Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity

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ABSTRACT  The analysis of plasma kinetics of the sympathetic neurotransmitter norepinephrine can be used to estimate sympathetic nervous "activity" (integrated nerve firing rate) for the body as a whole and for individual organs. In 12 patients with cardiac failure (left ventricular ejection fraction 10% to 39%), the mean arterial plasma norepinephrine concentration was 557 ± 68 pg/ml (mean ± SE) compared with 211 ± 21 pg/ml in 15 subjects without heart failure (p < .002). The difference was due to both increased release of norepinephrine to plasma (indicating increased "total" sympathetic activity) and reduced clearance of norepinephrine from plasma. The increase in sympathetic activity did not involve all organs equally. Cardiac (32 ± 9 vs. 5 ± 1 ng/min; p < .002) and renal (202 ± 45 vs 66 ± 9 ng/min; p = .002) norepinephrine spillover were increased by 540% and 206%, respectively, but norepinephrine spillover from the lungs was normal. Adrenomedullary activity was also increased in the patients with heart failure, whose mean arterial plasma epinephrine concentration was 181 ± 38 pg/ml compared with 71 ± 12 pg/ml in control subjects (p < .02). There is marked regional variation, inapparent from measurements of plasma norepinephrine concentration, in sympathetic nerve activity in patients with congestive heart failure. The finding of increased cardiorenal norepinephrine spillover has important pathophysiologic and therapeutic implications.

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THE CONSENSUS that sympathetic nervous overactivity is important in the pathophysiology of congestive heart failure is based in part on observations that the sympathetic neurotransmitter norepinephrine is present in plasma at increased concentration.\textsuperscript{1} The plasma concentration of norepinephrine, however, is determined by the rates of both release of norepinephrine to plasma and removal of norepinephrine from plasma. Norepinephrine plasma clearance might be reduced, and the plasma concentration thereby increased, because of the reduced cardiac output and organ blood flows that accompany congestive heart failure.

A second difficulty in the study of the role of the sympathetic nervous system in congestive heart failure has been the inability of clinical research methods to estimate sympathetic activity in internal organs. Microneurographic electrophysiologic methods are available for the clinical study of nerve firing rates in subcutaneous nerves supplying skin and skeletal muscle,\textsuperscript{2} but the nerves to internal organs are not accessible for such testing.

Measurement of sympathetic transmitter release is an alternative technique for quantifying sympathetic nerve activity in internal organs. We have developed a method\textsuperscript{3} using infusions of tritiated norepinephrine to determine both the rate at which norepinephrine spills into plasma and its rate of clearance from plasma. The technique has recently been applied to determine the rates of norepinephrine spillover from individual organs.\textsuperscript{4}

Although only a small fraction of the norepinephrine...
released from sympathetic nerves spills over to plasma, the regional rates of norepinephrine spillover from the heart, spleen, and lungs have been shown to correlate well with the rates of direct sympathetic nerve stimulation.

In this study, we have applied this technique to the study of congestive heart failure to determine (1) the extent to which the increased plasma levels of norepinephrine in patients reflect reduced clearance (rather than increased release) of norepinephrine and (2) whether sympathetic activity in patients with heart failure is uniformly increased or varies in different organs. We measured total norepinephrine spillover and clearance and the rates of spillover of norepinephrine from the heart, kidneys, and lungs. Cardiorenal sympathetic activity was the particular focus of the study because of the possible relevance of cardiac sympathetic stimulation to inotropic support of the failing heart, disease progression, and sudden death of renal sympathetic activity to salt and water retention in edematous states.

Methods

Study population. We studied 12 patients with clinical evidence of congestive heart failure (table 1). All had left ventricular ejection fractions (measured by radionuclide ventriculography) less than 40%. No patient had suffered recent myocardial infarction or unstable angina pectoris and none had uncorrected valvular heart disease or significant noncardiac disease. There were 15 control subjects, including five normal men (aged 26 to 46 years) and 10 patients (five men, five women, aged 42 to 63 years) with chest pain but normal left ventriculography, of whom five had coronary artery disease. The mean age of the patients was 59.3 ± 3.0 (SE) years and that of the control subjects was 47.5 ± 3.8 years. All subjects and patients gave written informed consent for the study, which was approved by the Alfred Hospital Ethics Review Committee.

Norepinephrine spillover and clearance. At steady-state during the infusion of \(^{3}H\)-norepinephrine, the total spillover of norepinephrine (NE) to plasma and total plasma norepinephrine clearance can be calculated.1

\[
\text{spillover} = \frac{\text{infusion rate (dpm/min)}}{\text{plasma NE specific activity (dpm/pg)}}
\]

and

\[
\text{clearance} = \frac{\text{infusion rate (dpm/min)}}{\text{plasma \(^{3}H\)-NE concentration (dpm/ml)}}
\]

where dpm = disintegrations per minute of tritiated norepinephrine.

The mean of the concentrations in three to seven arterial samples collected during each study was used for the calculation of norepinephrine specific activity, spillover rate, and clearance.

The rate of spillover of norepinephrine from individual organs was calculated from the Fick principle, corrected for the fractional extraction of norepinephrine:

\[
\text{NE spillover} = \frac{(\text{NE}_A - \text{NE}_V) + \text{NE}_V \times \text{ETR}}{\text{flow}}
\]

where \(\text{NE}_A\) and \(\text{NE}_V\) are the arterial and venous concentrations of endogenous norepinephrine and \(\text{ETR}\) is the fractional extraction of \(^{3}H\)-norepinephrine by the organ.

Regional clearance of norepinephrine is given by the product of \(^{3}H\)-norepinephrine extraction and plasma flow.

Protocol. Subjects were studied in the supine position 5 days after discontinuation of dietary sodium restriction and medications (other than digoxin). Studies were performed in an air-conditioned room (temperature 22.8° ± 0.56°C) after an overnight fast. Tea, coffee, cigarettes, and alcohol were not taken in the 12 hr preceding the study. After a priming bolus of 12 μCi of \(1-\text{ring-2,5,6-}^{3}H\)-norepinephrine (New England Nuclear, specific activity 40 to 50 μCi/mmol) and 120 mg of p-aminohippurate (PAH) via a peripheral vein, infusions were commenced that maintained plateau plasma concentrations during the study.3,4,11 Tritiated norepinephrine was infused at 0.7 μCi/m²/min and PAH at 5 mg/m²/min. The duration of infusions was 1.5 hr (total dose of tritiated norepinephrine approximately 80 μCi/m²). The tritiated norepinephrine was prepared in vials containing 0.2M acetic acid British Pharmacopoeia; (50 μCi/ml) and diluted 1:12.5 in normal saline (infuse concentration 4 μCi/ml) for infusion. Chromatographic testing for stability disclosed no detectable degradation over 3 hr under these conditions. A 21-gauge cannula was inserted percutaneously into a radial artery for pressure monitoring and blood sampling. No. 7F catheters were introduced percutaneously from an antecubital vein. The coronary sinus, renal vein, and pulmonary artery were catheterized sequentially with fluoroscopic monitoring. At each site venous blood samples were taken for measurement of catecholamines. Radial artery samples were obtained simultaneously. Cardiac output and coronary sinus blood flow were measured by thermodilution.12 Paired arterial and venous samples were collected for the estimation of renal plasma flow from the clearance of PAH.

Assays. Blood samples were transferred immediately on to ice and were centrifuged on completion of the study. Paired arterial and venous samples were assayed in duplicate at the same time. PAH was estimated colorimetrically, as described below. Plasma samples for catecholamine assays were kept frozen at −70°C. Catecholamines were measured by the method of Peuler and Johnson,13 which uses the enzymatic transfer (by catechol-o-methyl transferase) of tritium from a methyl donor to the plasma norepinephrine to be assayed.

Blood flow measurements. Cardiac output was calculated as the mean of at least three determinations by thermodilution, with 8 ml bolus injections of 5% dextrose at room temperature. Coronary sinus blood flow was measured as the mean of dupli-
cate determinations by thermodilution, with rapid infusions of 5% dextrose at room temperature. For the determination of renal plasma flow, standard curves relating optical density to the concentration of PAH were constructed with the subject’s plasma. The concentrations of PAH in duplicate experimental samples were determined from the curves and used to calculate total body clearance, which was corrected for fractional extraction to derive plasma flow. Blood and plasma flows were interconverted with the subject’s arterial hematocrit.

Statistics. Results have been expressed in the text as mean ± SEM. Because much of the data was not normally distributed, statistical significance was assessed by the Mann-Whitney U test (two-tailed). This test has 95% of the power of the t test when applied to normally distributed data. The null hypothesis was rejected if p < .05.

Results

Hemodynamics. Without vasodilator and diuretic treatment, the patients with heart failure experienced weight gain and increased breathlessness. Right heart catheterization was performed in all patients and demonstrated low cardiac index (2.36 ± 0.18 liters/min/m², normal range 2.71 to 3.92 liters/min/m²) and high systemic vascular resistance (table 2). Mean arterial pressure was similar in patients and control subjects. Coronary sinus blood flow was similar in the two groups, but renal blood flow was reduced in the patients with heart failure to 65% of the value in the control subjects (table 2, p < .02).

Norepinephrine spillover. Total norepinephrine spillover (figure 1) was 572 ± 86 ng/min in the patients with heart failure, 84% higher than that in control subjects (311 ± 34 ng/min; p < .02). In the patients with heart failure, norepinephrine spillover from the heart and kidney was increased to 640% and 306%, respectively, of the control values (p < .002 and p = .002, respectively). The rate of spillover from the lungs was similar in the two groups. Increased spillover from the heart and kidneys accounted for 62% of the increase in total spillover in the patients. Spillover from the gastrointestinal tract and liver, skin, and skeletal muscle was not measured.

Catecholamine clearance. The mean plasma norepinephrine clearance (figure 2) of the patients with heart failure (1.05 ± 0.07 liters/min) was 67% of the control value (1.57 ± 0.13 liters/min; p < .002). Mean pulmonary clearance was 56% of the control value and renal clearance was 74% of control. The fractional extraction of norepinephrine in transit through the heart, kidneys, and lungs was similar in the two groups (table 3). There was no consistent abnormality of regional epinephrine clearance (table 3). The tendencies toward lower cardiac and higher renal clearance of norepinephrine than of epinephrine did not reach significance.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<td>Hemodynamics in subjects with and without congestive heart failure</td>
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<table>
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<tr>
<th>Parameter</th>
<th>CHF</th>
<th>Control</th>
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<tbody>
<tr>
<td>Cardiac index (liters/min/m²)</td>
<td>2.36 ± 0.18</td>
<td>3.32 ± 0.13(^b)</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>95 ± 5</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>Systemic vascular resistance</td>
<td>1627 ± 84</td>
<td>1138 ± 48(^b)</td>
</tr>
<tr>
<td>Coronary sinus blood flow index</td>
<td>68 ± 11</td>
<td>68 ± 10</td>
</tr>
<tr>
<td>Renal blood flow index (ml/min/m²)</td>
<td>381 ± 44(^A)</td>
<td>600 ± 47</td>
</tr>
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\(^{A}\)p < .02
\(^{b}\)Laboratory normal value. Not all control subjects underwent right heart catheterization.

FIGURE 1. Total and regional norepinephrine spillover in patients with congestive heart failure and in control subjects. \(^{*}\)p < .02; \(^{**}\)p < .002.
Plasma catecholamine concentrations. The mean arterial plasma epinephrine concentration was 181 ± 38 pg/ml in patients with heart failure and 71 ± 12 pg/ml in control subjects (p < .02). There was a similar average difference (557 ± 68 vs 211 ± 21 pg/ml; p < .002) in the plasma norepinephrine concentration, which was 164% higher in the patients with heart failure (figure 2). The higher arterial plasma norepinephrine concentration in patients with heart failure was attributable to reduced plasma norepinephrine clearance (which alone would increase the concentration by 50%), and increased norepinephrine spillover (which would account for an 84% increase in plasma concentration). The predicted combined effect on the plasma norepinephrine concentration of the observed changes in spillover and clearance is given by the equation:

\[
[NE]_{CHF} = \frac{\text{control spillover} + \text{difference in spillover}}{\text{control clearance} + \text{difference in clearance}}
\]

For the observed values of spillover and clearance, the predicted plasma norepinephrine concentration in patients with heart failure is 545 pg/ml, which is close to the observed value of 557 ± 68 pg/ml.

Discussion

Norepinephrine spillover and plasma norepinephrine concentration. The increase in plasma norepinephrine concentration that we found in patients with congestive heart failure was proportionately greater than the increase in total norepinephrine spillover in the same patients. This finding demonstrates the importance of norepinephrine clearance as a determinant of the plasma norepinephrine concentration. In situations (such as congestive heart failure) where clearance is likely to be abnormal, the rate of norepinephrine spillover is more accurate than the plasma concentration as an index of sympathetic tone.14 In this study, the observed increase in the plasma norepinephrine concentration was caused by an interaction between increased nor-
epinephrine spillover and reduced clearance, the latter presumably the result of reduced pulmonary and visceral plasma flow. The reported prognostic significance of the plasma norepinephrine concentration in patients with cardiac failure\(^\text{16}\) may in part reflect this dependence of the plasma concentration on the adequacy and distribution of cardiac output.

**Effect of age on norepinephrine spillover.** The mean age of our patients was 12 years older than that of our control subjects. Plasma norepinephrine concentrations increase with age, by approximately 13% per decade.\(^\text{17-19}\) In our experience, this relationship reflects reduced clearance with increasing age,\(^\text{19}\) although others have reported that total norepinephrine spillover is higher in the elderly.\(^\text{20}\) Although age may affect norepinephrine kinetics to some degree, it is most unlikely that the marked increases we have demonstrated in total, cardiac, and renal spillover in heart failure are the result of this influence.

**Regional norepinephrine spillover in patients with congestive heart failure.** The increase in sympathetic tone in patients with congestive heart failure did not affect all organs equally: total norepinephrine spillover was increased by 80%, but spillover rates from the heart and kidney were increased by approximately 500% and 200%, respectively, while pulmonary spillover was normal. Increased norepinephrine spillover from the heart and kidney accounted for 62% of the increase in total spillover in patients with heart failure. We did not measure norepinephrine spillover from the hepatomesenteric circulation, skeletal muscle, or skin. These inputs contribute approximately 34% of overall norepinephrine spillover to plasma in healthy subjects,\(^\text{21}\) so increased sympathetic activity in these regions could contribute substantially to the increased total norepinephrine spillover in cardiac failure. There is microneurographic evidence of increased rates of discharge in sympathetic nerves supplying skeletal muscle in patients with heart failure.\(^\text{22}\)

**Interpretation of increased regional norepinephrine spillover.** The high rates of cardiac and renal norepinephrine spillover in patients with congestive heart failure point to increased sympathetic nerve activity in those organs. Because infused norepinephrine, like endogenous transmitter, is taken up mainly by neurons,\(^\text{23}\) the normal fractional extraction of tritiated norepinephrine across the heart and kidneys in our patients showed that neuronal uptake was normal, despite previous reports of some impairment in patients with cardiac failure.\(^\text{24, 25}\) The rate at which norepinephrine diffuses into plasma may depend in part on organ plasma flow.\(^\text{11, 26}\) If such a relationship does exist, the 35% reduction in renal flow in our patients with heart failure may have caused us to underestimate the degree of renal sympathetic stimulation. This difficulty does not apply to our data for the heart, where flow was normal. Our evidence for increased cardiac sympathetic nerve activity extends previous observations\(^\text{24, 27}\) of increased cardiac arteriovenous differences in plasma norepinephrine concentration in patients with heart failure.

**Mechanism of increased norepinephrine spillover.** The cause of the increased cardiac and renal sympathetic activity in patients with congestive heart failure remains unclear. Stimulation of cardiac mechanoreceptors by the cardiac dilatation and high intracavitary pressures that accompany heart failure might be expected to reduce efferent sympathetic nerve activity, especially to the kidney.\(^\text{28}\) However, these receptors adapt to long-term stimulation (by reduced rates of discharge), so the reflexes may not be active in patients with chronic heart failure.\(^\text{29}\) Unloading of arterial baroreceptors by reduced arterial pressure is an alternative mechanism for increased sympathetic nerve activity, but arterial pressure was normal in the patients we studied. Increased cardiac sympathetic activity might result from stimulation of sympathetic cardiocardioreflexes, but these also reduce renal sympathetic nerve activity.\(^\text{28}\)

Sodium depletion, from diuretic administration or dietary sodium restriction, increases total and renal norepinephrine spillover.\(^\text{4, 30}\) but both these measures had been discontinued 5 days before testing in our patients. Our patients were retaining sodium at the time of study, but this may be an effect\(^\text{10}\) rather than a cause of increased sympathetic stimulation. Dietary sodium loading does not increase norepinephrine spillover.\(^\text{30}\)

It is unlikely that our results were influenced by the patients’ therapy. Digoxin tends to decrease cardiac sympathetic nerve activity,\(^\text{31}\) and vasodilator and diuretic agents were discontinued. Finally, the increase in cardiac norepinephrine spillover was too great to be accounted for by the increased ventricular mass that accompanies left ventricular failure, and it was not a consequence of changes in coronary blood flow, which was normal.

**Myocardial norepinephrine content, norepinephrine spillover, and myocardial adrenoceptors.** Increased cardiac neural activity may explain the reduced myocardial catecholamine concentrations seen in patients with heart failure,\(^\text{32-34}\) with greatly increased cardiac norepinephrine turnover ultimately depleting tissue stores, although a reduction in tyrosine hydroxylase activity,\(^\text{34}\) affecting norepinephrine synthesis, could contribute.
It seems likely that the intense myocardial sympathetic stimulation implied by our results contributes to the myocardial β-adrenergic receptor downregulation\textsuperscript{15} that accompanies severe congestive heart failure. Bevan and Su\textsuperscript{36} have estimated that in blood vessels with neuroeffector gaps of 2000 Å stimulated at 10 Hz (about five times basal discharge), intrasynaptic norepinephrine concentrations of up to 10\textsuperscript{-6}M occur. Cardiac sympathetic neuroeffector junctions are narrower, and the potential for diffusion of transmitter is much less than in blood vessels, so the intrasynaptic concentrations in our patients, also at apparent stimulation rates approximately five times normal, are likely to be at least as high. The plasma concentrations of norepinephrine found in patients with heart failure are less than in blood vessels, so the intrasynaptic concentrations and do not produce physiologic effects.\textsuperscript{37} Circulating norepinephrine is unlikely to be responsible for cardiac β-adrenoreceptor downregulation.

**Pulmonary norepinephrine spillover.** The lungs contributed a large proportion of the total norepinephrine spillover to plasma, even though there was net extraction of norepinephrine in the lungs. It is not surprising, given the rich sympathetic innervation of the pulmonary vasculature\textsuperscript{38} and the unique position of the lungs, which receive the entire cardiac output, that considerable norepinephrine spillover occurred. The fact that spillover was normal in our patients with heart failure, who were not hypoxic, is consistent with the overriding importance of alveolar oxygen content as a determinant of pulmonary arterial resistance.\textsuperscript{39}

**Implications.** This study has shown that in resting patients with congestive heart failure there is increased spillover of norepinephrine from the heart and kidneys. The cause is not impaired neuronal uptake but increased sympathetic neural discharge. The consequences of these pathophysiologic changes in regional sympathetic nervous function have not been explored. Sympathetic nerves directly innervate the distal renal tubule and have an important influence on sodium balance.\textsuperscript{40} Increased renal sympathetic nerve stimulation may be a mechanism of salt and water retention in patients with cardiac failure and in edematous states in general.\textsuperscript{40, 41}

The increased cardiac sympathetic nervous activity in patients with cardiac failure may provide inotropic support. However, knowledge of the process of receptor downregulation and conflicting reports of the effects of adrenergic blockade in patients with heart failure\textsuperscript{41-43} invite scepticism concerning the importance of any beneficial effect. Moreover, intense cardiac sympathetic stimulation may be disadvantageous, by accelerating myocardial degeneration\textsuperscript{8} and promoting arrhythmias and sudden death.\textsuperscript{9}

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