Relationship among neuropeptide Y, catecholamines and haemodynamics in congestive heart failure

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Introduction

Cardiac mechanical function is mainly regulated by the autonomic nervous system, and recently, it has been shown that the heart is innervated by peptidergic neurons that may act as neurotransmitters or as neuromodulators[1-9]. Neuropeptide Y (NPY), originally isolated from porcine brains[10], has been demonstrated to coexist with noradrenaline in peripheral sympathetic nerves, and with epinephrine, in the adrenal medulla[7-10]. NPY-like immunoreactivity has been found in nerves within the myocardium and around cardiac blood vessels, suggesting that this peptide could participate in inotropic and chronotropic responses and may be an important regulator of coronary vasomotor tone[11]. Indeed, this peptide's potent vasoconstrictor effect and a potent inotropic effect on coronary vessels has also been reported[11,12]. A potent vasoconstrictor action on coronary vessels has also been reported in humans[12]. In patients with congestive heart failure (CHF), a high circulating norepinephrine concentration reflecting chronic sympathetic system hyperactivity has been demonstrated. In these patients, when compared to controls, a higher plasmatic NPY concentration has also been reported[13,14]. For these reasons, the chronic increase in both NPY and norepinephrine concentrations could lead to a potentially deleterious action on ventricular function. The present study was designed to assess at baseline, in patients with stable congestive heart failure, plasma norepinephrine and NPY concentrations both in the femoral artery and in the coronary sinus and to examine their relationship to haemodynamic parameters. Furthermore, concentration changes of norepinephrine and NPY were assessed during an inotropic intervention which decreased the sympathetic tone[15].

Methods

PATIENTS

Sixteen men and two women (age: 46 ± 15 years) in functional New York Heart Association class II or III, with dilated cardiomyopathy, as documented by echocardiography and previous episodes of congestive heart failure, participated in this study. Idiopathic dilated cardiomyopathy was the cause of congestive heart failure, all patients were in sinus rhythm and had had a previous normal coronary angiogram. Cardiac drugs were discontinued 48 h before entering the study, the research protocol of which was approved by the ethical committee of Henri Mondor University Hospital.
The development of an immunoradiometric assay (IRMA) for hNPY. This technique has been described previously. In brief, the affinity constants and the difference in the epitope binding regions of NPY02 and NPY05 allowed the development of an IRMA for hNPY determination. The use of NPY02 as the capture antibody and NPY05 as the indicator led to the best results. The standard curves were generated using hNPY diluted in NPY-free plasma. The sensitivity, i.e. the hNPY concentration resulting in an increase in counts per min bound that was 3 SD higher than the mean of the binding in 12 replicates of hNPY-free plasma, was 0·5 pmol. l−1. Values less than 0·5 pmol. l−1 were assigned this value. The mean plasma NPY concentration in normal subjects was found to be 2·4 ± 2·7 pmol. l−1 in a previous study. Plasma NPY was undetectable (<0·5 pmol. l−1) in 67% of these subjects. The 95th percentile value in the normal group was 7·5 pmol. l−1, and this value was defined as the upper limit of the normal range.

BASELINE HAEMODYNAMIC PARAMETERS

Patients fasted for at least 12 h before the procedure, which involved recording the following haemodynamic parameters during right (Swan–Ganz thermodilution catheter: Edwards Laboratory, Irvine, California) and left catheterization (5 Fr micromanometer-tipper catheter; Millar Industries, Houston, TX, U.S.A.): mean right atrial pressure, LV end-diastolic and systolic pressures, the first derivative of LV pressure (peak positive LV dP. dt−1) and mean aortic pressure. Cardiac output was determined by the thermodilution method (cardiac output computer 9520 A, Edwards Laboratory, Irvine, California). The cardiac index was calculated as cardiac output over the body-surface area. Systemic vascular resistance (UI) was calculated as the difference between mean arterial and mean right atrial pressures divided by the cardiac index.

To obtain coronary sinus blood samples, a N° 7 Fr NIH catheter was inserted into the femoral artery and positioned in the coronary sinus.

DETERMINATION OF PLASMA CATECHOLAMINE AND NPY CONCENTRATIONS

To determine NE, E and NPY plasma concentrations, samples were drawn from the femoral artery (through the femoral sheath) and from the coronary sinus.

NOREPINEPHRINE AND EPINEPHRINE

Plasma epinephrine and norepinephrine were determined in duplicate for each sample by a double-isotope radioenzymatic assay. In our laboratory, the assay was linear for norepinephrine between 0 and 59·1 nmol. l−1 and between 0 and 29·4 nmol. l−1 for epinephrine. The sensitivity of the assay was 0·15 nmol. l−1 for norepinephrine and epinephrine. Intra-assay coefficient of variation was 8% and inter-assay coefficient of variation was 12%.

NPY

Blood samples were collected in 75 g·l−1 EDTA, immediately centrifuged, and the plasma stored at −20 °C. Two monoclonal NPY antibodies (Mabs: NPY02 and NPY05) were produced and used for development of an immunoradiometric assay (IRMA) for hNPY. This technique has been described previously. In brief, the affinity constants and the difference in the

Table 1 summarizes haemodynamic measurements in all 18 patients at baseline and at maximal dobutamine infusion.

### Table 1. Haemodynamic parameters at baseline and at maximal dobutamine infusion rate

<table>
<thead>
<tr>
<th></th>
<th>n = 18</th>
<th>CI (l. min−1·m−2)</th>
<th>LVEDP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>SVR (UI)</th>
<th>HR (b. min−1)</th>
<th>dP. dt−1 (mmHg s−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td></td>
<td>2·34 ± 0·74</td>
<td>16·6 ± 7·2</td>
<td>81·6 ± 13</td>
<td>32·6 ± 11</td>
<td>82 ± 13</td>
<td>804 ± 229</td>
</tr>
<tr>
<td>Dobutamine</td>
<td></td>
<td>3 ± 0·77</td>
<td>9·7 ± 3·9</td>
<td>83·6 ± 15·6</td>
<td>26·6 ± 5</td>
<td>84 ± 15</td>
<td>1147 ± 295</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>NS</td>
<td>0·05</td>
<td>&lt;0·01</td>
<td></td>
</tr>
</tbody>
</table>

CI = cardiac index; LVEDP = left ventricular end-diastolic pressure, MAP = mean aortic blood pressure; SVR = systemic vascular resistance, HR = heart rate; dP. dt−1 = peak positive left ventricular dP. dt−1. Results are expressed as mean ± standard deviation.

### INFUSION OF DOBUTAMINE

Dobutamine was administered directly into the left coronary artery after baseline haemodynamic measurements and blood samples were obtained. This permitted direct inotropic action with only slight changes in systemic vascular resistance and heart rate. Dobutamine was administered consecutively during 5 min periods at incremental infusion rates of 25, 50, 100 and 200 µg·min−1. Infusions were given at a constant rate using an infusion pump (Vial-Medical, Grenoble, France).

Catecholamine and NPY concentrations were obtained at the maximal dobutamine infusion rate, both at the peripheral and coronary sinus levels. The NPY concentration was also measured from a new sample drawn 10 min after the end of the intracoronary dobutamine infusion.

### Statistical analysis

All data were expressed as the mean ± standard deviation. Paired t-tests were used to compare different variable changes in patients before and during dobutamine infusion. Linear regression analysis was used to assess the relationship between two parameters.

### Results

#### HAEMODYNAMIC PARAMETERS

Table 1 summarizes haemodynamic measurements in all 18 patients at baseline and at maximal dobutamine
Neuropeptide Y in patients with congestive heart failure

Figure 1 Concentrations of neuropeptide Y (a) and norepinephrine (b) in the femoral artery at baseline and at the maximal dobutamine intracoronary infusion rate. Base = concentrations at baseline, D = concentrations at the maximal dobutamine infusion rate. *P < 0.01.

infusion rate. Cardiac index and peak positive left ventricular dP/dt increased by 28 and 42% (P < 0.01) respectively. Left ventricular end-diastolic pressure and systemic vascular resistance decreased by 42 and 18% (P < 0.01) respectively. Heart rate and mean arterial pressure did not change.

Figure 1

PLASMA CATECHOLAMINE AND NPY CONCENTRATIONS

At baseline. In the femoral artery, NE and E concentrations were 6.48 ± 4.5 nmol l⁻¹ and 1.04 ± 0.74 nmol l⁻¹ respectively. NPY concentration was 2.15 ± 0.97 pmol l⁻¹. In the coronary sinus, NE concentration was 10.78 ± 5.42 nmol l⁻¹ and NPY concentration was 1.97 ± 0.63 pmol l⁻¹.

At maximal dobutamine infusion rate. In the femoral artery (Fig. 1), NE concentration decreased to 4.82 ± 2.69 nmol l⁻¹ (P < 0.001) while E concentration remained unchanged: 0.99 ± 0.61 nmol l⁻¹. NPY concentration did not change: 2.4 ± 0.99 pmol l⁻¹. In the coronary sinus, NE (10.13 ± 6.79 nmol l⁻¹) and NPY (2.14 ± 0.9 pmol l⁻¹) concentrations remained unchanged.

Ten minutes after the end of the dobutamine infusion, while haemodynamic values returned to baseline, NPY concentration, 2.58 ± 0.34 pmol l⁻¹ in the femoral artery, was the same as the baseline value.

RELATIONSHIP BETWEEN HORMONAL DOSAGES AND HAEMODYNAMIC PARAMETERS

NE was negatively correlated with LVEF (r = −0.61, P < 0.01), cardiac index (r = −0.54, P < 0.05) and with the net increase in LV dP/dt during the dobutamine infusion (ΔdP/dt) (r = −0.5, P < 0.05), while it was positively correlated with LV end-diastolic pressure (r = 0.48, P < 0.05) (Fig. 2). E concentration was not correlated with any haemodynamic variable. No correlation was found between NPY and haemodynamic parameters.

Discussion

The present study examined the relationship between NPY concentrations, catecholamine concentrations and haemodynamics both at baseline and during haemodynamic improvement, which decreased plasma peripheral norepinephrine concentration in patients with congestive heart failure. At baseline, peripheral nor-epinephrine concentrations were elevated, while NPY concentrations did not differ from those found in patients without heart failure. When the haemodynamic situation improved, norepinephrine concentration decreased in the femoral artery, while NPY concentration did not change. Only norepinephrine concentrations were found to be correlated with haemodynamic parameters.

NPY has been recently identified and found to be widely distributed throughout the central and peripheral nervous system of a variety of mammals including humans. This peptide has been shown to be stored, with catecholamines, in peripheral sympathetic nerves and in the adrenal medulla. Additionally, NPY-like immunoreactivity has been recently found in nerves within the myocardium and around coronary blood vessels. The physiological role of this peptide remains unclear. A potent vasoconstrictor effect, as well as negative inotropic action have been evidenced both in vitro and animal studies. In humans, NPY has been shown to have a potent vasoconstrictor action on coronary
arterioles leading to myocardial ischaemia, and suggests that this peptide could have a potential pathogenic role in angina pectoris. Systemic administration of NPY induced hypertension by increasing systemic vascular resistance.

Relations between NPY concentration and sympathetic tone have been previously addressed. Studies have demonstrated that NPY is released into the circulation by sympathetic stimulation. Both reflex sympathetic activation and electrical nerve stimulation of the adrenal medulla have been shown to cause a noticeable increase in NPY and catecholamine outputs. In normal subjects, it has been demonstrated that norepinephrine and NPY plasma concentrations were correlated and that NPY concentration increased during prolonged exercise and surgical stress. Congestive heart failure is a particular state of activation of the sympathetic nervous system. Indeed, congestive heart failure is characterized by high levels of circulating norepinephrine which are released from terminal sympathetic nerves and it is generally agreed that norepinephrine venous plasma concentrations provide a useful index of average sympathetic nervous activity. High catecholamine levels have been shown to be harmful to failing hearts, and recently it has been speculated that NPY could have a detrimental effect on the pathogenesis of heart failure, due to its vasoconstrictor and negative inotropic properties. Using a radioimmunoassay technique, Maisel et al. found that NPY concentrations were significantly higher in patients with congestive heart failure than in controls. However, while there was no correlation between the level of circulating norepinephrine and NPY concentrations in patients with congestive heart failure, this correlation was present in normal subjects, as reported in other studies. During exercise, norepinephrine concentration increased both in patients with congestive heart failure and in normal subjects, while a significant increase in NPY concentration occurred only in the latter. More recently, an increased NPY plasma concentration was observed in coronary care patients with clinical evidence of congestive heart failure. Only acute heart failure was considered and only 25% of these patients had an increase in circulating NPY concentration. In these studies, even if the systemic plasma NPY concentration was significantly higher than in normal subjects, it was not sufficiently high to induce any systemic effect or direct cardiac action.

In the present study, all patients had a chronic stable heart failure. Although norepinephrine concentration was high, NPY concentration was the same as in controls. This finding was unexpected in this particular case of chronic activation of the sympathetic system, NPY being supposedly released into the circulation upon sympathetic stimulation. These results differ from those obtained by Maisel et al. Reasons for these differences may be due either to a different patient population, with higher catecholamine concentrations in our study, or to different NPY dosage techniques. In the present study, NPY was measured by a radioimmunoassay technique which provided a high specificity for mature hNPY based on epitope recognition and related
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peptide assay results. This technique was performed initially to assess neuroendocrine tumours and good reproducibility and specificity was observed[17]. The discrepancy observed in our population of stable chronic heart failure patients, at the peripheral level, between high norepinephrine concentrations and rather normal NPY concentrations may result from differences of availability of norepinephrine and NPY at nerve endings. Bearing a peptide, released NPY is, after synthesis in the body cell, resupplied to nerve endings by axonal transport[23,29]. This characteristic is likely to limit the amount of NPY available for terminal release when compared with norepinephrine which is stored in nerve endings and obtained from both local synthesis and reuptake. This could explain the lack of increased NPY concentration. A major point of this and Maisel et al.’s study is the lack of NPY variation during sympathetic tone changes. Our data demonstrated a decrease in norepinephrine concentration, but not in NPY when sympathetic tone decreased. This could not be attributed to a longer NPY half-life since, 10 min after the end of the dobutamine infusion, NPY concentration remained unchanged[30]. This suggests that the interaction between norepinephrine and NPY is complex, and that their release from nerve endings is not closely correlated. It has been shown previously that the heart contains large amounts of NPY. In our study, NPY coronary sinus concentration did not differ from peripheral concentration. This suggests that in patients with heart failure, NPY concentration in the coronary circulation is not elevated enough to induce any effect on cardiac contractility and coronary vascular tone. Additionally, this result is consistent with the data of Anderson et al. who showed that, when measured in ventricles of patients undergoing cardiac transplantation, NPY myocardial concentration is low when compared to normal hearts[30].

In conclusion, the present data show that in patients with stable congestive heart failure, peripheral NPY concentrations are too low to induce any deleterious effect directly on vascular tone or cardiac contractility. A local role of NPY in neurotransmission may not, however, be ruled out in the pathogenesis of congestive heart failure.

References


