular

Table IV. Uptake of [^3H]NE by Fresh Tissue Slices Obtained from Sham-operated Dogs and Dogs with RHF

	Specific ^3H uptake	
	Sham	RHF
	fmol/mg/15 min	
Right ventricle	86.5±5.2 (23)	42.1±5.0* (30)
Left ventricle	73.3±4.7 (23)	79.1±4.2 (29)
Renal cortex	50.8±3.2 (6)	47.5±4.6 (7)

Values (mean±SE) are specific ^3H uptake (^3H uptake at 37°C minus ^3H uptake at 4°C) by the tissue slices over 15 min in an incubation medium containing 50 nM [^3H]NE. The number of experiments is given in parentheses. * $P < 0.0001$ compared with the sham group.

Table V. Binding Characteristics of [^3H]Mazindol to Myocardial Crude Membrane Preparations

	Sham (16)	RHF (20)
B_{max} (*fmol/mg protein*)		
Right ventricle	477±41	203±22*
Left ventricle	601±46	521±37
K_d (*nM*)		
Right ventricle	12.4±1.7	11.3±1.5
Left ventricle	11.8±1.9	10.5±1.1

Values are mean±SE. The number of experiments is given in parentheses. * $P < 0.0001$ compared with the sham group.

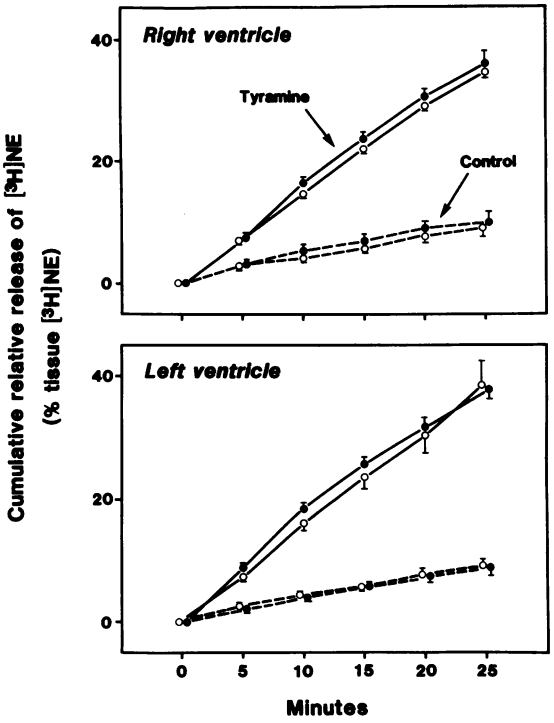

Figure 3. Cumulative relative release of ^3H after addition of either tyramine or deionized water (*control*) from the right and left ventricular slices of the sham-operated (*open circles, n* = 5) and RHF dogs (*closed circles, n* = 6). The tissue slices were preincubated in a [^3H]NE containing solution. Bars indicate SE.

beta-receptor density correlated with neither plasma NE concentration nor the duration of heart failure in the RHF dogs. Because the precise timing of onset of RHF could not be defined, the duration of RHF was approximated by the interval between the second surgery and sacrifice (35–124 d; 66±4 d).

Discussion

As we have shown previously in dogs with RHF (19), our present study confirms that beta-adrenoceptor number is reduced in the failing right ventricle, but is relatively unchanged in the left ventricle. Similar chamber-specific changes have been reported in patients with right ventricular failure secondary to primary pulmonary hypertension (18). The RHF dogs have been extensively studied; they exhibit an increase in plasma catecholamines, a decrease in myocardial NE stores, a reduced responsiveness to beta-receptor agonists (19), and other neurohormonal and reflex changes (30–34) that occur in patients with congestive heart failure.

Specific ^3H uptake activity, defined as the difference in radioactivity between tissue slices incubated in a [^3H]NE-containing solution at 37°C (total ^3H uptake) and those at 4°C (nonspecific ^3H uptake), was taken to approximate NE uptake activity in the present study. Ascorbic acid and nialamide were

Table VI. Correlation Coefficients and Coefficients of Determination of Biochemical and NE Changes in the Failing Right Heart

Variables	Correlation coefficient (r)	Coefficient of determination (r^2)
Beta-receptor density vs. NE uptake activity	0.818*	0.668
Beta-receptor density vs. NE uptake site number	0.728*	0.530
NE uptake activity vs. NE uptake site number	0.881*	0.775
Beta-receptor density vs. tissue [NE]	0.441‡	0.195
Beta-receptor density vs. RHF duration	−0.073	0.005
Beta-receptor density vs. arterial [NE]	0.080	0.006

* Values that are statistically significant at $P < 0.001$. ‡ The value that is statistically significant at $P < 0.05$.

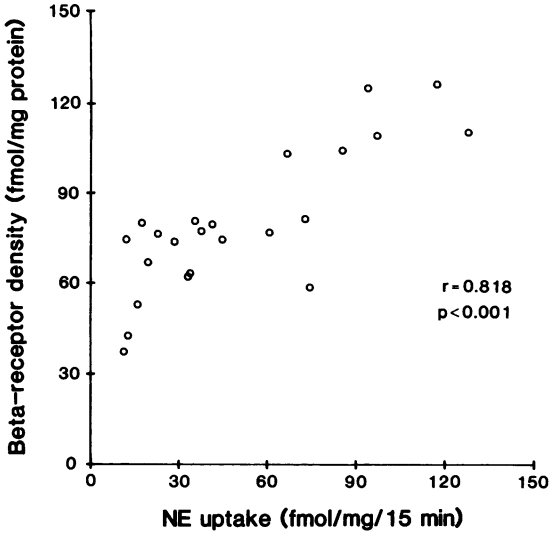

Figure 4. Correlation between beta-receptor density and NE uptake activity (specific ^3H uptake) of the right ventricles of RHF dogs.

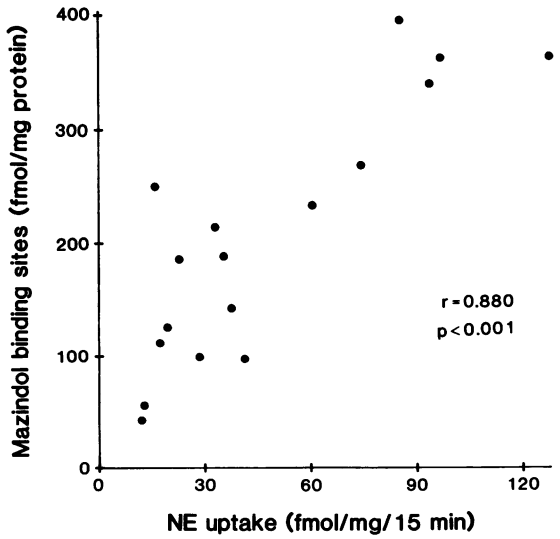

Figure 5. Correlation between mazindol binding sites (NE uptake carrier site density) and NE uptake activity (specific ^3H uptake) of the right ventricles of RHF dogs.

added to our incubation medium to retard spontaneous oxidation of NE and the activity of monoamine oxidase. Sharma and Banerjee (24) have shown that under the same experimental conditions, > 90% of the tissue radioactivity derives from the unmetabolized catecholamine. These findings are consistent with the observation that NE metabolites account for < 4% of the total ^3H recovered in a similar incubation medium (35). Furthermore, as shown by Sharma and Banerjee (24), we have confirmed that the temperature-dependent accumulation of ^3H in heart muscle can be either abolished or markedly reduced by ouabain, a Na^+-K^+ ATPase inhibitor, and desipramine, a NE uptake-1 carrier inhibitor (unpublished data). On the other hand, propranolol, phentolamine, and prazocin exert no effects on specific ^3H uptake. These findings indicate that the specific ^3H uptake is an active, energy-dependent process that requires normally functioning NE uptake-1 carrier sites, and that binding of [^3H]NE to alpha- and beta-adrenoceptors contributes negligibly to the total tissue ^3H radioactivity.

Our present study indicates no difference in either spontaneous or tyramine-induced release of NE between the normal and failing right ventricles. Similarly, using an in vivo labeling technique, Spann et al. (36) found the absolute levels of specific activity and the rates of disappearance were identical in the left ventricles of normal guinea pigs and those with heart failure produced by aortic constriction. The relative net turnover rates of NE were the same in the normal and failing hearts. The investigators concluded that NE release was not increased in heart failure. These results suggest that the primary defect of NE metabolism of the failing ventricle lies in the NE uptake mechanism.

Compared with sham animals, RHF dogs showed an average of 51% reduction of NE uptake activity and a 26% decrease in beta-receptor number in the failing right ventricle. Like that of beta-receptor number, this change in NE uptake was not only organ specific but also chamber specific, because neither the left ventricle nor the kidney showed abnormal NE uptake activity. Our [^3H]mazindol binding data further indicate that the diminished NE uptake activity was associated, at least in part, with a reduction in the number of NE uptake-1 carrier.

Our results further show that myocardial beta-receptor number correlated significantly with NE uptake activity and NE uptake-1 carrier density in the failing heart. Although the associations do not establish a cause and effect relationship, the coefficient of determination indicates that NE uptake activity contributed statistically to 66.8% of the changes in total beta-receptor number in the study. Recent evidence indicates that the decrease in beta-receptor number in the failing human heart is caused predominantly by a selective downregulation of beta-1 receptors (37). Unlike beta-1 receptors, myocardial beta-2 receptors do not change significantly in heart failure. If the human findings could be extrapolated to the experimental animals, the reduction of total beta-receptor number in our animals probably also was due to a selective decrease in beta-1 receptors. The percent decrease of beta-1 receptor density would then be expected to be greater than that of total beta-receptor density, and the beta-1 receptor number might be more closely correlated with NE uptake activity than was the total beta-receptor number. However, because we employed the nonselective antagonist alprenolol, our present study does not allow us to discriminate between the beta-receptor subtypes. Further investigations might be needed to establish the quantitative correlation between NE uptake activity and beta-1 receptor number.

The mechanism by which NE uptake-1 carrier density was reduced is not known. However, it cannot be explained by an increase in muscle mass alone, because right ventricular weight increased only 26%. NE uptake-1 carrier sites may decrease in number either because of loss of adrenergic nerve fibers in the failing myocardium or because of a specific mechanism of NE uptake-1 carrier downregulation. It is not known whether the number of NE uptake-1 carrier sites can be regulated by concentrations of either synaptic NE or other neurotransmitters.

Decrease in tissue NE uptake alone probably is inadequate to increase synaptic NE and cause beta-receptor downregulation. Studies have shown that in hearts devoid of NE uptake function such as occurs after either surgical or pharmacological denervation (13), myocardial beta-receptor number either shows no changes (38) or actually increases (39, 40).

Increased circulating NE is an index of heightened sympathetic tone, and has often been implicated to be the cause of beta-adrenergic subsensitivity and beta-adrenoceptor downregulation in congestive heart failure. However, circulating NE is not a precise measure of adrenergic synaptic NE directly surrounding beta-receptors. In tissues with a competent adrenergic neuronal uptake system, synaptic NE is taken up avidly by nerve endings and, even in the presence of excessive sympathetic stimulation, may not increase to a level sufficiently high to reduce beta-receptor number. In vitro studies have shown that beta-receptor downregulation by agonists occurs in a concentration-dependent, and time-dependent fashion (41, 42). The initial reaction after exposure to beta-agonists involves an uncoupling of beta-receptors from adenylate cyclase; reduction of cell surface beta-receptor number occurs later and only if a critically high agonist level is reached. In contrast, synaptic NE is expected to increase markedly after sympathetic stimulation in tissues with suppressed NE uptake. In our present study despite an increase in circulating NE levels, beta-receptor number was reduced only in the right ventricle, where a subnormal NE uptake activity was also documented. Neither the left ventricle nor the kidneys showed a depressed NE uptake activity or a reduction of beta-receptor number in RHF. This hypothesis is also consistent with the findings that myocardial beta-receptor number does not decrease after chronic intravenous administration of NE in normal dogs (11, 12).

Our study shows no significant correlation between right ventricular beta-receptor number and arterial NE concentration in RHF dogs. Likewise, Limas et al. (43) found no significant correlation between arterial NE and myocardial beta-receptor number in patients with moderate to severe congestive heart failure. The latter investigators, however, noted that the two variables correlated significantly in patients with mild to moderate heart failure. The relationship between plasma NE and myocardial beta-receptor number was also studied in patients with dilated cardiomyopathy (44). There was a weak correlation ($r = -0.42$) between arterial NE and myocardial beta-receptor number, but a tighter correlation ($r = -0.64$) existed between coronary sinus NE and myocardial beta-receptor, suggesting that changes of myocardial beta-receptor are causally related to cardiac-derived NE. Furthermore, using autoradiography, Murphree et al. (45) found that beta-receptor

density was lower in the subendocardium than the subepicardium of tissue slices taken from patients with severe heart failure. This transmural gradation of beta-receptor density suggests that myocardial beta-receptor density is influenced by some local tissue factors. Further studies are warranted to determine whether NE uptake is more severely depressed, or whether interstitial NE is higher, in the subendocardial layer than the subepicardial layer.

NE was depleted in both the right and left ventricles of the RHF dogs. Similar results have been previously reported in human and experimental heart failure (46, 47). This change appears to be specific to the heart, because NE concentration did not change significantly in the renal cortex. The decrease in myocardial NE probably is caused, at least in part, by the defective NE uptake mechanism (15, 36). The lack of correlation between NE uptake activity and myocardial NE depletion in the left ventricles of RHF dogs in our present study, however, suggests that NE depletion cannot be accounted for by abnormal NE uptake alone. Alternatively, myocardial NE depletion could have resulted from decreased NE synthesis, as heart failure has been shown to be associated with a decrease in cardiac tyrosine hydroxylase activity (48–50), or other rate-limiting steps for cardiac NE synthesis (51).

The dissociation constant for [^{3}H]DHA did not change significantly in either the right ventricle or renal cortex with the development of RHF. However, the dissociation constant of myocardial beta-receptors was slightly greater in the left ventricle of the RHF dog than the sham-operated control. The significance of this small increase in dissociation constant is not known. However, in dogs with left ventricular failure produced by aortic constriction, a much greater increase in dissociation constant has been noted (52). An increase in dissociation constant also occurs after chronic administration of exogenous NE (12), and probably is caused by loss of high-affinity beta-receptors (52, 53).

Recently, the beta-receptor antagonist metoprolol has been found to increase lymphocyte and myocardial beta-receptor number in patients with congestive heart failure (54, 55), suggesting beta-receptor downregulation may be causally related to agonist stimulation. In addition, metoprolol therapy has been shown to improve patients' heart failure symptoms, exercise capacity, and left ventricular function (56, 57). Most of these studies, however, were conducted in a small number of patients and were not placebo controlled. The question whether beta-receptor blockade in congestive heart failure reverses myocardial beta-receptor downregulation and increases myocardial inotropic responses to adrenergic stimulation deserves further extensive studies.

In summary, the present study indicates that right ventricular beta-receptor density correlated with the number of tissue NE uptake-1 carrier sites. We also showed that the defect in NE uptake mechanism was found only in the failing right ventricle. This chamber-specific reduction of NE uptake carrier sites may be expected to increase synaptic NE concentration, particularly in the presence of excessive sympathetic stimulation, and to cause agonist-induced homologous desensitization. Our results further suggest that the lack of correlation between beta-receptor density and arterial NE concentration does not necessarily refute a role of agonist-induced homologous desensitization. Direct correlation between synaptic NE concentration and myocardial beta-receptor number is needed. Further experiments are warranted to determine whether the beta-receptor downregulation in heart failure is causally related to beta-agonist stimulation.

Acknowledgments

The authors thank Drs. J. Franklin Richeson, William B. Hood, Jr., and John R. Sladek, Jr. for their helpful advice, and constructive comments in the preparation of the manuscript. We also thank Mary Ann Evans, Lisa Guthinger, and Lynette Knapp for their expert assistance.

The study was supported in part by the Public Health Service grants HL-35194, and HL-39260 and a grant-in-aid from the American Heart Association.

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