Pathophysiologic and Prognostic Considerations in Circulatory Insufficiency in Congestive Heart Failure: Receptor Function

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We measured plasma concentrations of norepinephrine, cyclic AMP, cyclic GMP, atrial natriuretic peptides (ANP) and beta-adrenoceptor density (Bmax) and affinity (Kd) of lymphocytes in patients with congestive heart failure and correlated these parameters with symptoms and hemodynamic indices.

Plasma concentration of norepinephrine, cyclic AMP, cyclic GMP and ANP significantly increased in patients with congestive heart failure. Plasma concentrations of norepinephrine were related to the severity of the heart failure, plasma cyclic AMP concentrations, and pulmonary artery pressures. Cyclic AMP concentrations fell rapidly after treatment of acute left ventricular failure. Peripheral blood lymphocytes were stimulated by isoproterenol, and cyclic AMP level in lymphocytes was assayed. In normal subjects the generation of cyclic AMP after stimulation decreased with age. The response of lymphocytes in patients of NYHA classes III and IV was significantly lower than in the normal age-matched controls. A significant correlation between plasma norepinephrine concentration and increase of lymphocyte cyclic AMP was demonstrated. From these results it was suggested that beta-adrenergic receptors in congestive heart failure were desensitized. Beta receptor numbers of lymphocytes significantly decreased in NYHA class III and IV, but did not decrease in class I and II. There was no significant difference in Kd associated with congestive heart failure. Plasma concentrations of cyclic GMP also depended on the severity of heart failure and the pulmonary artery pressure, and decreased sharply with treatment, although remaining at a high value. A significant correlation between the cyclic GMP and ANP concentration was found in patients with congestive heart failure. The concentration of ANP significantly correlated with pulmonary wedge pressure and pulmonary arterial pressure and inversely correlated with cardiac index and ventricular ejection fraction. Thus, beta-adrenoceptors in congestive heart failure are down-regulated and the measurement of Bmax in lymphocytes is a useful index for the management of patients with congestive heart failure. Plasma concentration of norepinephrine, cyclic AMP, cyclic GMP, and ANP can be used as objective indices of severity of congestive heart failure.

In congestive heart failure, the sympathetic nervous system is activated and the released norepinephrine stimulates beta-adrenergic receptors and increases the contractility and frequency of contraction of cardiac muscle. Chidsey et al reported that urinary excretion of norepinephrine was increased in heart failure. Studies by Thomas and Marks and ourselves showed that the plasma concentration of norepinephrine increased in patients with congestive heart failure, sug-

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gesting that the sympathetic nervous activity of these patients is increased.

The plasma concentration of cyclic adenosine monophosphate (AMP), which is the second messenger for beta adrenergic agents and many other hormones, appears to reflect the changes in tissue since the nucleotides in plasma are in a dynamic steady state relation with its intracellular pools. Cyclic guanosine monophosphate (GMP), like cyclic AMP, is present in plasma and these plasma concentrations could serve as indices for severity of congestive heart failure. In the present study, therefore, we measured plasma concentrations of norepinephrine, cyclic AMP, cyclic GMP, atrial natriuretic peptides (ANP) and beta-adrenoceptor density (Bmax) and affinity (Kd) of lymphocytes in patients with congestive heart failure and correlated these parameters with symptoms and hemodynamic indices.

**Patients and Methods**

Eighty five normal subjects (54 men and 31 women, mean age 47 years) and 138 patients with congestive heart failure were studied. The patients were classified according to the functional New York Heart Association classification: 17 patients in class I, 37 in class II, 55 in Class III, and 29 in class IV. For the study of plasma concentration of norepinephrine, 102 normal subjects and 203 patients with congestive heart failure were studied (NYHA classification: 34 patients in class I, 51 patients in class II, 74 patients in class III, and 44 patients in class IV). Plasma concentrations of cyclic nucleotides were also estimated in some of these subjects.

The study of the generation of cyclic AMP in lymphocytes by beta-adrenergic stimulation involved 31 normal volunteers, aged 22–73 years (mean age 45.1), 24 males and 7 females. The group with heart diseases consisted of 29 patients, aged 20–75 years (mean age 50.5), 19 males and 10 females. Patients were classified by the functional classification of NYHA. Five patients were in class I, 6 in class II, 14 in class III, and 4 in class IV.

For the study of ANP, 36 healthy subjects (18 men and 18 women), and 24 patients with congestive heart failure (12 men and 12 women, mean age 53 years) were studied. The patients with heart failure were classified according to their subjective symptoms with the functional classes of NYHA: 8 patients in class I, 6 in class II, 7 in class III, and 3 in class IV.

Cardiac catheterization of patients with congestive heart failure was carried out with an intracardiac catheter in the morning after overnight fasting. Pulmonary capillary wedge, pulmonary arterial pressure and left ventricular end-diastolic pressures were measured with a catheter. Mean pressure was obtained by electrical integration of the pulse contours. Cardiac output was measured with the thermodilution technique by a cardiac output computer using iced injectate. Left ventricular ejection fraction was estimated echocardiologically by the simplified two-dimensional method with two-dimensional phased array sector scanner (Toshiba, SDS-21A).

In the patients who did not undergo cardiac catheterization, blood samples were collected in the morning after an overnight fast. The subjects were allowed a 20 min rest period in the supine or Fowler’s position and then blood samples were collected. These were collected into a chilled tube packed in ice with 20 μl of 0.5 mole acetic acid and immediately centrifuged at 4°C. Plasma was separated and frozen at –20°C until assayed. Plasma cyclic nucleotide concentrations were simultaneously measured in duplicate by the radioimmunoassay method of Cailla et al. as modified by Honma et al. The radioactivity was determined in an automatic well type gamma counter (Aloka ARC 251). In this assay, cyclic nucleotides in a 100 μl aliquot of plasma were directly succininated without prior deproteinization and then were bound to the antibody in an imidazole buffer. The assay’s total sensitivity increased appreciably by the use of this buffer. The recovery of cyclic AMP was 105% and that of cyclic GMP 93% when compared with known added cyclic nucleotides.

The concentration of plasma norepinephrine was measured by a sensitive radioenzymatic method by Henry et al. modified by Lake et al. In this assay, norepinephrine was N-methylated by phenylethanolamine-N-methyltransferase, purified from bovine adrenal medulla and 3H-S-adenosyl-methionine to form 3H-epinephrine, which was selectively isolated and measured by liquid scintillation spectrometry (Beckman LS-335). In this assay, the recovery of norepinephrine from alumina with acetic acid was about 40%. Coefficients of variation of intra-assay and interassay of norepinephrine were both within 10%.

For the assay of the generation of cyclic AMP in lymphocytes by beta-adrenergic stimulation,
lymphocytes were isolated from 20 ml heparinized blood, using the density gradient method of Boyum. This isolation procedure yielded a cell preparation of 90–95% lymphocytes. Trypan blue exclusion tests showed greater than 95% viability. Duplicated samples of cell suspension were incubated with DL-isoproterenol HCL for 10 min at 37°C and, after the incubation, the cells were centrifuged immediately and pellets were frozen in liquid nitrogen. After thawing the pellets with 6% TCA, the cells were homogenized and centrifuged. The TCA was subsequently removed by ether extraction from the supernatant fluid, and the samples were assayed for cyclic AMP.

For the beta-adrenergic receptor binding assay of lymphocytes, (−)-[125I]-cyanopindolol was used as a ligand, and 10⁻⁴ M (±)-propranolol as a displacer. For the nonspecific binding assay, 500 μl of lymphocytes preparation was mixed with 100 μl of (−)-[125I]-cyanopindolol and 100 μl of (±)-propranolol (final concentration 1.4 x 10⁻⁵ M). These mixtures were incubated at 37°C for 60 min, filtered and washed. The washed filters were placed in a polyethylene tube, and their radioactivity was determined by a gamma counter (Aloka Autowell Gamma System). Numbers of beta-adrenergic receptor (Bmax) and their affinities (Kd) were calculated by Scatchard analysis.

Plasma concentrations of ANP were determined by radioimmunoassay according to the method of Miyata et al. One ml of plasma was mixed with an equal volume of 4% acetic acid and ANP was extracted by 'Sep-pak' C-18 column. The column was eluted with 10 ml of 4% acetic acid, followed by 3 ml of acetonitrile.

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Fig.1. Plasma concentration of norepinephrine in patients with congestive heart failure.

Fig.2. Plasma concentration of cyclic AMP in patients with congestive heart failure.

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TABLE 1 BASAL LEVEL OF CYCLIC AMP IN LYMPHOCYTES AND GENERATION AFTER STIMULATION WITH ISOPROTERENOL

<table>
<thead>
<tr>
<th>Nr</th>
<th>Age (yr)</th>
<th>Basal</th>
<th>After stimulation</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>31</td>
<td>45.1 ± 2.6</td>
<td>0.72 ± 0.07</td>
<td>2.89 ± 0.22</td>
</tr>
<tr>
<td>NYHA Class I</td>
<td>5</td>
<td>32.6 ± 4.7</td>
<td>0.88 ± 0.18</td>
<td>5.10 ± 0.13</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>45.5 ± 2.9</td>
<td>0.77 ± 0.12</td>
<td>3.41 ± 0.35</td>
</tr>
<tr>
<td>III</td>
<td>14</td>
<td>56.1 ± 3.4</td>
<td>0.79 ± 0.07</td>
<td>1.97 ± 0.24</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>59.0 ± 6.7</td>
<td>0.66 ± 0.13</td>
<td>1.12 ± 0.12</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM.
*p < 0.02. Cyclic AMP increases in lymphocytes in classes III and IV were smaller than in normal, age-matched controls.

Fig.3. Correlation between plasma norepinephrine concentration and increase of cyclic AMP of lymphocytes in patients with congestive heart failure.

in 0.5% acetic acid (6:4 v/v). The eluent was lyophilized and dissolved in 0.4 ml Tris-HCl buffer pH 7.4 with 0.1% bovine serum albumin. Radioimmunoassay was performed by the delayed assay at 4°C for 48 hours, and B/F separation was achieved by the second antibody method. Recovery of α-ANP from plasma ranged from 80–90%, and the coefficient variations were less than 10%.

Statistics
A one way analysis of variance was used to determine any significant differences in the group means. If the analysis of variance indicated a significant difference, a 2 tail, paired or unpaired t test was applied when appropriate. Probabilities were considered significant at the 0.05 level. Regression lines were fitted by the method of least squares. All values were expressed as mean ± standard error of mean.

RESULTS
Plasma concentrations of norepinephrine were

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Fig. 4. Beta-adrenoceptor density of lymphocyte in patients with congestive heart failure.

Fig. 5. Plasma concentration of cyclic GMP in patients with congestive heart failure.

0.200 ± 0.02 μg/l in the normal subjects, 0.17 ± 0.04 in patients in NYHA class I, 0.30 ± 0.03 in class II, 0.37 ± 0.03 in class III and 0.93 ± 0.07 in class IV as shown in Fig. 1.

Figure 2 shows the mean plasma concentration of cyclic AMP in patients with congestive heart failure according to the severity (NYHA class) of their symptoms. Plasma concentrations of cyclic AMP were 17.5 ± 0.4 nmol/l in the normal subjects, 18.5 ± 1.4 in patients in NYHA class I, 21.1 ± 1.1 in class II, 25.7 ± 0.9 in class III, and 29.6 ± 2.3 in class IV. Cyclic AMP concentrations were significantly higher in patients in class II, III and IV than in normal subjects (p < 0.01) and in patients in class III (p < 0.001) and IV (p < 0.05) than in those in class II. A significant correlation between plasma norepinephrine and cyclic AMP concentration was found in normal subjects and patients with congestive heart failure (r = 0.91, p < 0.005). Pulmonary artery pressure in patients with congestive heart failure was significantly correlated with plasma cyclic AMP concentration (r = 0.47, p < 0.005). Serial changes of cyclic AMP in patients with
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Fig. 6. Plasma concentration of ANP in patients with congestive heart failure.

Fig. 7. Correlation between plasma concentration of ANP and ejection fraction.

acute left heart failure were measured. The plasma concentration of c-AMP was $42.75 \pm 5.31$ nmol/l at the onset of acute heart failure (NYHA class IV). The plasma concentration of cyclic AMP was appreciably lower at about 20 nmol/l after the second day of initial improvement (NYHA class III or II).

Peripheral blood lymphocytes from patients with congestive heart failure were stimulated by isoproterenol, and cyclic AMP concentration in lymphocytes was assayed. Mean basal concentrations of cyclic AMP in unstimulated lymphocytes are shown in Table I. There were no significant differences between groups. Mean concentration of cyclic AMP in lymphocytes after in vitro stimulation with isoproterenol and the increases of cyclic AMP above basal level are also shown in Table I. The increase of cyclic AMP in class III

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and IV were significantly lower than in normal, age-matched control (p < 0.02 and p < 0.01, respectively). Those in class I and II were high than normal but not significantly. As shown in Fig. 3, a significant inverse correlation between plasma norepinephrine concentration and the logarithm of increase of lymphocyte cyclic AMP was demonstrated in patients with congestive heart failure (r = -0.77, p < 0.001).

Bmax of lymphocytes significantly decreased in NYHA class III (4.58 ± 0.63 fmol/10^6 cells) and in class IV (4.60 ± 0.68), but did not decrease in class I (8.76 ± 0.97) and class II (7.48 ± 0.78) compared with control group (7.24 ± 0.41) as shown in Fig. 4. While, Kd showed no significant difference among congestive heart failure. Plasma concentration of norepinephrine showed a negative correlation with Bmax (r = -0.89).

Plasma concentrations of cyclic GMP were 3.9 ± 0.2 nmol/l in normal subjects, 65 ± 0.8 nmol/L in patients in NYHA class I, 9.5 ± 0.9 nmol/l in class II, 13.1 ± 0.8 in class III, and 12.8 ± 1.4 nmol/l in class IV. Cyclic GMP concentrations were significantly higher in patients in all classes than in the normal subjects (p < 0.001) and those in class II (p < 0.05), III (p < 0.001), and IV (p < 0.005) than in those in class I; the mean concentration in patients in class III (p < 0.05) was also significantly higher than in those in class II as shown in Fig. 5. Mean pulmonary artery pressure in patients with congestive heart failure was significantly correlated with plasma cyclic GMP concentration. Pulmonary artery pressure in patients with congestive heart failure was significantly correlated with plasma cyclic GMP concentration (r = 0.65, p < 0.02). Serial changes of cyclic GMP in patients with acute left heart failure were measured. The plasma concentration of C-GMP was 35.58 ± 14.0 nmol/l at the onset of acute left heart failure. The plasma concentration of cyclic GMP was appreciably lower at about 20 nmol/l after the second day of initial improvement and remained at a higher level than the normal value for more than seven days after the initial improvement.

As shown in Fig. 6, plasma concentrations of ANP were 131 ± 12 pg/ml in normal subjects, 199 ± 42 pg/ml in patient in HYHA class I, 598 ± 94 in class II, 1523 ± 193 in class III, 2734 ± 404 in class IV. A significant correlation between the cyclic GMP and ANP concentration was found in patients with congestive heart failure (r = 0.83, p < 0.01). The concentration of ANP significantly correlated with pulmonary wedge pressure (r = 0.62, p < 0.05) and pulmonary arterial pressure (r = 0.62, p < 0.05), and inversely correlated with cardiac index (r = -0.67, p < 0.05) and ventricular ejection fraction (r = -0.54, p < 0.05) as shown in Fig. 7.

**DISCUSSION**

In previous reports plasma norepinephrine concentrations were elevated in patients with congestive heart failure as a result of activation of the sympathetic nervous system. Thomas et al demonstrated that the plasma norepinephrine level correlated directly with the functional cardiac state and reflected the degree of cardiac decompensation. Our results also showed that patients in class II, III, and IV have higher plasma norepinephrine concentrations than normal subjects. The contractile response of myocardium to beta-agonist was found to be depressed in congestive heart failure. The cyclic AMP performs a regulatory role in contractility, though its actions may be much more complex than originally expected. Thomas reported that peripheral blood lymphocytes from patients with advanced heart failure and high plasma norepinephrine concentration failed to generate normal amount of cyclic AMP and speculated that beta-adrenergic receptors were desensitized in these patients. We obtained the same results. In addition, our results showed a significant inverse relation between plasma norepinephrine concentration and cyclic AMP increase in lymphocytes of patients with congestive heart failure. This suggests more strongly that the sustained high level of plasma norepinephrine may contribute to the decreased lymphocyte beta-response. Furthermore, our findings showed that Bmax of lymphocytes from the patients with severe congestive heart failure significantly decreased. A negative correlation between plasma concentration of norepinephrine and Bmax was demonstrated (r = -0.89). Recently correlations among myocardial and lymphocyte Bmax, of beta-adrenoceptor as well as myocardial contractility were reported. These results suggest that beta-adrenoceptors are down-regulated in congestive heart failure and the measurement of Bmax in lymphocytes is a useful index for the management of patients with congestive heart failure.

The present findings also show that plasma concentration of cyclic AMP and cyclic GMP were significantly increased in patients with
congestive heart failure. Mean pulmonary artery pressure was significantly correlated with plasma cyclic AMP as well as cyclic GMP concentration in those patients. Hamet and et al.\textsuperscript{17} and Winquist et al.\textsuperscript{18} have shown that ANP markedly increased urinary cyclic GMP excretion and renal tissue content of cyclic GMP as well as vascular cyclic GMP levels by activation of particulate guanylate cyclase. In this report, the plasma concentration of ANP was determined in healthy subjects and in patients with congestive heart failure. Plasma concentration of ANP was closely correlated with functional classes of heart failure (NYHA), mean pulmonary capillary wedge pressure and mean pulmonary arterial pressure, and inversely correlated with cardiac index and left ventricular ejection fraction. There was also a significant correlation between plasma ANP and cyclic GMP concentration. These results suggest that increased atrial pressure stimulates the release of ANP in congestive heart failure and that increased plasma cyclic GMP levels in patients with congestive heart failure may be explained by the increase of ANP level. Release of ANP into the circulation may be a physiological defense mechanisms against congestive heart failure because ANP has diuretic, vasodilative and anti-aldosterone effects.

In summary, plasma concentrations of norepinephrine, cyclic AMP, cyclic GMP and ANP significantly increased in patients with congestive heart failure. Plasma concentrations of these chemical parameters were related to the severity of the heart failure and pulmonary artery pressure. A significant correlation between plasma norepinephrine concentration and increase of lymphocyte cyclic AMP was demonstrated. Beta-adrenoceptors are down-regulated in congestive heart failure and the measurement of Bmax in lymphocytes is a useful index for the management of patients with congestive heart failure. Increased atrial pressure stimulates the secretion of ANP and plasma concentration of ANP and cyclic GMP could be used as an objective index of the severity of congestive heart failure.

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