Neurochemical Evidence of Cardiac Sympathetic Activation and Increased Central Nervous System Norepinephrine Turnover in Severe Congestive Heart Failure

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Objectives. The aim of this study was to characterize cardiac sympathetic nervous function in patients with severe heart failure and to investigate the influence of the cause of heart failure, hemodynamic variables and central nervous system catecholamine release on cardiac sympathetic tone.

Background. Although heart failure is generally accompanied by sympathoexcitation, the integrity of cardiac sympathetic nerve function in heart failure remains controversial, particularly in relation to nerve firing activity and to the capacity of sympathetic nerves to recapture norepinephrine. Additionally, the location of the afferent and central neural pathways implicated in heart failure-induced sympathoexcitation remains unclear.

Methods. Radiotracer techniques were applied in 41 patients with severe heart failure and 15 healthy control subjects to study the biochemical aspects of whole body and cardiac sympathetic activity. Hemodynamic indexes of cardiac performance were measured in the heart failure group, and their association with sympathetic activity was studied. Jugular venous catechol spillover was measured to study the central noradrenergic control of sympathetic outflow.

Results. Sympathoexcitation was evident in the heart failure group, reflected by a 62% increase (p < 0.001) in total body and a 277% increase (p < 0.001) in cardiac norepinephrine spillover rates. These changes were accompanied by significant increases in the cardiac spillover of the norepinephrine precursor dihydroxyphenylalanine, the sympathetic cotransmitter neuropeptide Y and the extraneuronal metabolite 3-methoxy-4-hydroxyphenylglycol. The level of cardiac sympathetic activity was significantly correlated (r = 0.59, p < 0.001) with the mean pulmonary artery pressure. An increase in the spillover of dihydroxyphenylalanine and 3-methoxy-4-hydroxyphenylglycol from the brain was present, suggesting activation of central noradrenergic neurons.

Conclusions. Cardiac sympathetic activation is present in severe heart failure, bearing a close relation with pulmonary artery pressures, independent of heart failure etiology. Activation of noradrenergic neurons in the brain is also present and may be the underlying central nervous mechanism of the sympathoexcitation observed in heart failure.

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nerve firing, perhaps giving rise to an overestimation of the extent of neural activation.

The physiologic implications of these observations, however, remain somewhat unclear when sympathetically mediated neuroeffector responses are attenuated in heart failure, perhaps attributable to downregulation of beta-adrenoceptors or abnormalities of G proteins (12,13). In contrast, chronic high levels of norepinephrine as present in heart failure may exert cardiotoxic actions (14) and play a facilitative role in the genesis of ventricular arrhythmias (15). Accordingly, a more complete understanding of the cardiac sympathetic profile of patients with heart failure would have implications for devising optimal treatment strategies.

Although a detailed understanding of the mechanisms that integrate afferent hemodynamic signals, central processing, and efferent neural outflow for arterial blood pressure control has emerged (16,17), in heart failure the afferent stimulus for chronic overall and cardiac sympathetic activation remains largely unknown (18). Leimbach et al. (3) have observed a significant positive correlation between left ventricular filling pressures and muscle sympathetic activity, perhaps suggesting a role for cardiopulmonary baroreceptors. Additionally, it is unclear whether the peripheral sympathoexcitation that occurs in heart failure is driven by activation of central neural controlling processes, similar to those in the brainstem that participate in arterial blood pressure control.

To resolve these insufficiently clarified issues of cardiac sympathetic function in heart failure, we studied a large cohort of patients with severe heart failure (of various etiologies) by examining the spillover from the heart to plasma of the sympathetic neurotransmitter norepinephrine, its precursor and metabolites and that of the sympathetic co-transmitter neuropeptide Y. To examine the central controlling processes of cardiac sympathetic activity in heart failure, we measured the overflow of norepinephrine and related catechols (dihydroxyphenylalanine, dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol) from the brain because of the important contribution of central monoaminergic neurons to the control of sympathetic nervous outflow. Further, we explored the relationship between sympathetic activity and hemodynamic indexes that might represent afferent inputs to the central nervous system.

Methods

Study patients. The study group comprised 41 patients with severe heart failure (age 51 ± 1 years) and 15 healthy volunteers (age 46 ± 5 years, p = NS). The patients with heart failure were all in New York Heart Association functional class III or IV (average left ventricular ejection fraction determined by radionuclide ventriculography 18.5 ± 1.1%) and were undergoing assessment for suitability as candidates for heart transplantation. Of these patients, 20 had ischemic cardiomyopathy (demonstrated angiographically) and the 21 remaining patients in the nonischemic group included 15 patients with idiopathic dilated cardiomyopathy, 2 with alcoholic cardiomyopathy and 1 patient each with heart failure due to hypertension, valvular heart disease, doxorubicin toxicity or restrictive cardiomyopathy. In all cases, the patient's heart failure was considered to be of such severity that discontinuation of medications was not appropriate. Medications were generally comparable in all patients and included furosemide (98%), an angiotensin-converting enzyme inhibitor (98%) and warfarin. Thirty-four patients were receiving digoxin, whereas the remaining seven had not received digoxin for ≥1 month. The healthy control subjects were recruited by advertisement in the general community. The study was performed with the approval of the Alfred Hospital Ethics Review Committee and all subjects gave written informed consent.

Catheterization protocol. All studies were performed in the morning after a light breakfast. All subjects had refrained from smoking and from consuming caffeinated beverages over the 12 h before the procedure. Under local anesthesia, the radial artery was cannulated (3F, 5 cm, Cook) for arterial pressure monitoring and blood sampling. In the healthy volunteers, a venous introducer sheath was placed in the antecubital fossa. In the patients with heart failure, the sheath was placed in either the antecubital fossa or the right internal jugular vein.

In the patients with heart failure, a pulmonary artery thermodilution catheter (7F, Arrow, Arrow International) was advanced to the pulmonary circulation for the measurement of right heart pressures, pulmonary capillary wedge pressure and cardiac output. Subsequently in these patients and the healthy control subjects, a coronary sinus thermodilution catheter (Webster CCS 7/8U 90A, Webster Laboratories) was positioned in the coronary sinus under fluoroscopic control for blood sampling and determination of coronary sinus blood flow (19). In 11 patients with heart failure and 14 healthy control subjects, the coronary sinus catheter was also manipulated into the right internal jugular vein above the confluence of the facial veins for thermodilution measurement of jugular venous blood flow and blood sampling for the determination of plasma catecholamines in the venous effluent from the brain.

Neurochemical measures of sympathetic activity. Whole body sympathetic activity. The norepinephrine isotope-dilution technique as previously described by this laboratory (20,21) was employed to provide a biochemical index of "global" sympathetic nerve activity. In brief, tritiated levor-(7-3H)-norepinephrine (New England Nuclear) was infused continuously at a rate of 0.5 to 1 μCi/min through a peripheral vein for ≥60 min to achieve steady-state plasma concentrations. Once steady-state conditions had been achieved, the total norepinephrine spillover to plasma and norepinephrine plasma clearance were calculated as previously described (20).

Cardiac sympathetic activity. An extensive neurochemical assessment of cardiac sympathetic nerve function was performed by measuring the transcadiac spillover of nor-
epinephrine, its precursor dihydroxyphenylalanine, the intraneuronal metabolite of norepinephrine dihydroxyphenylglycol and an extraneuronal metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylglycol. Additionally, the cardiac release of neuropeptide Y, which is coreleased with norepinephrine from intraneuronal vesicular stores (22-24), was also measured as a further guide to cardiac sympathetic nervous activity. The regional spillover of norepinephrine from the heart was determined by using the modified Fick principle, which relies on a correction for the fractional extraction of tritiated norepinephrine across the vascular bed concerned (21,25). The spillover of neuropeptide Y and dihydroxyphenylalanine was determined by calculating the product of their respective venoarterial concentration gradients and the coronary sinus plasma flow, whereas dihydroxyphenylglycol spillover was computed by using coronary sinus blood flow because this lipophilic catechol is evenly distributed between plasma and red blood cells (26). Release of 3-methoxy-4-hydroxyphenylglycol across the heart was determined in the same manner (for 25 patients with heart failure and 9 control subjects) used to determine the dihydroxyphenylglycol spillover, on the basis of preliminary observations from our laboratory suggesting that 3-methoxy-4-hydroxyphenylglycol is also evenly distributed between plasma and red blood cells.

Central nervous catecholaminergic activity. On the basis of an extensive body of experimental evidence (27-30) suggesting that noradrenergic nuclei in the forebrain play an important stimulant role in regulating sympathetic nervous outflow, we used radiotracer methods to test for the presence of increased central noradrenergic activity in patients with heart failure. Recent clinical research (31) supports the application of this approach to assessing central noradrenergic activity by demonstrating the existence of a direct relation between norepinephrine release from the brain and peripheral sympathetic nervous activity. The turnover of norepinephrine in the central nervous system was assessed indirectly by measuring the rate of spillover of norepinephrine and the related catechols dihydroxyphenylalanine, dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol into an internal jugular vein (25,32). The tip of the coronary sinus thermodilution catheter was placed beyond the mandibular angle, proximal to the entry of any facial veins, to minimize the contamination of the cerebral venous effluent. The catheter was used for sampling jugular venous blood and for blood flow determination by the thermodilution technique (32). The spillover of norepinephrine, dihydroxyphenylalanine, dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol was calculated according to those methods used to determine their spillover from the heart.

Biochemical assays. Blood samples were collected into ice-chilled tubes containing an anticoagulant, ethyleneglycol-bis (β-amino-ethyl ether) N,N′ tetraacetic acid (EGTA) and reduced glutathione to prevent oxidation. After centrifugation, plasma samples were stored at −80°C until assayed. Norepinephrine, dihydroxyphenylalanine and dihydroxyphenylglycol concentrations were determined by high performance liquid chromatography with electrochemical detection as previously described (33). The plasma-specific activity of tritiated norepinephrine and dihydroxyphenylglycol was determined by liquid scintillation spectroscopy after fractional collection of the eluant from the electrochemical detector cell. Plasma concentrations of 3-methoxy-4-hydroxyphenylglycol were determined using a technique adapted from Eisenhofer et al. (34). In brief, 3-methoxy-4-hydroxyphenylglycol was extracted from ultrafiltered plasma samples by the addition of ethyl acetate, followed by drying under nitrogen. 3-methoxy-4-hydroxyphenylglycol was then reconstituted and underwent liquid chromatography with electrochemical detection. Plasma neuropeptide Y levels were determined by direct immunoassay using antiseraum raised in rabbits immunized with synthetic neuropeptide Y as previously described (23,35).

Statistical methods. Data are presented as mean value ± SEM. Where normally distributed, group data were compared using an unpaired Student t test. Data showing nonnormal distribution were compared using the Mann-Whitney U test. Relations between continuous variables were examined using the least squares method of linear regression. Variables that were significantly related to the cardiac norepinephrine spillover rate in a univariate analysis were entered into a stepwise multivariate analysis (SPSS-PC) to detect the independent variables that were significantly related to the cardiac norepinephrine spillover rate. These included the mean pulmonary artery pressure, left ventricular ejection fraction, mean arterial blood pressure and cardiac output. The pulmonary capillary wedge pressure was not entered because of a significant relation (r = 0.93, p < 0.001) with the mean pulmonary artery pressure. The criteria were p < 0.05 for entry and p < 0.10 for removal. Analysis of covariance was used to compare the slopes and intercepts of the relation for two continuous variables between groups, according to previously described methods (36). The null hypothesis was rejected where the p value was < 0.05.

Results

Hemodynamic profiles. Patients with congestive heart failure demonstrated a significantly abnormal hemodynamic profile consistent with the severity of their heart failure. The pulmonary capillary wedge pressure was 21.5 ± 1.3 mm Hg, mean pulmonary artery pressure 29.7 ± 1.6 mm Hg and cardiac output was 3.9 ± 0.1 liters/min. The mean arterial blood pressure in the patients with heart failure was 79.4 ± 1.7 mm Hg compared with 96.1 ± 1.8 mm Hg in the control subjects (p < 0.001). In the patients with heart failure, coronary sinus blood flow tended to be higher (212 ± 15 ml/min) than that in control subjects (168 ± 17 ml/min) (p = 0.06 [NS]). The thermodilution-derived unilateral internal jugular venous blood flow was not significantly different between patients with heart failure (409 ± 68 ml/min, n = 11) and control subjects (446 ± 46 ml/min, n = 14). When
Table 1. Neurochemical Profile of Severe Heart Failure

<table>
<thead>
<tr>
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<th>Control Subjects</th>
<th>Patients With Heart Failure</th>
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<tbody>
<tr>
<td>Arterial NE (pmol/liter)</td>
<td>1,397 ± 165</td>
<td>3,296 ± 342*</td>
</tr>
<tr>
<td>Total NE SR (pmol/min)</td>
<td>3,354 ± 335</td>
<td>5,448 ± 363*</td>
</tr>
<tr>
<td>Total NE clearance (liter/min)</td>
<td>2.51 ± 0.16</td>
<td>1.74 ± 0.08*</td>
</tr>
<tr>
<td>Cardiac NE SR (pmol/min)</td>
<td>105 ± 19</td>
<td>394 ± 46*</td>
</tr>
<tr>
<td>Cardiac tritiated NE extraction</td>
<td>0.78 ± 0.04</td>
<td>0.56 ± 0.02*</td>
</tr>
<tr>
<td>Cardiac DOPA SR (pmol/min)</td>
<td>110 ± 9</td>
<td>201 ± 29†</td>
</tr>
<tr>
<td>Cardiac DHPG SR (pmol/min)</td>
<td>682 ± 86</td>
<td>659 ± 79</td>
</tr>
<tr>
<td>Cardiac tritiated DHPG SR (dpm/min)</td>
<td>3,928 ± 685</td>
<td>7,472 ± 1,043†</td>
</tr>
<tr>
<td>Cardiac MHPG SR (pmol/min)</td>
<td>252 ± 73</td>
<td>564 ± 78‡</td>
</tr>
<tr>
<td>Cardiac NPY SR (pg/min)</td>
<td>−834 ± 926</td>
<td>1,759 ± 786f</td>
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*p < 0.001. †p < 0.01. ‡p < 0.05. DHPG = dihydroxyphenylglycol; DOPA = dihydroxyphenylalanine; MHPG = 3-methoxy-4-hydroxyphenylglycol; NE = norepinephrine; NPY = neuropeptide Y; SR = spillover rate.

Examined by etiology of heart failure, the hemodynamic profiles of the patients with ischemic cardiomyopathy closely resembled those of patients with heart failure of nonischemic origin, only the cardiac output differing significantly (ischemic vs. nonischemic etiology (3.7 ± 0.2 vs. 4.2 ± 0.2 liters/min, p < 0.05). The coronary sinus blood flow was not significantly different in these two groups (196 ± 24 vs. 227 ± 20 ml/min), although it tended to be lower in those of patients with an ischemic etiology of heart failure.

Neurochemical measures of whole body and cardiac sympathetic activity. The arterial concentration of norepinephrine was significantly increased in patients with heart failure (3,296 ± 242 vs. 1,397 ± 165 pmol/liter, p < 0.001). In patients with heart failure, the whole body spillover rate of norepinephrine was increased by 62% (5,448 ± 363 vs. 3,354 ± 335 pmol/min, p < 0.001) and the rate of clearance of norepinephrine from plasma was significantly reduced by 28% (1.74 ± 0.08 vs. 2.51 ± 0.16 liters/min, p < 0.001).

Patients with heart failure had a 277% increase in the rate of norepinephrine spillover to plasma from the heart compared with values in control subjects (394 ± 46 vs. 105 ± 19 pmol/min, p < 0.001). There was an accompanying significant increase in the cardiac release of dihydroxyphenylalanine, tritiated dihydroxyphenylglycol (the tritiated intraneuronal norepinephrine metabolite) and 3-methoxy-4-hydroxyphenylglycol (the extraneuronal metabolite of norepinephrine), consistent with increased cardiac sympathetic nervous activity (Table 1). The fractional extraction of tritiated norepinephrine across the heart was significantly depressed in subjects with heart failure (0.56 ± 0.02 vs. 0.78 ± 0.04, p < 0.001). The concentration of neuropeptide Y (which is coreleased with norepinephrine from sympathetic nerve terminals) in arterial plasma was modestly elevated in the patients with heart failure (273 ± 38 vs. 187 ± 29 pg/ml, p = 0.07). However, cardiac release of neuropeptide Y was significantly elevated compared with that in control subjects, in whom net extraction was evident (Table 1).

Influence of cause of heart failure and therapy on sympathetic activity. To examine the effect of potential influences of etiology on cardiac sympathetic nervous function, we compared the variables of cardiac sympathetic neural integrity among patients with failure of ischemic and nonischemic background (Fig. 1). The spillover of norepinephrine into plasma across the heart was similar in patients with ischemic (348 ± 57 pmol/min) and nonischemic (438 ± 71 pmol/min) heart failure. The fractional extraction of tracer norepinephrine across the heart was also similar in patients with ischemic and nonischemic heart failure. The production of the related catechols, dihydroxyphenylalanine, dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol was also similar (Fig. 1). Although the cardiac production of tritiated dihydroxyphenylglycol was not significantly different, a trend toward higher production was present in the nonischemic group. Because the ischemic heart failure cohort had a significantly lower cardiac output and a tendency toward a lower left ventricular ejection fraction than that of patients with idiopathic cardiomyopathy, we used an analysis of covariance to compare the cardiac production of norepinephrine and tritiated dihydroxyphenylglycol with the
Cardiac output as a covariate. In this analysis, the cause of heart failure was further demonstrated not to be of significance in determining the rate of norepinephrine spillover from the heart.

Although the medications prescribed were very similar among the patients with heart failure, 7 of the 41 patients were not receiving digoxin. Because of this difference and previous suggestions that digoxin might influence sympathetic nervous function (37–39), we compared the neurochemical profiles of patients with heart failure according to the presence or absence of digoxin therapy. The hemodynamic profile of those subjects treated with digoxin was not significantly different from those of patients not receiving cardiac glycosides. Furthermore, the arterial norepinephrine concentration (3,292 ± 281 vs. 3,310 ± 434 pmol/liter) and the whole body spillover rate for norepinephrine (5,055 ± 410 vs. 5,138 ± 735 pmol/min) were similar in those patients receiving digoxin and those not. In the heart, the fractional extraction of norepinephrine across the heart was not different according to the presence (0.55 ± 0.03) or absence (0.57 ± 0.03) of digoxin therapy. Although the spillover of norepinephrine from the heart was not significantly influenced by digoxin treatment, there were trends toward higher spillover rates for norepinephrine and related catechols in those patients not receiving digoxin. These trends could not be further resolved by analysis of covariance with either the pulmonary arterial pressures or cardiac output as the covariate.

Hemodynamic correlates of sympathetic activity. Consistent with a possible underlying reflex mechanism for the significant increase in cardiac sympathetic activity in the setting of heart failure, there was a modest significant positive univariate correlation (Fig. 2) of cardiac norepinephrine spillover with both the mean pulmonary artery pressure \( r = 0.59, p < 0.001 \) and the pulmonary capillary wedge pressure \( r = 0.50, p < 0.01 \). In addition, there was a weaker negative correlation with cardiac output \( r = -0.32, p < 0.05 \). Stepwise multiple regression analysis identified the mean pulmonary artery pressure as the only hemodynamic factor that contributed significantly to a regression model of the cardiac norepinephrine spillover rate. Cardiac production of tritiated dihydroxyphenylglycol and dihydroxyphenylalanine \( r = 0.48, p < 0.01 \) and \( r = 0.35, p < 0.05 \) was also significantly related to both the pulmonary artery pressure and wedge pressure. Of note, there was no demonstrable relation between the mean arterial pressure and cardiac norepinephrine spillover, whereas the whole body norepinephrine spillover was weakly negatively correlated with systemic arterial pressure \( r = -0.36, p < 0.05 \). Left ventricular ejection fraction had no relation with measurements of either cardiac or whole body sympathetic activity.

Central nervous catecholaminergic activity. We examined the spillover of norepinephrine and related lipophilic catechols from the brain in 11 patients with heart failure and 14 control subjects. The spillover of norepinephrine into the cerebrovascular circulation was not significantly different in the heart failure (112 ± 34 pmol/min) and control (93 ± 61 pmol/min) groups (Fig. 3). However, the spillover of dihydroxyphenylalanine was significantly elevated in patients with heart failure (558 ± 118 pmol/min vs. 173 ± 94 pmol/min, \( p < 0.05 \)). In conjunction with this finding, the spillover of endogenous dihydroxyphenylglycol (404 ± 110 vs. 192 ± 76 pmol/min) and labeled dihydroxyphenylglycol (3,362 ± 1,931 vs. 1,182 ± 648 dpm/min) showed a trend toward higher levels in patients with heart failure \( (p = NS) \). The spillover of the extraneuronal norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol into the cerebral circulation was significantly elevated in patients with heart failure (2,369 ± 577 vs. 789 ± 146 pmol/min, \( p = 0.02 \) [Fig. 3]).

**Discussion**

In the current study, we examined the whole body sympathetic, cardiac sympathetic and central catecholamin-
ergic neurochemical profile of patients with severe heart failure. The heart failure cohort comprised patients with cardiomyopathy of various underlying causes and was of such severity that all patients were under active consideration for heart transplantation. In comparison with previous studies examining sympathetic activity (2,11,40), the current patient group had more severe depression of left ventricular ejection fraction despite the continuation of antifailure therapy during neurochemical assessment.

Neurochemical profile of whole body and cardiac sympathetic activity. Patients with heart failure demonstrated marked elevation of whole body and cardiac sympathetic activity compared with levels in a control group of healthy volunteers of comparable ages. Sympathetic activation was reflected peripherally by elevation of arterial plasma norepinephrine, which, as described previously (2,41) and confirmed in the current study, is due to both an increase in the whole body spillover rate of norepinephrine and a coexistent decrease in the norepinephrine clearance rate. Cardiac sympathetic activation was evident in the threefold elevation in the rate of spillover of norepinephrine to plasma. In association with this, the cardiac spillover of both dihydroxyphenylalanine, the norepinephrine precursor, and the extraneuronal norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol was significantly elevated in those with heart failure. The cardiac release of neuropeptide Y, which coexists with norepinephrine in the terminals of noradrenergic nerves (22-24,42), was also significantly increased in the patients with heart failure. Although previous workers demonstrated an elevation of neuropeptide Y levels in arterial plasma samples (43,44) and the myocardium (12) of patients with heart failure, to our knowledge the current study is the first to document increased cardiac neuropeptide Y release in patients with heart failure.

Although the rate of cardiac norepinephrine release measured in the current heart failure cohort is consistent with rates previously reported from our laboratory (2,11), certain more subtle differences in the neurochemical profile of sympathetic nervous function in our and other studies (11,40) deserve consideration. Most notable among these differences is the degree of reduction in norepinephrine extraction across the coronary circulation. Norepinephrine extraction, as measured by the removal of labeled norepinephrine from plasma, across the heart, is predominantly related to the activity of the uptake-1 carrier, which under normal conditions is responsible for removing about 70% to 80% of the norepinephrine transiting the coronary circulation (40,45). Aside from uptake-1 activity, the extraction of norepinephrine is also known to be dependent on the presence of diffusion barriers, relative blood flow and the activity of extraneuronal metabolic processes (46). In our study, norepinephrine extraction from plasma by the heart was reduced by 28% compared with reductions in previous reports of 14% (11) and 53% (40). In contrast to the reduction in norepinephrine extraction across the heart, and suggesting that this was not due to faulty neuronal uptake of norepinephrine, there was a significant increase in the intraneuronal production of tritiated dihydroxyphenylglycol, which is dependent on uptake-1 activity in the heart (47). Although it has been suggested that the reduced norepinephrine reuptake could account for a significant proportion of the increased rate of spillover of norepinephrine into the coronary circulation of patients with heart failure (40), the 275% increase in norepinephrine spillover in the current study is of a substantially greater magnitude than any possible attenuation of neuronal extraction and hence must reflect to a major extent the influence of increased nerve firing.

The mechanism for the attenuation in norepinephrine extraction in heart failure is unclear, although several potential explanations are apparent. First, myocardial interstitial changes have been described to varying degrees in heart failure (48); these may increase the diffusional barrier for radiolabeled norepinephrine between the coronary circulation and sympathetic nerve terminals. Second, it may be a consequence of sympathetic activation itself. In acute studies (47) involving electrical sympathetic nerve stimulation, the fractional extraction of tracer across the heart has been shown to decrease, limiting its capacity to reflect true uptake-1 activity under such conditions. Such studies do not, however, mimic the effect of chronic cardiac sympathetic stimulation, as in heart failure, and it is conceivable that chronic sympathetic overactivity might result in some diminution of uptake-1 activity. The possibility that the higher coronary sinus blood flow in the heart failure group contributed to the observed reduction in tracer extraction could not be excluded, although previous workers (49) have suggested that the relation is significant only at the extreme ranges of blood flow. Regardless of the state of neuronal norepinephrine reuptake in the failing heart, the increased spillover of dihydroxyphenylalanine, 3-methoxy-4-hydroxyphenylglycol and neuropeptide Y do confirm that sympa-
thetic nerve firing and norepinephrine synthesis and release are increased.

Influence of etiology on heart failure. In the current study, we compared the cardiac sympathetic profile of patients with heart failure of ischemic and nonischemic etiology because of previous suggestions (12) that neurochemical differences exist between these two forms of cardiomyopathy. We found no differences in estimates of cardiac release or synthesis of norepinephrine or its subsequent intraneuronal or extraneuronal metabolism. Additionally, extraction of titrated norepinephrine across the heart was not influenced by the etiology of the cardiomyopathy. Bristow et al. (12) previously examined ex vivo failing hearts of ischemic and nonischemic etiology and found no differences in the tissue content of myocardial catecholamines. In contrast, tissue they obtained from nonischemic failing hearts showed a more marked attenuation of beta-adrenoceptor density, whereas the ischemic myocardium demonstrated a greater degree of receptor uncoupling. Although the latter finding may reflect the influence of chronic ischemia (50,51), it is difficult to attribute the differences in beta-adrenoceptor density to differing levels of cardiac sympathetic activity when we find no demonstrable differences in norepinephrine release. We could not, however, exclude regional variations in sympathetic nerve activity within the myocardium.

Influence of medications. One major difference between the present study and those performed previously (2,11,40) was our continuation of antifail ure therapy during neurochemical testing because of the severity of the cardiac failure. As a consequence, this study was also able to document that sympathoexcitation is sustained despite optimal heart failure therapy. Anti-failure medications were typically furosemide, an angiotensin-converting enzyme inhibitor, warfarin and, in most cases, digoxin. We found no significant influence of digoxin on whole body or cardiac sympathetic activity when comparing patients with similar hemodynamic profiles. Previously, digoxin has been reported to have a range of effects on sympathetic nervous traffic and transmitter release. Digoxin augments baroreceptor sensitivity in heart failure (52-54) and may enhance baroreceptor-induced sympathoinhibition during physical or chemical stimulation (38). Ferguson et al. (37,55) reported that intravenous administration of a short-acting cardiac glycoside, deslanoside, reduced muscle sympathetic nervous activity at rest. This effect was presumed to be a reflection of increased baroreceptor sensitivity rather than a positive inotropic effect because administration of dobutamine did not result in sympathoinhibition. Apart from baroreceptor-mediated effects, digoxin has been reported in animal studies (59) to release norepinephrine from sympathetic nerve terminals. In the present study, we did not observe any apparent effect of long-term digoxin administration (at clinically relevant doses) on the spillover of norepinephrine or related metabolites from the heart or on arterial plasma norepinephrine concentrations, whole body norepinephrine spillover or norepinephrine clearance, thus supporting the findings of Goldsmith et al. (56).

Identification of possible neural reflex processes. Despite a general consensus that sympathetic excitation occurs in heart failure, the location of the afferent receptors and central pathways involved in eliciting heightened sympathetic activity remain controversial. In the periphery, Leimbach et al. (3) reported that muscle sympathetic nervous activity was most closely related to left ventricular filling pressure. Similarly, Ferguson et al. (37) reported a direct relation between muscle sympathetic activity and pulmonary artery pressures. Others (58) have suggested a link between muscle sympathetic nervous activity and skeletal muscle metaboreceptor activation acutely during exercise, which may have some relevance in heart failure.

Cardiac sympathetic tone is normally regulated by a complex interplay of afferent neural signals predominantly arising from arterial and cardiopulmonary baroreceptors and processed in the cardiovascular centers of the central nervous system. Distension of the left atrium and pulmonary veins has been shown to result in a positive chronotropic response, the afferent pathway lying in the vagus with the efferent response being mediated by cardiac sympathetic nerves (59). Less attention, however, has been directed toward the effects of cardiac distension on cardiac sympathetic nervous activity in structures other than the sinoatrial node. Kurz et al. (60) found that left atrial or pulmonary venous distension with balloon catheters increased left ventricular contractility and that this increase was mediated through cardiac sympathoexcitation. In keeping with these findings and those of the microneurographic studies, we observed a direct correlation, although of limited strength, between the rate of norepinephrine spillover from the failing heart and both mean pulmonary artery pressure and pulmonary capillary wedge pressure. Mechanoreceptors have been described (61) in the pulmonary artery, although their physiologic significance remains controversial. Right ventricular pressure receptors have also been described (62) but probably play little role in cardiovascular control.

To increase our understanding of the role of the brain in the peripheral sympathoexcitation observed in heart failure, we performed jugular venous sampling to measure the spillover of norepinephrine, its precursor dihydroxyphenylalanine and the metabolites dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol into the internal jugular vein to indirectly study norepinephrine synthesis and release in the brain. The source of monoamines released into the human cerebral circulation has been principally ascribed to brain neurons rather than to cerebrovascular sympathetic nerves because overflow is not reduced by ganglion blockade (32). This technique has previously provided evidence of a direct relation between central norepinephrine release and sympathetic outflow in healthy subjects (31) and is in agreement with animal studies (27-30,63,64) that demonstrate an excitatory influence of noradrenergic nuclei in the brain on sympathetic nervous outflow. We found significant
increases in the spillover of the norepinephrine precursor dihydroxyphenylalanine and the extraneuronal metabolite 3-methoxy-4-hydroxyphenylglycol from the brain in patients with heart failure. There was also a nonsignificant increase in the release of endogenous dihydroxyphenylglycol in the heart failure group. Norepinephrine spillover was not elevated. The apparent discrepancy between the latter finding and the observed increase in efflux of the related monoamines probably reflect the presence of a partial diffusional block to norepinephrine (65), whereas the other monoamines are more lipophilic.

Conclusions. End-stage heart failure is characterized by a considerable mortality rate due either to a progressive decline in pump function or to sudden death, the latter usually caused by a ventricular arrhythmia. Cardiac sympathetic overactivity has been implicated in the genesis of ventricular arrhythmias, and in the present study we confirmed the presence of heightened cardiac sympathetic tone in patients with severe heart failure independent of the etiology of their cardiomyopathy or the presence of digoxin therapy. We found cardiac norepinephrine spillover rates to be significantly correlated with pulmonary artery pressures, possibly representing an afferent stimulus for sympathetic stimulation. We also found increased cerebral neuronal catecholamine synthesis and release in patients with heart failure. However, the fundamental links between the hemodynamic afferent stimulus, central neural processing and sustained elevation of peripheral sympathetic outflow remain unclear.

References

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