Augmented Sympathetic Neurotransmitter Activity in the Peripheral Vascular Bed of Patients with Congestive Heart Failure and Cardiac Norepinephrine Depletion

By Richard S. Kramer, M.D., Dean T. Mason, M.D., and Eugene Braunwald, M.D.

SUMMARY
Since there is an overall augmentation of sympathetic nervous activity in patients with congestive heart failure while adrenal medullary function is normal and neurotransmitter stores are diminished in the heart, investigation was directed to the functional status of adrenergic receptors and available norepinephrine stores in the peripheral vascular beds. Blood flow, determined plethysmographically, and vascular resistance, calculated in the calf, following intra-arterial injections of tyramine and norepinephrine in eight patients with congestive heart failure were compared to the responses in nine patients with heart disease but without heart failure. The absolute increase in vascular resistance produced by graded doses of norepinephrine was greater in patients with heart failure. However, the relative augmentation of vascular resistance produced by any dose of norepinephrine was essentially identical in the two groups. In contrast, the vasoconstrictor response to a standard dose of the indirectly acting sympathomimetic agent, tyramine, was markedly enhanced ($P < 0.01$) in cases of heart failure, both when this increased response was considered in terms of the relative rise in vascular resistance, and in the quantity of injected norepinephrine required to produce a similar elevation of resistance. Concentration of norepinephrine in atrial tissue, determined at the time of cardiac surgery a few days after completion of the pharmacological studies, was significantly lower in the patients with heart failure. It is concluded that the quantity of endogenous sympathetic neurotransmitter available for release by tyramine from nerve endings in the peripheral arteriolar bed in the calf is not reduced and may even be augmented in patients with heart failure, and that the elevated levels of plasma norepinephrine in the heart failure state are derived, at least in part, from labile adrenergic stores in the peripheral vascular bed.

Additional Indexing Words:
Sympathetic nervous system  Peripheral vascular norepinephrine
Arteriolar resistance, calf  Calf blood flow  Plethysmography
Blood pressure  Tyramine

There is now considerable evidence that sympathetic activity is augmented in patients with congestive heart failure.$^{1,2}$ Associated with this overall increase of sympathetic function in the body, which is reflected in increased urinary excretion of norepinephrine,$^{3}$ is normal adrenal medullary activity, reflected in normal excretion of epinephrine,$^{3}$ and a depletion of neurotransmitter stores in the heart.$^{4}$ These observations

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suggest, therefore, that the elevated levels of plasma and urinary norepinephrine which occur in heart failure\(^6\) may ultimately be derived from sympathetic nerve endings located in tissues other than the heart.

Since the augmentation of sympathetic activity in heart failure does not appear to be uniform throughout the cardiovascular system, it became of interest to determine the functional status of adrenergic receptors and available norepinephrine stores in the sympathetic nerve endings of the peripheral vascular bed in patients with heart failure. Accordingly, the present investigation was undertaken to characterize sympathetic activity and labile norepinephrine stores in a specific vascular bed, the resistance vessels in the calf. This was accomplished by comparing the response of these vessels to tyramine, a drug which constricts vascular smooth muscle by releasing norepinephrine stores from sympathetic nerve endings,\(^6\) and to norepinephrine injected directly into the arterial circulation.

**Methods**

Studies were carried out on 17 patients with heart disease, 14 of whom underwent open-heart operations within 4 days of the study. The patients were divided into two groups. In group I were nine patients (five men and four women between 19 and 34 years of age) who had never experienced heart failure and were considered to be in functional Class I, according to the criteria of the New York Heart Association. Six of these patients were operated upon following the study; three had foramen secundum atrial septal defects and one each had incomplete atrio-ventricular canal, discrete subaortic stenosis, and tetralogy of Fallot. Two of the three patients who were not operated upon had idiopathic supraventricular arrhythmias, and one had undergone correction of pulmonic stenosis 6 months earlier. The eight patients in group II, four men and four women, were between 24 and 58 years of age; four had mitral valvular disease, two had combined aortic and mitral valvular lesions, and one each had ventricular septal defect, and idiopathic hypertrophic subaortic stenosis. Each of these eight patients was in functional Class IV, was receiving digitalis and diuretics, and had reached optimal compensation at the time of study.

The venous occlusion plethysmographic tech-

\[ \text{nique for the measurement of blood flow in the calf, as described in detail previously, was utilized.} \]

Changes in limb volume were determined by means of the mercury-in-rubber strain gauge placed on the midcalf. The lower extremity was positioned horizontally at the level of the right atrium. Arterial pressure was measured through an indwelling needle placed in the common femoral artery. To eliminate the circulation to the foot, a sphygmomanometric cuff around the ankle was inflated to a level exceeding systolic arterial pressure. A second sphygmomanometric cuff, 20 cm wide, was placed around the thigh, and venous occlusion of the calf was produced by inflating this cuff suddenly to a pressure below the diastolic arterial pressure. Blood flow in the calf was calculated from the rate of change of calf circumference during venous occlusion\(^7,8\) and was expressed in ml/100 g of tissue/min. Vascular resistance in the calf was calculated as the ratio of mean arterial pressure to calf blood flow and was expressed in units of mm Hg/ml/100 g/min.

With the patient in a stable, resting state, four consecutive control measurements of blood flow and arterial pressure were made at 5-min intervals. Tyramine,\(^*\) 100 \(\mu g/m^2\) BSA, was then rapidly injected through the femoral arterial needle. Venous occlusion curves were recorded at 20-sec intervals during the next 5 min; mean arterial pressure was recorded continuously. Fifteen minutes after all effects of tyramine had disappeared, the effects of graded doses of \(\text{l-}
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norepinephrine\(^\dagger\) were determined in an identical manner. The doses routinely employed were 0.0625 \(\mu g/m^2\), 0.25 \(\mu g/m^2\), 0.5 \(\mu g/m^2\), and 1.0 \(\mu g/m^2\), and 10 to 15 min were permitted to elapse between injections. The maximal change observed following each injection is reported.

The cardiac concentration of norepinephrine was determined in right or left atrial appendages removed immediately prior to institution of cardiopulmonary bypass in each of the 14 patients who underwent surgical treatment several days after the study. These specimens were rinsed immediately in saline solution, gently blotted dry, and quickly frozen with dry ice. The specimens were then weighed and homogenized with 5% trichloroacetic acid. The norepinephrine in the tissue extract was adsorbed onto aluminum oxide, eluted with acetic acid, and oxidized with potassium ferricyanide to the trihydroxyindole.

\*Obtained from Mann Research Laboratories, New York, New York.

\daggerObtained from Winthrop Laboratories, New York, New York.
Norepinephrine dose-response curves, in patients without heart failure (Group I) and patients with heart failure (Group II). The average peak values of vascular resistance (±SEM) elicited by intra-arterial injections of graded doses of norepinephrine are shown. The probability values indicate the significance of the difference of the responses between the two groups. The mean maximal resistance in response to tyramine (open circles and vertical broken lines) is positioned on the appropriate curve, providing an expression of the tyramine response in terms of “norepinephrine-equivalent dose.”

The concentration of norepinephrine was determined spectrophotofluorometrically and expressed as µg/g of heart muscle.4

Results

During the control period prior to administration of the drugs, the four consecutive measurements of mean arterial pressure, calf blood flow, and calculated vascular resistance varied less than 10% in each of the 17 patients. No significant alteration in calf blood flow occurred as the result of the insertion of the arterial needle or the rapid injection of saline. The reliability and reproducibility of the acute venous occlusion method for the assessment of limb blood flow and arteriolar resistance has been substantiated previously.7

Patients without Heart Failure (Group I)

In the patients in group I the control mean arterial pressure averaged 87.4 ± 2.1 mm Hg (SEM), calf blood flow averaged 4.01 ± 0.38 ml/100 g/min, and calculated vascular resistance averaged 23.2 ± 2.0 mm Hg/ml/100 g/min. Following administration of tyramine, the arteriolar resistance rose by an average of 4.3 ± 0.3 mm Hg/ml/100 g/min to a peak value of 27.5 ± 2.2 mm Hg/ml/100 g/min. The relationship between the response of vascular resistance to tyramine and to increasing doses of norepinephrine is shown in figure 1. The increase in resistance above the control produced by 100 µg/m² BSA of tyramine was less than that observed following the injection of 0.0625 µg/m² BSA of norepinephrine, which raised resistance to 32.7 ± 2.0 mm Hg/ml/100 g/min.

Patients with Heart Failure (Group II)

In the patients in group II, the control mean arterial pressure did not differ significantly from that in group I and averaged 85.3 ± 4.5 mm Hg. The calf blood flow averaged only 2.07 ± 0.27 ml/100 g/min and was significantly lower (P < 0.01) than that observed in the patients in group I, while the resting vascular resistance, 45.2 ± 5.2 mm Hg/ml/100 g/min, was significantly higher (P < 0.01). In these patients the standard dose of tyramine produced a maximal elevation of calf vascular resistance of 39.2 ± 4.5 mm Hg/ml/100 g/min to 84.4 ± 9.8 mm Hg/ml/100 g/min, a rise that was significantly greater, both absolutely and relatively (P < 0.01), than that observed in the patients in group I (fig. 1). While the absolute increase of vascular resistance in response to each dose of norepinephrine was significantly greater than in the patients in group I, the relative increases did not differ in the two groups. The injection of the standard dose of tyramine (100 µg/m² BSA) resulted in an increase in vascular resistance which was, on the average, similar to that resulting from the injection of 0.25 µg/m² BSA of norepinephrine (fig. 1).

Analysis of the Effects of Tyramine and Norepinephrine on Vascular Resistance

Since the action of tyramine on vascular resistance is mediated by the release of endogenous norepinephrine, the functional
were determined and found to be essentially identical in the two groups of patients (fig. 2). In contrast, the relative increments of vascular resistance elicited by the standard dose of tyramine differed greatly and averaged $19 \pm 1.0\%$ in patients in group I and $87 \pm 1.5\%$ in patients with heart failure.

**Concentration of Norepinephrine in Cardiac Tissue**

In the six patients in group I in whom atrial tissue was obtained, the concentration of norepinephrine in the atrium averaged $1.54 \pm 0.26 \mu g/g$. This was significantly lower ($P < 0.01$) than the concentration in the eight patients in group II, in whom it averaged $0.30 \pm 0.06 \mu g/g$ (fig. 3).

**Discussion**

Blood flow in the lower extremities was lower and vascular resistance higher in the patients with heart failure than in the patients

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Increase CVR to Tyramine (%)</th>
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<tbody>
<tr>
<td>GROUP I</td>
<td>$19 \pm 1.0$</td>
</tr>
<tr>
<td>GROUP II</td>
<td>$87 \pm 1.5$</td>
</tr>
</tbody>
</table>

$p < .01$

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**Figure 2**

Norepinephrine dose-response curves for the two groups of patients, with response expressed exponentially as the mean per cent increment above basal calf vascular resistance (CVR) produced by graded doses of norepinephrine (NE). The resultant overlapping suggests a fundamental similarity of arteriolar response to exogenous norepinephrine among the two groups of patients. In contrast, the mean per cent increment of calf vascular resistance after tyramine injection was significantly greater in the heart failure group (inset).

**Figure 3**

Concentrations of norepinephrine in biopsies of the atrial appendage obtained prior to cardiopulmonary bypass in the two groups of patients. The horizontal lines represent average values.
with heart disease without functional impairment (fig. 1). The heart failure state is believed to produce increased peripheral vascular resistance,1,7 presumably by arteriolar constriction due to augmented activity of the sympathetic constrictor nerves,1,2,9,10 and by altering directly the mechanical properties of the vessel wall.11 In addition, the absolute increase in resistance produced by any given dose of norepinephrine was greater in patients with heart failure (fig. 1). It is likely that this resulted, at least in part, from the elevated arteriolar tone existing in the basal state in these patients. Since, according to the Poiseuille relationship, the resistance to flow through a vessel is inversely proportional to the fourth power of its radius, a given decrement in the radius of an already constricted vascular segment results in a disproportionate increase in resistance. Furthermore, the lowered calf blood flow in the basal state in the patients in group II tends to enhance the effective concentration of vasoactive drugs at the receptor sites, and perhaps thereby increases the effectiveness of drug-receptor interaction. However, the relative effects of given doses of norepinephrine were identical in both groups of patients (fig. 2). The differences in resistance between the two groups of patients prior to the injection of the drugs are not considered to affect the interpretation of the results which were designed to compare the effects of two vasoconstrictor agents, tyramine and norepinephrine, in individual patients, and the effects of different base-line levels would be expected to alter the response to both agents in a similar manner.

The major finding in this study was that in patients with heart failure and depletion of cardiac catecholamine stores the vasoconstrictor response to a standard dose of tyramine is greatly enhanced, both when this response is considered in terms of the relative increase in calculated vascular resistance which it produced and in terms of the quantity of norepinephrine which had to be injected to produce a similar augmentation of resistance (figs. 1 and 2). This observation indicates that the quantity of endogenous sympathetic neurotransmitter available for release by tyramine from nerve endings in the peripheral vascular bed is not reduced and may actually be appreciably increased in chronic heart failure.

In agreement with this postulation is the clinical finding that overall sympathetic activity in the body is enhanced in patients with heart failure, despite the fact that their cardiac stores of norepinephrine are reduced (fig. 3). This increased sympathetic activity is reflected in the obvious reduction in cutaneous blood flow,12 the presence of venoconstriction,1,7,13 the well-documented elevation of renal vascular resistance14 which can be reduced by sympathetic blockade, and the augmented urinary excretion of norepinephrine.3

The mechanism responsible for the increased quantities of norepinephrine in the adrenergic nerve endings which can be released by tyramine in patients with heart failure is not clear. However, the recent work of Spector and associates may be relevant; these investigators have provided evidence for the existence of a feedback mechanism which couples the biosynthesis of norepinephrine to the activity of the rate-limiting biosynthetic enzyme-tyrosine hydroxylase, which in turn is related to the level of sympathetic activity.15,18 Other studies in our laboratory have shown that the cardiac norepinephrine depletion which occurs in heart failure is accompanied by, and presumably secondary to, a marked reduction in tyrosine hydroxylase activity.17 Perhaps in the sympathetic neurons innervating the resistance vessels the augmented sympathetic activity occurring in heart failure stimulates production of norepinephrine, while the prolonged, more intense activity in the nerve endings in the heart ultimately reduces the concentration of the rate-limiting biosynthetic enzyme.

References


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