Norepinephrine Spillover to Plasma During Steady-State Supine Bicycle Exercise

Comparison of Patients With Congestive Heart Failure and Normal Subjects

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This study was performed to determine the relative contributions of plasma norepinephrine clearance and norepinephrine release to the increase in plasma norepinephrine concentration that occurs during exercise and to determine whether the high rates of cardiac norepinephrine release from the heart and kidney in patients with heart failure are associated with diminished reserve for regional sympathetic nervous stimulation. During supine steady-state bicycle exercise at 50% of maximum voluntary exercise capacity, the plasma norepinephrine concentration of six patients with congestive heart failure rose from 385 ± 88 to 2,200 ± 497 pg/ml, whereas that of nine normal subjects rose from 208 ± 21 to 882 ± 257 pg/ml. The change in plasma concentration in both groups was due to an increase in norepinephrine spillover to plasma without a change in plasma norepinephrine clearance. In patients with heart failure, cardiac spillover increased from 80 ± 26 to 528 ± 265 ng/min during exercise, and renal spillover rose from 146 ± 71 to 418 ± 69 ng/min. In the normal subjects, cardiac spillover rose from 5 ± 2 to 73 ± 23 ng/min, and renal spillover increased from 76 ± 27 to 275 ± 106 ng/min. There is no evidence of a reduced reserve for overall or regional sympathetic stimulation in patients with heart failure. Reduced reflex responses in these patients are more likely due to end-organ refractoriness than to inadequate stimulation. (Circulation 1988;78:516–521)

Elevated plasma norepinephrine concentration observed in patients with congestive heart failure is due to both reduced plasma norepinephrine clearance and increased spillover of norepinephrine to plasma. Because reduced plasma clearance of norepinephrine is an important determinant of the baseline plasma concentration of norepinephrine, it might also be an important determinant of the increase in plasma norepinephrine that occurs during exercise. One would expect this effect to be more marked in patients with heart failure because of the intensity of splanchnic vasoconstriction during exercise in these patients. There is also evidence of intense cardiorenal sympathetic nervous stimulation under resting conditions in patients with heart failure. We hypothesized that the very high resting rates of norepinephrine spillover to plasma from the heart indicated maximal cardiac sympathetic stimulation. An inability to increase cardiac norepinephrine release, as well as tissue refractoriness to norepinephrine, might contribute to the “chronotropic incompetence” observed in patients with heart failure. We were interested in examining the renal sympathetic response to exercise because of the abnormality we had previously observed in this vascular bed at rest and because this bed also has been reported to undergo significant vasoconstriction during exercise.

In the present study, we examined the changes in overall, cardiac and renal norepinephrine spillover and plasma norepinephrine clearance during supine bicycle exercise in patients with congestive heart failure and in normal subjects.

Subjects and Methods

Study Population

We studied six patients with clinical evidence of left ventricular failure (Table 1). All had left ventricular ejection fractions (measured by radionuclide angiography) less than 0.40. No patient had suffered recent myocardial infarction or unstable angina pectoris, and none had uncorrected valvular heart disease or significant noncardiac disease. The

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 TABLE 1. Study Population

<table>
<thead>
<tr>
<th>Patients with Normal subjects</th>
<th>Patients with Heart failure</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>Age (mean ± SEM yr)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6/3</td>
<td>6 ± 6</td>
</tr>
<tr>
<td>Patients with Heart failure</td>
<td>Sex (male/female)</td>
<td>6/0</td>
</tr>
<tr>
<td>Age (mean ± SEM yr)</td>
<td>62 ± 8</td>
<td>0 ± 9</td>
</tr>
<tr>
<td>Incidence</td>
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<td>2</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Coronary artery disease</td>
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<td>4</td>
</tr>
<tr>
<td>Idiopathic dilated cardiomyopathy</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>New York Heart Association Functional Class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Cardiopulmonary ratio (mean ± SEM %)</td>
<td></td>
<td>54 ± 5</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (mean ± SEM %)</td>
<td></td>
<td>28 ± 1</td>
</tr>
</tbody>
</table>

Exercise capacity of all patients was limited by breathlessness and muscle fatigue and not by angina pectoris. There were nine normal control subjects (six men, three women, aged 45-60 years) who underwent clinical, hematological, and biochemical screening, chest radiography, rest and exercise electrocardiography, and echocardiography before admission to the study. All patients and subjects gave written, informed consent for the study, which was approved by the Alfred Hospital Ethics Review Committee, Prahran, Australia.

Norepinephrine Spillover and Clearance

At steady state during the infusion of tritiated norepinephrine, the rate of spillover of norepinephrine into plasma and total plasma clearance can be calculated by:

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

and

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

where NE is norepinephrine, and dpm is disintegrations per minute of tritiated norepinephrine.

The mean concentrations in two to four arterial blood samples were used to calculate total norepinephrine specific activity, spillover, and clearance. The rate of norepinephrine spillover from individual organs was calculated by the Fick principle, which was corrected for the fractional extraction of [3H]norepinephrine:

\[
\text{spillover} = \left(\frac{\text{NE}_v - \text{NE}_a}{\text{NE}_a} + \text{NE}_a \cdot E_{\text{TRIT}}\right) \times \text{plasma flow}
\]

where \(\text{NE}_v\) and \(\text{NE}_a\) are the venous and arterial concentrations of endogenous norepinephrine, respectively, and \(E_{\text{TRIT}}\) is the fractional extraction of [3H]norepinephrine by the organ. Regional clearance of norepinephrine is given by the product of \(E_{\text{TRIT}}\) and plasma flow.

An assumption underlying the use of the tracer method for the calculation of norepinephrine spillover is that the concentrations of tracer and endogenous norepinephrine are at equilibrium. Although the accuracy of this assumption had been demonstrated previously for studies at rest,\(^6\) it was necessary to undertake repeated sampling during exercise to ascertain whether a new steady state had been reached. In five subjects (four normal and one with heart failure), arterial blood samples were collected after 5 and 10 minutes of exercise, and the following variables were determined: endogenous norepinephrine concentration (934 ± 483 and 1,006 ± 500 pg/ml), tritiated norepinephrine concentration (1,329 ± 177 and 1,390 ± 194 dpm/ml), and plasma norepinephrine specific activity (2.71 ± 0.75 and 2.84 ± 0.97 dpm/pg). Because the tritiated norepinephrine infusion rate was constant, calculated total norepinephrine spillover to plasma also did not change during this period. The coefficients of variation for repeated cardiac norepinephrine spillover measurements during exercise were 17% and 4% in two subjects, but the first subject had marked transient hypertension during the first minutes of exercise, during which his cardiac spillover was much higher than the two similar values recorded later. For all other subjects, exercise was associated with smooth changes in hemodynamics, which were stable before sampling was performed.

Study Protocol

After baseline investigations, all subjects underwent preliminary bicycle exercise in the supine position, usually on the day before the study. During this test, workload was increased from zero by 20-W increments each minute until further exercise was impossible. Normal subjects were limited by leg fatigue; patients developed breathlessness and leg fatigue.

After fasting overnight, the subjects were studied in the supine position in an air-conditioned room (temperature, 22.8 ± 0.6°C). Dietary sodium restriction and medications (other than digoxin) were discontinued 5 days before study, and tea, coffee, alcohol, and other stimulants were not taken for 12 hours before the study. Tritiated norepinephrine was prepared and infused as previously described to achieve a stable tracer concentration.\(^8\) The total duration of infusions was less than 3.5 hours, during which time no degradation of the infusate is detectable by chromatographic testing.\(^6\)

A 21-gauge cannula was inserted percutaneously into a radial artery for pressure monitoring and sampling, and 7F catheters were introduced percutaneously from one or both antecubital veins. The coronary sinus and renal vein were catheterized with fluoroscopic monitoring. At each site, venous
blood samples were collected for measurement of catecholamines. Arterial samples were collected simultaneously. One or two venous catheters were left in situ during exercise.

Supine bicycle exercise was performed at a single workload set at half that achieved during the baseline test. This was 65 ± 6 W for the normal subjects and 55 ± 5 W for the patients (2p = 0.05). Subjects exercised for 10–15 minutes. Hemodynamic variables were measured after 5 minutes, and sampling was performed thereafter. Cardiac output and coronary sinus blood flow were measured by thermodilution.9 Paired arterial and venous samples were collected for the calculation of renal plasma flow from the clearance of p-aminobenzylisopropylate, which has been shown to reach steady state under these conditions.6

Assays
Blood samples were kept on ice and were centrifuged immediately after the study. Plasma was kept frozen at –70°C, until assay for catecholamines, which was performed by the radioenzymatic method of Peuler and Johnson.11 p-Aminobenzylisopropylate was assayed colorimetrically from standard curves that were constructed from each subject’s plasma. Tritiated norepinephrine was extracted as previously described.6

Statistics
Group data are expressed as mean ± SEM. Relative changes from rest to exercise are expressed as median ratios. Because of the small numbers and skewed distributions of variables, comparisons between resting and exercise values within groups and comparisons between groups were made with the Mann-Whitney U test (two-tailed12). The null hypothesis was rejected when p was less than 0.05.

Results

Hemodynamics
Resting heart rate was 74 ± 6 beats/min in patients with heart failure and 64 ± 2 beats/min in normal subjects (2p < 0.02). Mean arterial pressure was similar in the two groups. Renal blood flow in patients with heart failure at rest was 72% of the value in normal subjects; the difference was not statistically significant in this small group. Coronary sinus blood flow was measured in only three patients.

During exercise (Figure 1), there was an increase in heart rate of 51 ± 7 beats/min in the patients and 58 ± 7 beats/min in the normal subjects (2p = 0.09). Mean arterial pressure rose by 14 ± 7 and 63 ± 10 mm Hg in the patients and normal subjects, respectively (2p = 0.002). Renal blood flow fell from 732 ± 148 to 637 ± 126 ml/min (12%) in the patients and from 1,182 ± 310 to 1,043 ± 238 ml/min (13%) in the normal subjects. Because perfusion pressure rose much more in the normal subjects, this indicates greater relative renal vasoconstriction in the normal subjects, especially because it is likely that the heart failure group also experienced a greater increase in venous pressure during exercise.

Norepinephrine Spillover
Total norepinephrine spillover at rest (Figure 2) was 544 ± 143 ng/min in the patients and 229 ± 30 ng/min in the normal subjects (2p < 0.02). Although the patients’ workload was slightly less than that of the normal subjects, the relative increase in overall spillover of norepinephrine to plasma did not differ significantly: fivefold in the patients and threefold in the
the control subjects. Resting cardiac spillover (Figure 3) in the normal subjects was similar to that found in our previous study. Cardiac spillover was markedly increased in all three patients with heart failure. During exercise, the patients' cardiac norepinephrine spillover increased sevenfold, whereas there was a 10-fold increase \((p<0.001)\) in cardiac spillover among the normal subjects. Cardiac norepinephrine spillover was 3% of total spillover at rest and 9% of total during exercise \((p = 0.03)\) in the normal subjects. Renal norepinephrine spillover (Figure 3) was also increased at rest in the patients with heart failure to 256% of that in the normal subjects \((p = \text{NS})\). The median increase in renal spillover during exercise in the patients was threefold, whereas that of the normal subjects was fourfold.

**Plasma Norepinephrine Clearance**

Total plasma norepinephrine clearance (Figure 2) was similar in the two groups, and there was no significant change during exercise in either group. In the patients, cardiac clearance of norepinephrine (Figure 4) was normal at rest but fell to zero during exercise, whereas in the normal subjects, there was no change. There was no difference between groups in resting renal norepinephrine clearance (Figure 4). During exercise, renal clearance was 56% of the resting value in the patients with heart failure and was 54% of the resting value in the normal subjects \((p = \text{NS})\).

**Plasma Catecholamine Concentrations**

The mean arterial plasma norepinephrine concentration (Figure 2) at rest was 385 ± 88 pg/ml in the patients with heart failure and 208 ± 21 pg/ml in the normal subjects \((2p<0.05)\). The elevation of the concentration in the patients was due to increased spillover of norepinephrine to plasma, rather than to impairment of plasma norepinephrine clearance. During exercise, the plasma concentration rose sixfold in the patients and threefold in the normal subjects (NS between groups). This increase was due to increased spillover, not to reduced clearance, of norepinephrine. The patients' resting plasma epinephrine concentration was 118 ± 36 pg/ml, and it rose to 512 ± 184 pg/ml during exercise. The resting plasma epinephrine concentration of the normal subjects was 92 ± 15 pg/ml, and it rose to 301 ± 145 pg/ml during exercise. There was no difference between groups in the ratios (3.9 and 1.7) of plasma concentrations at rest to those during exercise.

**Discussion**

The major conclusions from this study are that patients with heart failure are capable of increasing overall, cardiac, and renal sympathetic nervous activity and that the rise in plasma norepinephrine concentration that occurs during supine exercise is entirely due to increased release of norepinephrine. There is evidence of a marked, preferential increase in cardiac sympathetic activity during moderate exercise, but renal sympathetic stimulation increases only to the same extent as general sympathetic activity.

**Comparison With Previous Studies**

In a previous study, we reported that congestive heart failure was associated with both an increase in overall norepinephrine spillover and a reduction of plasma norepinephrine clearance. Similar findings have been reported by Davis and colleagues. In the present study, the patients had less severe cardiac failure as assessed by symptoms and ejection fraction. It is therefore not surprising that they also had a lower plasma norepinephrine concentration or that the increase in their plasma concentration above normal was the result of increased norepinephrine release, without impairment of norepinephrine clearance, which we believe is most likely a consequence of impaired visceral blood flow. It is interesting that the overall spillover rate of norepinephrine to plasma was similar in both the previous (severe heart failure) and the present (moderate heart failure) patients, that the resting cardiac spillover was extremely high, and that renal spillover was increased to a similar degree. Sympathetic overactivity is an early feature in the cardiomyopathic hamster model of congestive heart failure, but it has not been studied in mild human cardiomyopathy. In such patients, who probably have

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**Figure 3.** Regional norepinephrine spillover at rest (R) and during exercise (EX) in normal subjects (NORMAL) and in patients with congestive heart failure (CHF). NA, norepinephrine.

**Figure 4.** Regional norepinephrine clearance at rest (R) and during exercise (EX) in normal subjects (NORMAL) and in patients with congestive heart failure (CHF). NA, norepinephrine.
normal plasma norepinephrine clearance, the plasma norepinephrine concentration may be relatively low despite significant abnormality of overall or regional sympathetic activity.

**Workload and Sympathetic Response**

The absolute work performed by the patients in this study was slightly less than that performed by the normal subjects. This design was necessary to ensure that exercise would be of sufficient duration in each group to allow for sampling and hemodynamic measurements. The increase in plasma norepinephrine concentration at a fixed proportion of maximal workload has been reported as being impaired in patients with congestive heart failure, but that study relied on samples taken at several workloads, each workload being performed for 1 minute. Our study shows that the overall sympathetic response of patients during steady-state exercise was similar to that of normal subjects at a similar proportion of their maximum workload. This response resulted in higher, not lower, plasma norepinephrine concentrations in the patients with heart failure.

**Norepinephrine Kinetics After Perturbations of Steady-State Conditions**

The analysis of kinetics of tracer norepinephrine infusions is now established as a more precise index of overall and regional sympathetic nervous activity under resting conditions than the plasma norepinephrine concentration alone. The technique has also been applied to the analysis of the overall sympathetic response to upright tilt, sustained for 90 minutes. The present study represents a major extension of application of this technique to the analysis of overall and regional sympathetic responses to an intervention after a relatively short period for reequilibration. We have shown that, after the initial attainment of steady-state conditions at rest, the plasma concentrations of endogenous and tritiated norepinephrine were stable after 5 minutes of exercise; this response matched the time course of the hemodynamic response to exercise at a fixed load and probably represents a new steady state. Although total norepinephrine spillover to plasma accurately reflects the sum of regional spillover measurements at rest, the attainment of an overall steady state during exercise does not guarantee the attainment of steady state in each region. Our limited data suggest that cardiac spillover, like heart rate, reaches a new plateau after a few minutes of exercise. However, because of this potential limitation and the small number of patients in this study, our description of the regional response to exercise in heart failure is essentially qualitative.

**Cardiac Response to Exercise**

There was a marked increase in cardiac norepinephrine spillover during exercise in the normal subjects, which resulted in cardiac spillover accounting for 9% of the total spillover, compared with 3% of a lesser total at rest. This means that there was selective sympathetic stimulation of the heart during exercise. It is not possible to compare this with the response of the few patients in whom cardiac data were obtained, but this study is sufficient to exclude the possibility that patients with heart failure are unable to increase cardiac stimulation above their high basal levels. The inference that follows is that reduced cardiac responses to the stimuli of exercise result from end-organ refractoriness in patients with heart failure. Of note, the extraction of tritiated norepinephrine was markedly reduced during exercise in patients with heart failure. Given that infused norepinephrine is cleared mainly by neuronal uptake, there are three possible explanations for this finding. First, tritiated norepinephrine might be taken up and released by terminal vesicles at high rates of sympathetic stimulation; because the increased spillover during exercise in normal subjects only equaled that of the patients at rest, this effect would influence mainly the patients. Second, normal neuronal uptake may be saturable; again, such a phenomenon is more likely to have affected the patients than the normal subjects. Third, the finding may reflect impairment of neuronal uptake in patients with heart failure, which is not detectable at rest. This question cannot be resolved by present techniques.

**Renal Response to Exercise**

The changes in renal hemodynamics observed in this study are similar to those found previously in normal human subjects. However, they are greater than the values reported for exercising dogs. In dogs, but not in humans, splenic contraction makes a significant contribution to the redistribution of blood flow that occurs during exercise. Although there was a greater relative vasoconstriction in the normal subjects, this probably increased their renal vascular resistance to about the level observed at rest in the patients with congestive heart failure. Further vasoconstriction occurred in the patients with heart failure during exercise. These changes in vascular resistance were accompanied by a greater norepinephrine spillover at rest in the patients and by similar increases in spillover during exercise in each group. It is not possible to relate blood flow to spillover; we did not measure renin release or sodium excretion rates, both of which also may be affected by sympathetic stimulation. The falls in renal norepinephrine clearance during exercise were greater than would be explained by the changes in plasma flow alone. There is no apparent explanation for the decrease in renal extraction of tritiated norepinephrine at spillover rates that, in relation to organ plasma flow, are considerably less than those in the heart.
Importance of Other Vascular Beds

We hypothesized that reduced visceral blood flow during exercise\(^2\) would lead to reduced norepinephrine clearance during exercise in both experimental groups but to more of a reduction in the patients with heart failure. This hypothesis was based on the fact that the kidneys and liver are responsible for about 32% of total norepinephrine clearance.\(^7\) In fact, plasma clearance did not change in either group. Previous work from our laboratory\(^7\) has shown that the major sites of clearance of norepinephrine from plasma are the lungs and the liver. Although hepatic blood flow falls during exercise,\(^2\) cardiac output rises so that pulmonary norepinephrine clearance would increase unless fractional extraction fell markedly. Similarly, we have estimated that skeletal muscle at rest contributes about 11% of the total plasma norepinephrine clearance. The increased flow to exercising muscle may allow increased clearance in those regions during exercise, even if fractional extraction is low.

Implications

This study indicates that rates of sympathetic stimulation in patients with congestive heart failure, although increased, are capable of even further increase in response to the moderate stimulus of steady-state exercise at 50% of maximum voluntary exercise capacity. No sustained arrhythmias occurred during exercise in either group. We did not measure cardiac function, but it seems likely that the increased sympathetic stimulation of the heart contributes to increased rate and force of contraction during exercise.\(^24\)\(^,\)\(^25\) If cardiac responses to exercise are reduced in patients with heart failure, the cause is more likely to be tissue refractoriness than lack of stimulation. The effects of renal sympathetic stimulation, which might include renin release, renal sodium and water retention, and renal vasocostriction (general or selective) are also undefined.

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References

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