Cardiac neuropeptide Y and noradrenaline balance in patients with congestive heart failure

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Abstract

Objective—To measure plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity in relation to cardiac function in patients with congestive heart failure.

Design—Retrospective analysis of plasma noradrenaline concentrations and neuropeptide Y-like immunoreactivity in the arterial and coronary circulations, in patients with a high or low ejection fraction (31.3% (1.3%) or 17.7% (1.1%) respectively) and in healthy volunteers.

Setting—Cardiology department of a university hospital.

Patients—41 patients with congestive heart failure with various aetiologies. Ten healthy volunteers served as a reference group.

Main outcome measures—Concentrations of noradrenaline measured by high performance liquid chromatography and of neuropeptide Y-like immunoreactivity measured by radioimmunoassay. Cardiac index, pulmonary capillary wedge pressure, pulmonary vascular resistance, and systemic vascular resistance were derived by catheterisation of the right heart. Ejection fraction was measured by radionuclide angiography, cineangiography, or M mode echocardiography.

Results—There were pronounced and significant increases in circulating arterial concentrations of neuropeptide Y-like immunoreactivity and noradrenaline in both the high and low ejection fraction groups compared with the healthy subjects. In the patients myocardial release of neuropeptide Y-like immunoreactivity tended to be greater compared with normal subjects, but not significantly so. While normal subjects showed myocardial noradrenaline uptake, patients with congestive heart failure showed significant and progressive myocardial noradrenaline release. Arterial as well as coronary sinus concentrations of neuropeptide Y-like immunoreactivity correlated significantly with plasma noradrenaline concentrations from the respective sites. Plasma noradrenaline concentrations in the artery and coronary sinus were negatively correlated with ejection fraction and cardiac index; no such relations were found for concentrations of neuropeptide Y-like immunoreactivity.

Conclusions—Both circulating concentrations of neuropeptide Y-like immunoreactivity and noradrenaline are significantly increased in moderate to severe forms of congestive heart failure. Plasma concentrations of neuropeptide Y-like immunoreactivity correlated with plasma noradrenaline concentrations, but plasma noradrenaline concentrations alone correlated with ejection fraction and cardiac index. Thus plasma noradrenaline concentration seems to be a more sensitive index of cardiac dysfunction than the concentration of neuropeptide Y-like immunoreactivity in congestive heart failure.

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The 36 aminoacid peptide neuropeptide Y is stored with noradrenaline in cells in the peripheral and central nervous systems as well as in the adrenal medulla.^{1,2} In the human heart neuropeptide Y-like immunoreactivity is abundant in sympathetic nerve fibres associated with nodal tissue in direct contact with cardiac muscle fibres and around the coronary fibres.³

In some experimental studies neuropeptide Y reduces myocardial perfusion and exerts a negative inotropic effect,^{4,5} although other studies have failed to detect a direct action of the peptide on heart muscle.⁶ Neuropeptide Y is a potent constrictor of the coronary vascular bed.⁷⁻⁹ In many vascular regions, including the coronary circulation, it potentiates the contractile response to noradrenaline.¹⁰⁻¹²

In humans neuropeptide Y is a potent vasoconstrictor in the coronary circulation¹³ as well as in the peripheral vascular beds.¹⁴ In healthy volunteers the circulating concentration of neuropeptide Y-like immunoreactivity released from peripheral nerves or the adrenal medulla is < 30 pmol/l in the resting state.¹⁵⁻¹⁷ During sympathetic activation (physical exercise,^{15,16,18} cold pressor testing,¹⁸ or hypoxia,¹⁷ for example) circulating concentrations of neuropeptide Y-like immunoreactivity are substantially increased.

In patients with congestive heart failure cardiac noradrenaline spillover is increased from activation of the sympathetic nervous system.¹⁹ As neuropeptide Y is released with noradrenaline during strong sympathetic activation, our aim was to study myocardial as well as systemic release of neuropeptide Y in

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relation to noradrenaline during resting conditions in patients with congestive heart failure.

Patients and methods

SUBJECTS

Forty one patients with heart failure were studied; 31 were men and 10 women and their mean age was 51 years (range 17–72). The study protocol was approved by the ethics committee of the University of Gothenburg. Causes of heart failure were idiopathic dilated cardiomyopathy in 25 patients, ischaemic heart disease in 10, cardiomyopathy associated with rheumatic disease in two, alcoholic cardiomyopathy in one patient, uraemic cardiomyopathy in one, post-partum cardiomyopathy in one, and cardiomyopathy associated with muscular dystrophy in one patient. All patients had a clinical and echocardiographic picture resembling dilated cardiomyopathy. No patients had clinical signs of acute ischaemia or angina. The mean (SD) duration of symptoms of heart failure was 33 (36) months. Thirty seven patients were taking frusemide, 26 digoxin and six a β blocker. Twenty one patients were being treated with angiotensin converting enzyme inhibitors at the time of study; the 20 others were not taking such drugs. In five patients disease was classified as being New York Heart Association class II, in 29 as class III, and in seven as class IV. Ejection fractions were measured by radionuclide angiography, cine angiography, or M mode echocardiography. The patients were divided into two groups according to high and low ejection fraction separated by the median value of the group overall (25.7%). The low ejection fraction group consisted of 17 patients (range 11–25%) and the high ejection fraction group of 24 patients (range 26–52%). Furthermore, the patients were evaluated according to treatment with angiotensin converting enzyme inhibitors.

Healthy young volunteers ($n = 10$) who participated in a study evaluating the effects of dynamic exercise and hypoxia on release of neuropeptide Y-like immunoreactivity served as a normal reference group.¹⁷ They were men with a mean age of 25 (21–30). Blood samples (10 ml) for the measurement of peripheral concentrations of neuropeptide Y-like immunoreactivity and noradrenaline were obtained from the coronary sinus and the brachial artery.¹⁷

INVESTIGATIONS

The right heart was catheterised in the morning; subjects were in a fasting state and were not given premedication. A triple lumen Swan-Ganz pulmonary artery catheter and a Wilton-Webster coronary sinus catheter were introduced percutaneously through the internal jugular vein by the Seldinger technique. Correct positioning of the coronary sinus catheter was checked by fluoroscopic radiopaque injection and by tracings of flow curves. An arterial line was obtained through the radial artery. Pressures were obtained through fluid filled catheters and measured by

Statham P23 transducers (Cleveland, Ohio). Flow rates were measured by a thermodilution technique. Cardiac output was measured in the pulmonary artery and calculated by a digital computer (Cardiac Output Computer, WTI and Edwards Laboratories, Irvine, CA, USA). The coronary sinus catheter was attached to a Wheatstone bridge and changes in thermistor resistance caused by temperature changes were recorded on a Siemens-Elma Mingograph. Coronary sinus blood flow was calculated with the following formula²⁰:

$$F_B = F_I \times k \left(\frac{T_B - T_I}{T_B - T_M} - 1 \right)$$

where F_B = blood flow in the coronary sinus; F_I = infusion rate; T_B , T_I , T_M = temperature of blood, injectate, and mixture of blood and injectate, respectively; and k = a constant derived from the density and specific heat of saline solution and blood. Blood samples (10 ml) were obtained from the coronary sinus and the radial artery respectively for measurements of plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity. Pressures, flow rates, and blood samples were obtained at least 30 minutes after completion of catheterisation.

Plasma concentrations of neuropeptide Y-like immunoreactivity were measured by radioimmunoassay according to the method of Theodorsson-Norheim *et al.*²¹ The antiserum was raised in rabbits and showed no (0.1%) cross reactivity to structurally related peptides such as peptide YY or pancreatic polypeptide. The sensitivity of the method was around 10 pmol/l in a 0.5 ml plasma. Intra-assay coefficient of variation for concentrations below 50 pmol/l was 7%.²¹ Plasma noradrenaline concentration was analysed by high performance liquid chromatography with electrochemical detection.²² The sensitivity of noradrenaline assay was 0.1 nmol in 2 ml plasma. Intra-assay coefficient of variation for concentrations below 10 nmol/l was 5.6%.²²

Derived variables were calculated by the following equations: cardiac index = cardiac output/body surface area; stroke volume index = cardiac index/heart rate; pulmonary vascular resistance = (mean pulmonary artery pressure—pulmonary artery wedge pressure)/cardiac output $\times$ 80; systemic vascular resistance = (mean artery pressure—right atrial pressure)/cardiac output $\times$ 80; myocardial release of neuropeptide Y-like immunoreactivity and noradrenaline = (coronary sinus concentration—arterial concentration) $\times$ coronary sinus flow.

STATISTICAL ANALYSIS

The values in the text are means (SE). Data were calculated on a StatView program on a Macintosh computer. Linear regression was used to establish relations between the variables. Unpaired Student's t test was used for comparisons between the two groups. Two tailed p values < 0.05 were considered to be significant.

Table 1 Haemodynamics and plasma concentrations of neuropeptide Y-like immunoreactivity and noradrenaline in patients with congestive heart failure. Values are means (SE)

Variable	High ejection fraction (n = 24) group	Low ejection fraction (n = 17) group
Haemodynamics		
Ejection fraction (%)	31.3 (1.3)	17.7 (1.1)**
PCW (mm Hg)	17.2 (1.9)	18.8 (2.6)
Pulmonary vascular resistance (dyn.s.cm ⁻²)	2.5 (0.3)	3.0 (0.5)
Systemic vascular resistance (dyn.s.cm ⁻²)	1715.9 (115.8)	1591.5 (93.2)
Cardiac index (l/min)	2.4 (0.1)	2.0 (0.1)*
Neuropeptide Y-like activity		
Arterial (pmol/l)	31.5 (2.6)	36.3 (2.5)
Coronary sinus (pmol/l)	33.8 (2.5)	41.5 (3.6)
Difference (pmol/l)	2.3 (2.0)	4.7 (2.5)
Release (pmol/min)	0.39 (0.29)	0.74 (0.39)
Noradrenaline		
Arterial (nmol/l)	2.56 (0.29)	3.99 (0.54)*
Coronary sinus (nmol/l)	3.55 (0.51)	6.33 (0.89)**
Difference (nmol/l)	1.10 (0.26)	2.22 (0.39)*
Release (nmol/min)	0.22 (0.10)	0.36 (0.11)

*p < 0.05, **p < 0.01 between the two groups. PCW, pulmonary capillary wedge pressure.

Results

Table 1 shows mean haemodynamic values and plasma concentrations of neuropeptide Y-like immunoreactivity and noradrenaline in patients with congestive heart failure. The low ejection fraction group had lower cardiac indices, with higher concentrations of noradrenaline in arterial and coronary sinus blood. Arterial noradrenaline concentration was raised in both groups compared with the normal reference group, and it was significantly higher in the patients with a low ejection fraction than in those with a high ejection fraction (figure 1). Arterial concentrations of neuropeptide Y-like immunoreactivity were significantly higher in both high and low ejection fraction groups compared with the normal

Figure 1 Arterial and myocardial release of noradrenaline and neuropeptide Y-like immunoreactivity in patients with congestive heart failure with high and low ejection fraction compared with normal reference group. *p < 0.05, **p < 0.01, ***p < 0.01 v reference group; †p < 0.05 high ejection fraction v low ejection fraction.

controls (p < 0.05 and p < 0.01, respectively), but no significant difference was found between the two groups of patients (figure 1). Significant myocardial release of noradrenaline was found in both groups of patients

Figure 2 Correlations between plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity in artery and coronary sinus and between myocardial release of noradrenaline and neuropeptide Y-like immunoreactivity in patients with congestive heart failure.

Figure 3 Correlations between ejection fraction and plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity in artery and coronary sinus in patients with congestive heart failure.

Figure 4 Correlations between cardiac index and plasma concentration of noradrenaline and neuropeptide Y-like immunoreactivity in artery and coronary sinus in patients with congestive heart failure.

compared with normal controls ($p < 0.05$, $p < 0.001$ respectively). However, such significance was not found for the myocardial release of neuropeptide Y-like immunoreactivity, although it tended to be increased in the low ejection fraction group (fig 1).

Concentrations of neuropeptide Y-like immunoreactivity correlated significantly with concentrations of noradrenaline in both the artery ($r = 0.44$, $p < 0.01$) and coronary sinus ($r = 0.40$, $p < 0.05$) (fig 2). Except for two patients who showed borderline myocardial noradrenaline uptake, all other patients showed release of noradrenaline from the myocardium. On the other hand, only 60% of the patients (15) showed release of neuropeptide Y-like immunoreactivity from the myocardium, while 40% of the patients (10) showed uptake of the peptide. Because of the limited number of patients available (25) the correlation between myocardial release of neuropeptide Y-like immunoreactivity and noradrenaline was not significant ($r = 0.37$, $p = 0.07$) (figure 2).

Plasma concentrations of noradrenaline in the artery and coronary sinus were negatively correlated with ejection fraction ($r = -0.36$, $p < 0.05$), while the corresponding concentration of neuropeptide Y-like immunoreactivity correlated less well with ejection fraction ($r = -0.18$, $p > 0.05$) (fig 3). Arterial and coronary sinus concentrations of noradrenaline but not neuropeptide Y-like immunoreactivity correlated significantly with cardiac index ($r = -0.48$, $r = -0.47$ respectively, $p < 0.01$) (fig 4). Plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity in artery and coronary sinus did not correlate with pulmonary capillary wedge pressure, pulmonary vascular resistance, or systemic vascular resistance.

The changes in haemodynamics and plasma concentrations of neuropeptide Y-like immunoreactivity and noradrenaline in the patients according to treatment by angiotensin converting enzyme inhibitors are listed in table 2. Only pulmonary capillary wedge pressure differed significantly between the two groups, the group given angiotensin converting enzyme inhibitors having a higher pulmonary capillary wedge pressure ($p < 0.05$). Ejection fraction, cardiac index, plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity and myocardial release of the two hormones were similar in both groups.

Discussion

The main findings of this investigation were the appreciable increases in plasma concentrations of neuropeptide Y-like immunoreactivity and noradrenaline in peripheral arteries and the significant relation between circulating concentrations of noradrenaline and neuropeptide Y-like immunoreactivity in peripheral arteries and the coronary sinus in patients with congestive heart failure. Furthermore, circulating concentrations of noradrenaline correlated better with ejection fraction and

Table 2 Changes in haemodynamics, plasma concentrations of neuropeptide Y-like immunoreactivity and noradrenaline according to angiotensin converting enzyme inhibitor treatment in patients with congestive heart failure. Values are means (SE)

Variable	Treated (n = 21)	Not treated (n = 20)
<i>Haemodynamics</i>		
Ejection fraction (%)	27.0 (1.8)	24.5 (2.3)
PCW (mm Hg)	20.9 (2.3)	14.4 (1.9)*
Pulmonary vascular resistance (dyn.s.cm ⁻⁵)	2.9 (0.4)	2.4 (0.3)
Systemic vascular resistance (dyn.s.cm ⁻⁵)	1675.1 (124.0)	1647.6 (106.5)
Cardiac index (l/min)	2.1 (0.1)	2.4 (0.1)
<i>Neuropeptide Y</i>		
Arterial (pmol/l)	31.4 (2.1)	35.6 (3.3)
Coronary sinus (pmol/l)	34.1 (2.9)	39.7 (3.6)
Difference (pmol/l)	3.8 (2.4)	2.2 (2.5)
Release (pmol/min)	0.34 (0.31)	0.50 (0.37)
<i>Noradrenaline</i>		
Arterial (nmol/l)	3.51 (0.46)	2.66 (0.37)
Coronary sinus (nmol/l)	5.35 (0.82)	4.49 (0.72)
Difference (nmol/l)	1.79 (0.37)	1.62 (0.34)
Release (nmol/min)	0.34 (0.12)	0.23 (0.08)

*p < 0.05, between the two groups. PCW, pulmonary capillary wedge pressure.

cardiac index than did concentrations of neuropeptide Y-like immunoreactivity.

Because the pumping of the heart is reduced in congestive heart failure efferent sympathetic nerve activity in renal²³ and muscular sympathetic nerves²⁴ is exaggerated and plasma concentrations of noradrenaline are high.²⁵⁻²⁷ Plasma concentrations of noradrenaline are associated not only with the severity, but also with the prognosis of congestive heart failure.^{25, 28} Venous concentrations of neuropeptide Y-like immunoreactivity are raised in patients with congestive heart failure of various aetiology.^{29, 30} Our results accord with the idea that plasma concentrations of neuropeptide Y-like immunoreactivity are increased in patients with moderate to severe heart failure. Interestingly, plasma concentrations in forearm veins in patients in other studies ranged from 54 pmol/l³⁰ to over 100 pmol/l²⁹ by the same assay, which is higher than the concentrations of neuropeptide Y-like immunoreactivity that we found. This may indicate that venoarterial difference and peripheral release of neuropeptide Y-like immunoreactivity may exist in the forearm in congestive heart failure, although no clear cut venoarterial concentration differences were seen in normal subjects in the forearm at rest or during exercise.¹⁶ Moreover, the condition of most of our patients was stable with treatments such as diuretics, digoxin, angiotensin converting enzyme inhibitors, and β adrenergic blocking agents. These factors may also contribute to a lower plasma concentration of neuropeptide Y-like immunoreactivity that we found in our patients.

We used a normal reference group from a previous study¹⁷ because the techniques of assaying plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity and measuring haemodynamic variables were the same in the two studies. The study in normal subjects showed that during resting conditions baseline arterial concentrations of noradrenaline and neuropeptide Y-like immunoreactivity were 1.2 (0.2) nmol/l and 24.9 (1.3) pmol/l respectively.¹⁷ Myocardial release of noradrenaline and neuropeptide Y-like immunoreactivity were -0.09 (0.01) nmol/min and 0.16 (0.22) pmol/min.¹⁷ In the

current study, resting arterial concentrations of noradrenaline and neuropeptide Y-like immunoreactivity were significantly increased in the patients with congestive heart failure compared with normal controls. Most of the patients showed myocardial noradrenaline release and most (60%) showed myocardial release of neuropeptide Y-like immunoreactivity. As the peptide is not released until sympathetic nerve activity is intense in healthy subjects,¹⁶ its release from the myocardium therefore indicates the presence of high cardiac sympathetic activity in patients with congestive heart failure during resting conditions.

Concentrations of neuropeptide Y-like immunoreactivity correlated with noradrenaline concentrations in the coronary sinus, indicating that the peptide is released with noradrenaline from the myocardium in congestive heart failure. The net release of myocardial neuropeptide Y-like immunoreactivity correlated only marginally with the net release of noradrenaline ($r = 0.37$, $p = 0.07$), mainly because of limited data (from 25 patients), which may influence the statistical power. Interestingly, in a recent study in patients with idiopathic dilated cardiomyopathy the concentrations of noradrenaline, dopamine, and neuropeptide Y-like immunoreactivity in the failing ventricle were significantly decreased compared with those in control hearts.³¹ This is compatible with an augmented release of noradrenaline and neuropeptide Y-like immunoreactivity from the sympathetic nerve terminals in congestive heart failure and accords with our findings.

We analysed the data according to treatment with angiotensin converting enzyme inhibitors because this treatment causes short term decreases in plasma noradrenaline concentrations in heart failure^{32, 33} and is now widely used. Angiotensin converting enzyme inhibitors improve cardiac function and decrease in mortality in large clinical trials.^{28, 34, 35} As angiotensin II facilitates release of noradrenaline from sympathetic neurones in vitro,^{36, 37} it is reasonable to hypothesise that agents that block angiotensin II production would reduce noradrenaline release in the long term. The hypothesis is supported by the results of the studies of Left Ventricular Dysfunction trial, in which patients with a left ventricular ejection fraction of less than or equal to 35% and symptoms of congestive heart failure were treated by enalapril.³⁸ During a one year follow up, enalapril significantly decreased plasma noradrenaline concentrations in patients with congestive heart failure. Moreover, enalapril had the greatest effect in patients with the highest plasma noradrenaline concentrations before treatment.³⁸

The CONSENSUS trial showed that neurohumoral activation was reduced by enalapril. The authors concluded that the effects of enalapril on mortality were related to the counteraction of neuroendocrine activation.²⁸ A previous study showed, however, that there was a progressive neurohumoral activation over time in patients with chronic,

clinically stable congestive heart failure and the increase in plasma noradrenaline concentration did not seem to be prevented by long-term treatment with angiotensin converting enzyme inhibitors.³⁹ In our study, plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity were not different in patients taking diuretic and digoxin compared with those who were additionally taking an angiotensin converting enzyme inhibitor. This might be because angiotensin converting enzyme inhibitors reduce previously raised sympathetic activity, and the condition of our patients requiring such treatment was more severe as shown by their increased pulmonary wedge pressure. In interpreting our data one also has to consider that all patients were being treated medically, which might have changed the reactivity of the neurohormonal systems. Patients with the most severe disease may have had their hormone concentrations changed most by medical treatment, which would lessen any correlation between indices of congestive heart failure and hormone concentration. This is supported by the CONSENSUS trial that a composite index of hormones predicted mortality only in patients who were not taking angiotensin converting enzyme inhibitors.²⁸ We therefore considered it justified to relate circulating concentrations of noradrenaline and neuropeptide Y-like immunoreactivity to two major indices of cardiac function (ejection fraction and cardiac index). In our patients circulating concentrations of noradrenaline in the peripheral artery and coronary sinus correlated inversely with ejection fraction and cardiac index. Such significant relations were not seen for concentrations of neuropeptide Y-like immunoreactivity. Furthermore, we found that all patients with a net release of neuropeptide Y-like immunoreactivity from the myocardium also had a net release of noradrenaline while a substantial proportion of patients with a net myocardial release of noradrenaline did not have a net release of neuropeptide Y-like immunoreactivity. These data taken together seem to indicate that concentrations of neuropeptide Y-like immunoreactivity and its myocardial release represent a less sensitive index of cardiac function than noradrenaline concentration in patients with congestive heart failure.

In conclusion, in patients with congestive heart failure plasma concentrations of noradrenaline and neuropeptide Y-like immunoreactivity are significantly increased. Circulating concentrations and net myocardial release of noradrenaline and neuropeptide Y-like immunoreactivity are associated in patients with congestive heart failure. Unlike noradrenaline concentration, however, concentrations of neuropeptide Y-like immunoreactivity correlate poorly with cardiac function in patients with moderate and severe cardiac failure. Further studies during exercise, for example are needed to discover the full extent of the relation between these two sympathetic cotransmitters in congestive heart failure.

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