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Evidence for Reduction of Norepinephrine Uptake Sites in the Failing Human Heart

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Objectives. **This study investigated the role of neuronal uptake of norepinephrine (uptake-1) in human heart failure as a local factor for altering concentrations of norepinephrine at the cardiac myocyte membranes.**

Background. **Several beta-adrenergic neuroeffector defects occur in heart failure. Whether an alteration in norepinephrine uptake-1 occurs is still unresolved.**

Methods. **The role of norepinephrine uptake-1 was studied in electrically stimulated (1 Hz, 37°C) human ventricular cardiac preparations and isolated myocardial membranes.**

Results. **The effectiveness of norepinephrine in increasing the force of contraction was decreased in relation to the degree of heart failure. In contrast, the potency of norepinephrine was increased in failing hearts (New York Heart Association functional class IV) in relation to the concentrations producing 50% of the maximal effect (EC_{50}). The EC_{50} values for isoproterenol, which is not a substrate for norepinephrine uptake-1, were reduced in myocardium in functional classes II to III and IV compared with those in nonfailing myocardium. The uptake inhibitors cocaine and desipramine (3 μmol/liter) potentiated the positive inotropic effects of norepinephrine in nonfailing myocardium ($p < 0.05$) but not in functional class IV myocardium. Radioligand binding experiments using the uptake inhibitor hydrogen-3 mazindol revealed a significant decrease by ~30% in norepinephrine uptake-1 carrier density in functional classes II to III and IV myocardium versus nonfailing myocardium ($p < 0.05$).**

Conclusions. **In human heart failure, there is a presynaptic defect in the sympathetic nervous system, leading to reduced uptake-1 activity. This defect in the failing heart can be mimicked by the effects of uptake blocking agents, such as cocaine and desipramine, in the nonfailing heart only. Compromised norepinephrine uptake-1 in functional class IV cannot be further increased by cocaine and desipramine. The pathophysiologic consequences could be an increased synaptic concentration of norepinephrine predisposing to adenylyl cyclase desensitization.**

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In failing human myocardium, the blunted cardiac responsiveness to beta-adrenergic stimulation is due to a reduction in $beta_1$-adrenoceptor density (1,2) and an increase in G_i-alpha proteins (3–5), which could be induced by an increased level of circulating catecholamines (6–8), an increase in neuronal release of norepinephrine (9) or an impairment of neuronal uptake of norepinephrine, or a combination of these mechanisms. Cardiac neuronal uptake is the predominant mechanism for terminating the action of norepinephrine on beta-adrenoceptors when catecholamines are released by sympathetic nerve terminals (10). In patients with heart failure, data arguing against (11,12) or in favor (13) of reduced myocardial norepinephrine uptake exist. However, direct experimental evidence in isolated failing human myocardium in favor of reduced norepinephrine uptake as well as its potential functional consequences is limited. The purpose of this study was to use functional and biochemical techniques to investigate the role of neuronal uptake of norepinephrine (uptake-1) in human heart failure as a local factor for altering concentrations of norepinephrine at the cardiac myocyte membranes. Studies were performed in electrically stimulated human ventricular cardiac preparations and isolated myocardial membranes. Tissue was obtained from patients who underwent mitral valve replacement or heart transplantation. For comparison, tissue from nonfailing donor hearts was studied.

Methods

Myocardial tissue. Myocardium from terminally failing human hearts was obtained from patients after cardiectomy during cardiac transplantation. The patients had dilated or ischemic cardiomyopathy and were in New York Heart Association functional class IV on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before operation. During mitral valve replacement, tissue from moderately failing hearts was obtained. All patients gave written informed consent before operation. Clinical details of the patients included in the study are shown in Table 1. Flunitrazepam and pancuronium bromide with isoflurane were used for general anesthesia. Cardiac surgery was performed during

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Table 1. Clinical Characteristics Obtained at Preoperative Cardiac Catheterization for Patients Providing Study Myocardium Samples

Gender/Age (yr)	NYHA	Diagnosis	LVEDP (mm Hg)	LVEDV (ml)	EF (%)	CI (liters/min × m^2)
Mitral Valve Replacement						
F/57	II–III	MR	12	116	74	2.4
F/67	II–III	MR	10	263	45	2.1
F/46	II–III	MR > MS	15	149	58	3.1
M/44	II–III	MS > MR	10	76	60	1.7
M/46	II–III	MR	12	384	49	3.2
M/59	II–III	MR	20	62	53	1.4
F/65	II–III	MS	3	63	76	2.4
F/68	II–III	MS	15	259	54	3.1
Mean 56.5	II–III		12.1	171.5	58.6	2.4
±SEM ±3.5			±1.6	±39.1	±3.7	±0.2
Cardiac Transplantation						
M/55	IV	DCM	20	364	14	2.0
M/50	IV	DCM	22	424	15	2.2
M/47	IV	DCM	—	—	—	1.4
M/42	IV	DCM	14	290	21	3.2
M/51	IV	DCM	16	208	21	2.2
M/49	IV	DCM	41	532	14	1.9
M/39	IV	ICM	28	178	49	3.5
M/63	IV	ICM	—	—	—	—
M/48	IV	ICM	28	92	29	1.6
Mean 49.3			24.1	298.3	23.3	2.3
±SEM ±2.2			±2.6	±53.5	±4.4	±0.2

CI = cardiac index; DCM = dilated cardiomyopathy; EF = ejection fraction; F = female; ICM = ischemic cardiomyopathy; LVEDP = left ventricular end-diastolic pressure; LVEDV = left ventricular end diastolic volume; M = male; MR = predominant mitral regurgitation; MS = predominant mitral stenosis; NYHA = New York Heart Association functional class (II to III = moderately failing myocardium, IV = severely failing myocardium); — = data not available.

cardiopulmonary bypass with cardioplegic arrest during hypothermia. The cardioplegic solution (modified Bretschneider solution) contained (in mmol/liter) NaCl 15, KCl 10, $MgCl_2$ 4, histidine 180, tryptophan 2, mannitol 30 and potassium dihydrogen oxoglutarate 1. Nonfailing myocardium was obtained from five donors who were declared brain dead as a result of traumatic injury. These hearts could not be transplanted for technical reasons. Echocardiographic evaluation showed normal left ventricular function. Invasive hemodynamic measurements were not performed. None of the donors of the nonfailing hearts received sustained catecholamine treatment. However, bolus injections of catecholamines were documented in three patients during resuscitation after acute treatment of trauma. Thus, these hearts cannot be termed normal but only nonfailing. The reasons for not transplanting the hearts were fever, pericardial effusion or evidence for coronary heart disease. The use of human cardiac tissues was approved by the ethical committees of the Universities of Munich and Cologne.

Isolated cardiac preparation and measurement of force of contraction. Immediately after excision, papillary muscle strips were placed in ice-cold preaerated Tyrode solution and delivered to the laboratory within 10 min. The experiments were performed on isolated electrically driven muscle preparations. Muscle strips of uniform size with muscle fibers running approximately parallel to the length of the strips were dissected under microscopic control using scissors in aerated modified Tyrode solution (see composition later). Connective tissue was carefully trimmed away. The muscles were suspended in an organ bath (75 ml) maintained at 37°C and containing a modified Tyrode solution of the following composition (in mmol/liter): NaCl 119.8, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 1.8, $NaHCO_3$ 22.6, NaH_2PO_4 0.42, glucose 5.0, ascorbic acid 0.28, ethylenediaminetetraacetic acid (EDTA) 0.05. The bathing solution was continuously aerated with 95% oxygen and 5% carbon dioxide. The muscles were stimulated by two platinum electrodes using field stimulation from a Grass S88 stimulator (frequency 1 Hz, impulse duration 5 ms, intensity 10% to 20% greater than threshold). Each muscle was stretched to the length at which force of contraction was maximal. The force at rest was kept constant throughout the experiment. The resultant tension was measured isometrically with an inductive force transducer (W. Fleck, Mainz, Germany) attached to a Gould recorder. Preparations were allowed to equilibrate for at least 90 min, with the bathing solution changed once after ~45 min. Experimental details have been described elsewhere (3).

Membrane preparation and radioligand binding experiments. Left ventricular myocardial tissue was chilled in 30 ml of ice-cold homogenization buffer (20 mmol/liter of tris(hydroxymethyl)aminomethane [Tris] hydrochloride, 4 mmol/liter of EDTA and 1 mmol/liter of dithiotheitol, pH 7.4). Connective tissue was trimmed away; myocardial tissue was minced

with scissors; and membranes were homogenized with a motor-driven glass–Teflon homogenizer for 1 min. The membrane preparation was then homogenized by hand with a glass–glass homogenizer. The homogenate was spun at 484 *g* (Beckman JA 20 rotor) for 15 min. The supernatant was filtered through four layers of gauze, diluted with an equal volume of potassium chloride (1 mol/liter) and stored on ice for 10 min. This suspension was centrifuged at 100,000 *g* for 30 min. The pellet was resuspended in 50 volumes of incubation buffer (50 mmol/liter of Tris HCl, 120 mmol/liter of NaCl, 5 mmol/liter of KCl, pH 7.4) and homogenized for 1 min with a glass–glass homogenizer. This suspension was recentrifuged at 100,000 *g* for 45 min. The final pellet was resuspended in incubation buffer and stored at −80°C. Storage did not alter the results. Protein was measured according to Lowry et al. (14) using bovine serum as standard. The assays were performed in a total volume of 250 μl. The incubation was carried out at 22°C for 60 min. These conditions allowed complete equilibration of the receptor with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/C filters that were presoaked with 0.1% 3-([3-chelamidopryl]-dimethylamonio)-1-propansulfate (CHAPS) to reduce nonspecific binding. The filters were washed immediately three times with 6 ml of ice-cold incubation buffer to remove any unbound radioligand. All experiments were performed in triplicate. Radioactivity was determined in an LKB counter (Pharmacia LKB, Freiburg, Germany). Myocardial norepinephrine uptake carrier sites were studied using hydrogen-3 (H-3) mazindol (specific activity 24.7 Ci/mmol) as radiolabeled ligand (1 to 50 nmol/liter). Specific binding of H-3 mazindol was defined in the presence of desipramine (100 μmol/liter).

Materials. (*S*)-(−)-Norepinephrine was from Merck-Schuchardt (Hohenbrunn, Germany). Atropine sulfate and desipramine were purchased from Sigma Chemie GmbH (Deisenhofen, Germany). Cocaine solution was prepared in the pharmacy of the Klinikum Großhadern (University of Munich). Hydrogen-3 mazindol (specific activity 24.7 Ci/mmol) was from New England Nuclear-DuPont GmbH (Dreieich, Germany). All other compounds used were of analytic grade or the best grade commercially available. Only deionized and twice-distilled water was used throughout.

Statistical evaluation. All data are mean value ±SEM. Statistical significance was estimated with the Student *t* test for unpaired observations and analysis of variance. The Scheffé procedure for multiple comparisons was applied. A p value <0.05 was considered significant. The drug concentrations producing 50% of the maximal effect (EC_{50}) were graphically determined for each individual experiment.

Results

Effects of norepinephrine and isoproterenol. Figure 1 shows the positive inotropic effects of norepinephrine in isolated electrically driven papillary muscle strips from moderately (functional classes II to III) and severely (functional class IV) failing as well as nonfailing myocardium. Norepinephrine concentration dependently increased force of contraction in both groups. As expected, the positive inotropic effect of norepinephrine was reduced in papillary muscle strips from functional classes II to III and IV compared with that in nonfailing myocardium. To evaluate the potency of the beta-adrenoceptor agonist, concentration–response curves for norepinephrine are shown in percent of the maximal effect in each preparation. Unexpectedly, the potency of norepinephrine to increase force of contraction was increased in functional classes II to III and IV myocardium compared with nonfailing myocardium (Fig. 1, bottom). Correspondingly, the EC_{50} values were significantly lower in failing than in nonfailing myocardium (Table 2). The increased potency of norepinephrine despite reduced maximal effects could either reflect more efficient coupling of the remaining beta-adrenoceptors or a reduced inactivation of norepinephrine by uptake-1 in failing myocardium. To address the latter, we compared the effects of norepinephrine with those of isoproterenol, which is not a substrate for uptake-1 (15,16), under same experimental con-

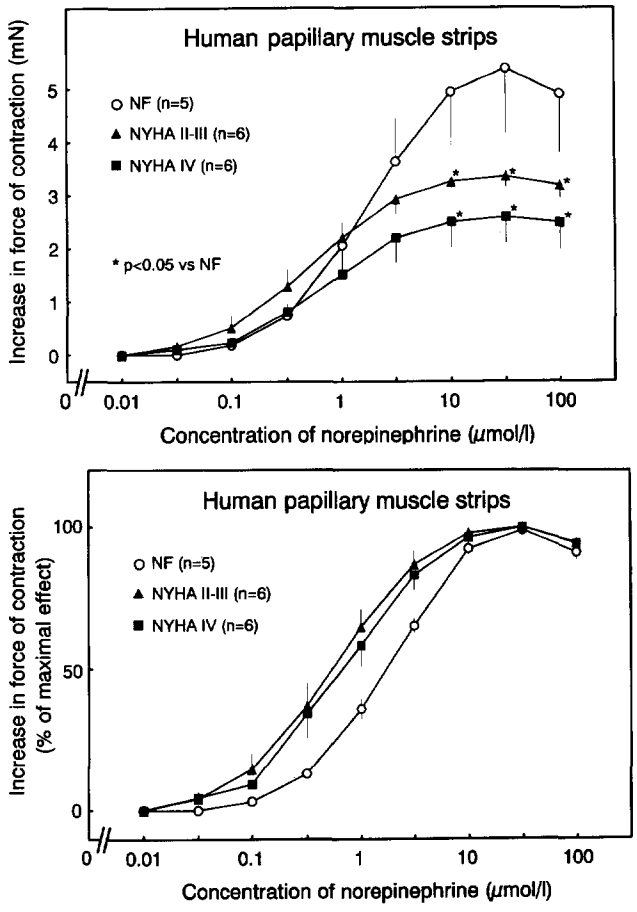


Figure 1. Concentration–response curves for the effect of norepinephrine (0.01 to 100 μmol/liter) on isometric force of contraction in isolated electrically driven papillary muscle strips from nonfailing donor hearts (NF) and from patients undergoing mitral valve replacement (New York Heart Association [NYHA] functional classes II to III) or cardiac transplantation (functional class IV).

Table 2. EC_{50} Values for Effect of Isoproterenol and Norepinephrine Alone or With Cocaine or Desipramine

Study Conditions	EC_{50} Values (μmol/liter)		
	NF	NYHA II–III	NYHA IV
Isoproterenol	0.016 (0.01–0.02)	0.026 (0.02–0.04)*	0.06 (0.04–0.08)*
NE	1.7 (1.21–2.43)	0.50 (0.22–1.14)*	0.59 (0.3–1.15)*
NE + cocaine	0.58 (0.36–0.94)†	0.28 (0.12–0.66)	1.1 (0.43–2.86)
NE + desipramine	0.51 (0.19–1.35)†	0.27 (0.11–0.67)	0.85 (0.13–5.45)

*$p < 0.05$ versus nonfailing myocardium (NF). †$p < 0.05$ versus norepinephrine (NE). EC_{50} values = concentration producing half-maximal effects (range); NYHA = New York Heart Association functional class (see Table 1).

ditions. The results are summarized in Figure 2. As with norepinephrine, the maximal effects of isoproterenol were reduced in relation to the severity of heart failure (Fig. 2, top). As shown in Figure 2, bottom, the effects of isoproterenol were less potent in failing than in nonfailing myocardium, also in relation to the stage of the disease. Consistently, the EC_{50} values were significantly higher in functional classes II to III and IV myocardium than in nonfailing myocardium. In summary, opposing results on the potency to increase force of contraction were obtained with isoproterenol, which is not a substrate for uptake-1 (15,16), and the physiologically occurring beta-adrenoceptor agonist norepinephrine, which is primarily inactivated by uptake-1, uptake into presynaptic stores (17).

To further substantiate the evidence for a reduced uptake-1 in failing myocardium, experiments with the uptake-1 inhibitors desipramine and cocaine were performed. Some difference in the effect of these uptake-1 inhibitors on the concentration–response curve of norepinephrine in the different groups could be expected if there was an alteration in norepinephrine carrier. Figure 3 shows concentration–response curves for norepinephrine alone and in the presence of desipramine (3 μmol/liter) or cocaine (3 μmol/liter) in cardiac preparations from nonfailing myocardium. At 3 μmol/liter of desipramine or cocaine, force of contraction was reduced by ~15% (not shown). Higher concentrations were not used because of the negative inotropic effects of the uptake-1 inhibitors. Cocaine and desipramine shifted the concentration–response curve of norepinephrine to the left in nonfailing myocardium. The EC_{50} values were significantly reduced compared with those for norepinephrine alone (Table 2). Figure 4 summarizes the data in functional class IV myocardium. Neither desipramine nor cocaine affected the concentration–

Figure 2. Concentration–response curves for the effect of isoproterenol (isoprenaline) (0.0001 to 1 μmol/liter) on isometric force of contraction in isolated electrically driven papillary muscle strips from nonfailing donor hearts (NF) and from patients undergoing mitral valve replacement (New York Heart Association [NYHA] functional classes II to III) or cardiac transplantation (functional class IV).

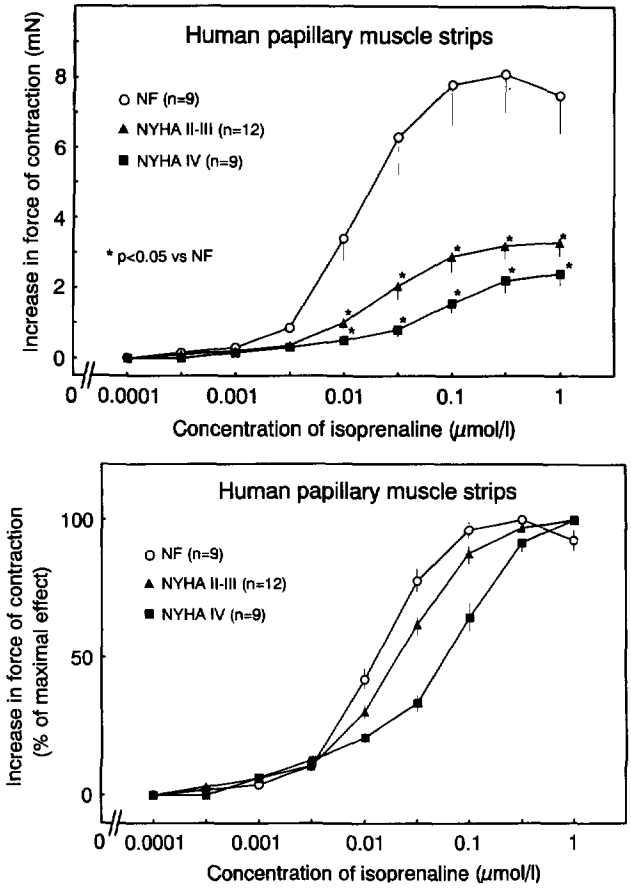
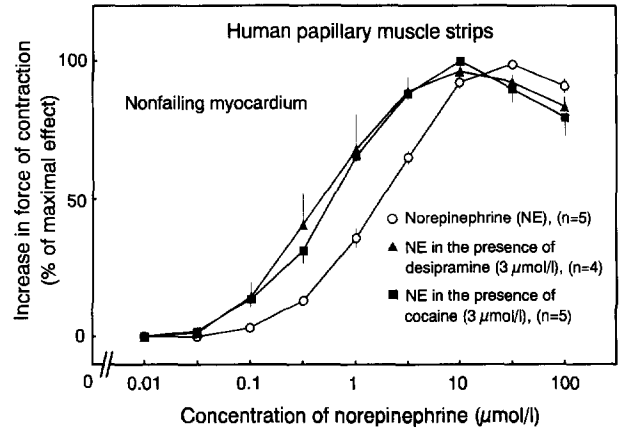


Figure 3. Concentration–response curves for the effects of norepinephrine (0.01 to 100 μmol/liter) alone and in the presence of cocaine (3 μmol/liter) or desipramine (3 μmol/liter) on isometric force of contraction in isolated electrically driven papillary muscle strips from nonfailing donor hearts.



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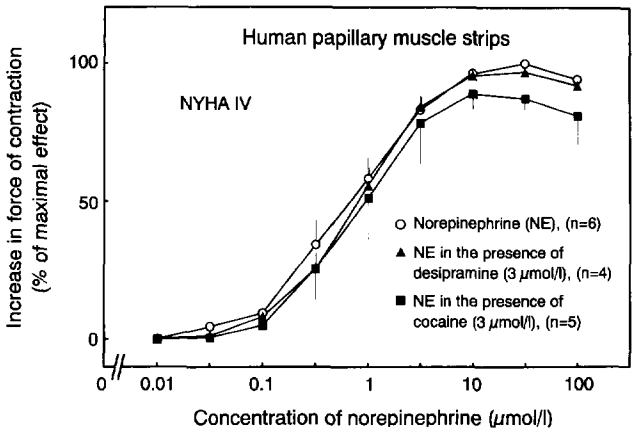

Figure 4. Concentration–response curves for the effects of norepinephrine (0.01 to 100 μmol/liter) alone and in the presence of cocaine (3 μmol/liter) or desipramine (3 μmol/liter) on isometric force of contraction in isolated electrically driven papillary muscle strips from patients undergoing heart transplantation (New York Heart Association [NYHA] functional class IV).

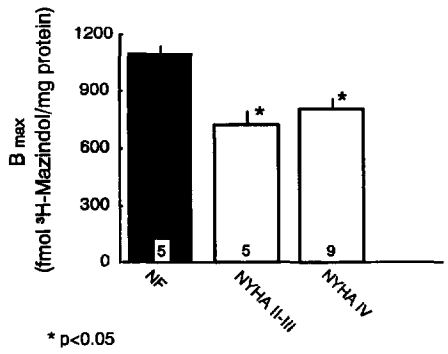

Figure 5. Density of norepinephrine uptake carrier sites in cardiac membranes from the human left ventricle from nonfailing (NF) donor hearts and from patients undergoing mitral valve replacement (New York Heart Association [NYHA] functional classes II to III) and heart transplantation (functional class IV). Densities were investigated with radioligand saturation experiments. See Methods.

response curves in functional class IV myocardium. The EC_{50} values of norepinephrine alone or in the presence of cocaine or desipramine in functional class IV myocardium were not significantly different from those in nonfailing myocardium in the presence of uptake-1 inhibitors. Thus, uptake-1 inhibition in nonfailing myocardium produced a situation similar to that in failing myocardium under control conditions.

Norepinephrine uptake carrier sites. To quantitate norepinephrine uptake carrier sites directly, radioligand binding experiments were performed in functional classes II to III and IV myocardium and in nonfailing myocardium. Hydrogen-3 mazindol binding experiments were performed as described elsewhere (18). Hydrogen-3 mazindol binding was strongly NaCl dependent. Maximal specific binding was obtained in the presence of 120 mmol/liter of NaCl (not shown). Radioligand saturation binding experiments (in the presence of 120 mmol/liter of NaCl) revealed concentration-dependent binding of the radioligand. Scatchard (19) analysis of the binding data showed monophasic binding with a specific binding of ~30% at the dissociation constant (K_D) (not shown). The density of norepinephrine uptake carrier sites obtained with these techniques is shown in Figure 5. There was a reduction by ~30% in uptake sites in functional classes II to III and IV myocardium compared with nonfailing myocardium ($p < 0.05$). The absolute numbers and K_D values are summarized in Table 3.

Discussion

It is widely accepted that there is a general activation of the sympathetic nervous system in heart failure (6–8,20), which results in increased circulating norepinephrine levels at rest and during exercise (6–8) and correlates with the prognosis of patients with heart failure (6,8). In addition, Swedberg et al. (9) observed an increased release of norepinephrine from the myocardium of patients with heart failure compared with control subjects. The latter finding could result from increased activity of myocardial sympathetic neurons or impaired uptake of norepinephrine from the synaptic cleft, or both. Further evidence for local alteration of myocardial norepinephrine (i.e., uptake, release and synthesis) has been reported. There are reduced stores of myocardial norepinephrine (18,21,22) and a reduced myocardial turnover, including defects in catecholamine synthesis (23,24) as well as reduced effects of the indirectly acting sympathomimetic drugs tyramine (25) and dopexamine (26). Controversy exists about the involvement of norepinephrine uptake site mechanisms in heart failure. Rose et al. (13) reported a reduction in the rate of norepinephrine uptake and of norepinephrine release with a multiple indicator technique and reported a reduction in norepinephrine release from the myocardium of patients with dilated cardiomyopathy. However, Hasking et al. (12) provided evidence for unchanged norepinephrine uptake as judged from the kinetics of tritiated norepinephrine constantly applied to the myocardium of patients with heart failure. In agreement with the latter report, Meredith et al. (11) reported data on the cardiac outflow of the norepinephrine precursor dihydroxyphenylalanine, the intraneural metabolite of norepinephrine dihydroxyphenylglycol and of tritium-labeled norepinephrine. The latter report was in

Table 3. Binding Characteristics of Hydrogen-3 Mazindol to Human Left Ventricular Membrane Preparations

	B_{max} (fmol H-3 mazindol bound/mg protein)	K_D (nmol/liter)
NF (n = 5)	1,102 ± 37	27.0 (22.1–33.1)
NYHA II–III (n = 5)	724 ± 77*	36.4 (22.4–59.2)
NYHA IV (n = 9)	809 ± 52*	28.4 (23.7–34.1)

*$p < 0.05$ versus nonfailing myocardium (NF). Data presented are mean value ± SEM or value (range). B_{max} = maximal number of binding sites; K_D = dissociation constant; NYHA = New York Heart Association functional class (see Table 1).

favor of an increased neuronal firing rate of sympathetic nerves in the presence of unchanged neuronal uptake.

The discrepancies among available studies are apparently influenced by the different techniques used and the problem of differentiating between sympathetic activity and catecholamine uptake-1 in vivo.

Cardiac uptake-1 and contractility. To overcome the problems inherent in in vivo studies, we investigated the effects of norepinephrine compared with isoproterenol, which is not a substrate of uptake-1, uptake into presynaptic stores (15,16) in isolated human myocardium in vitro. The efficiency of both beta-adrenoceptor agonists in increasing force of contraction was reduced in relation to severity of heart failure. These findings were reported earlier by several groups (1–3,27) and might be related to postsynaptic alterations in beta-adrenergic signal transduction involving beta-adrenoceptor down-regulation or uncoupling (1–3,28) and increased levels of myocardial G_i-alpha (4–6). However, the opposite results were obtained with the two beta-adrenergic agonists when their potency was compared. As expected, and as previously reported (1), the potency of isoproterenol was observed to be reduced in failing myocardium. This could be the result of beta-adrenoceptor uncoupling and down-regulation (1,2,27,28). However, the potency of norepinephrine to increase force of contraction was increased in failing human myocardium.

This finding is compatible with the concept that exogenously applied norepinephrine is not inactivated because of a reduced capacity for norepinephrine uptake. If this were the case, one would expect that the concentration–response curve of norepinephrine would be affected differently by norepinephrine uptake-1 inhibitors, such as desipramine or cocaine. Indeed, cocaine and desipramine shifted the concentration–response curves of norepinephrine to the left in nonfailing myocardium but did not affect the effect of norepinephrine in failing human myocardium. Thus, another discrepancy exists between the in vivo data reported by Meredith et al. (11) and the in vitro data on isolated myocardium reported in the present study, some of which could be due to the fact that Meredith et al. (11) studied in vivo norepinephrine uptake in patients with moderate heart failure. Thus, norepinephrine uptake in the whole heart, including the vasculature and the arteries, is determined by using the techniques of Meredith et al. (11). The experiments performed in the present study exclusively address alterations in norepinephrine uptake in left ventricular myocardium. Conclusions drawn from these data cannot be uncritically extrapolated to other parts of the heart, such as the arteries or vasculature. Therefore, the different results indirectly indicate evidence of differences in norepinephrine uptake or even sympathetic innervation of different parts or components of the failing human heart. Finally, Meredith et al. (11) studied patients with hypertension or ischemic heart disease, or both, at the stage of moderate heart failure. In the present study, we evaluated myocardium from patients with terminal heart failure due to ischemic dilated cardiomyopathy as well as myocardial tissue from patients with mitral valve replacement. Therefore, the underlying cardiac disease could also have affected these differences.

Norepinephrine uptake carrier sites in isolated myocardial membranes. To substantiate functional findings with a biochemical technique, we labeled norepinephrine uptake-1 carrier sites with the radioligand H-3 mazindol. This compound has been reported to label catecholamine uptake carrier sites in the brain (18). In the present study, we observed a significant reduction by ~40% in H-3 mazindol binding sites in left ventricular particulate fractions from failing hearts compared with fractions from nonfailing hearts. In right heart failure in dogs caused by tricuspid valve avulsion, Liang et al. (29) reported a significant reduction by ~50% of H-3 mazindol binding sites in the right but not left ventricle. The reduction of binding sites closely was correlated with the depressed norepinephrine uptake activity in the same hearts. These investigators concluded that local mechanisms could be related to the impairment of norepinephrine uptake confined to the right ventricle in their model. Norepinephrine uptake inhibitors failed to influence the concentration–response curve for norepinephrine, although the number of H-3 mazindol binding sites was reduced by 30%. Thus, a large portion of uptake-1 carriers in the present study of human heart might not participate in active norepinephrine inactivation.

Study limitations. A potential limitation of the radioligand binding experiments is the use of a high concentration of desipramine (100 μmol/liter) to determine nonspecific binding in experiments characterizing norepinephrine uptake carrier sites in human ventricular tissue. Thus it is possible that desipramine displaces H-3 mazindol binding from nonspecific binding sites. However, we could not reproduce the data of Liang et al. (29), who reported numbers of H-3 mazindol binding sites similar to those herein by using 0.3 μmol/liter of desipramine. Thus, H-3 mazindol binding to human myocardial membranes is more similar to binding sites in striatal membranes, where ~30 to 100 μmol/liter of desipramine is necessary to displace H-3 mazindol binding (18). Such difficulties in quantifying norepinephrine uptake carrier sites by binding experiments emphasize the necessity of applying functional as well as biochemical methods to investigate reduced norepinephrine uptake-1 in failing myocardium.

Evidence for a reduction in norepinephrine uptake effects has been observed in the transplanted human heart. After transplantation, degeneration of the postganglionic ventricular nerve terminal has been observed in histologic (30) and immunohistologic (31) investigations. Consistently, as in the failing human heart (21,22), norepinephrine depletion occurs in the transplanted heart (26,32). Failing myocardium is also similar to transplanted heart myocardium with regard to uptake mechanisms. In heart transplant recipients the effect of epinephrine on myocardial force of contraction was observed to increase in vivo, whereas the effect of isoproterenol was unchanged (33). Desipramine treatment in healthy volunteers enhanced the effect of epinephrine but was ineffective in heart transplant recipients (33). To our knowledge these are the first

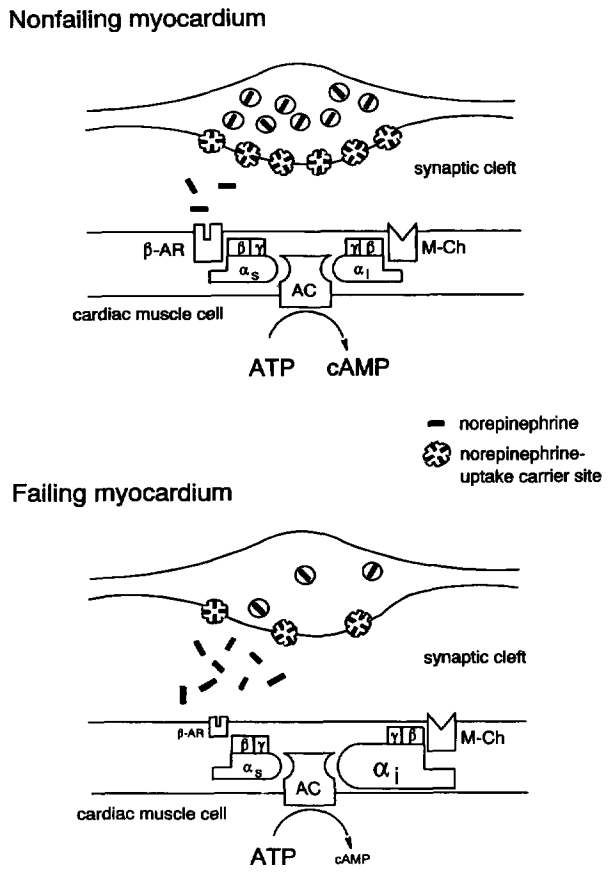

Figure 6. Scheme of functional defects in the failing human myocardium. Down-regulation of cardiac beta-adrenoceptors (β-AR) and an increase in inhibitory guanine binding proteins (G_i-alpha) lead to a reduced formation of the intracellular second messenger cyclic adenosine monophosphate (cAMP). In addition to these postsynaptic changes, there could be a presynaptic defect in the sympathetic nerve system consisting of a reduced density of norepinephrine uptake carrier sites. The consequence could be an increase in the concentration of norepinephrine in the synaptic cleft. AC = adenylyl cyclase; ATP = adenosine triphosphate; M-Ch = m-cholinoceptors; α_i (α_s) = G_i (G_s)-protein subunit alpha; β (γ) = G-protein subunit beta (gamma).

findings to demonstrate the similarity of the failing and the transplanted human heart.

Clinical implications. In patients with heart failure as well as in heart transplant recipients, there will be a higher ratio of inotropic to vasconstrictor effects (i.e., a higher ratio of beta-adrenergic to alpha-adrenergic stimulation). These findings indicate some important consequences in the initiation of postsynaptic adenylyl cyclase desensitization of the failing heart. A defective norepinephrine uptake could contribute to high concentrations of norepinephrine in the synaptic cleft. In addition, elevated plasma norepinephrine concentrations contribute to beta-adrenoceptor down-regulation even in situations in which the release of norepinephrine from the sympathetic cardiac neurons is low because cardiac norepinephrine is not inactivated by uptake-1. The latter hypothesis has been substantiated in the dog model. Norepinephrine infusion failed to reduce beta-adrenoceptor numbers in dogs with an intact myocardial norepinephrine uptake mechanism (34), but beta-adrenoceptor down-regulation was observed in dogs after cardiac denervation (34), a condition that is known to impair myocardial norepinephrine uptake (35). Thus, defective norepinephrine uptake into presynaptic stores is likely to contribute to adenylyl cyclase desensitization, which is regarded as an important mechanism of myocardial dysfunction in heart failure (Fig. 6).

Conclusions. Functional and biochemical evidence indicate that norepinephrine uptake is impaired in failing human hearts. This defect is likely to alter the profile of the hemodynamic response to norepinephrine in vivo and might play a pathophysiologically important role in generating postsynaptic alterations, such as beta-adrenoceptor down-regulation and increased G_i-alpha levels, leading to adenylyl cyclase desensitization.

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