PHARMACODYNAMICS AND DRUG ACTION

Venous neuropeptide Y receptor responsiveness in patients with chronic heart failure

Background: **Chronic heart failure is associated with increased sympathetic nerve activity and elevated plasma neuropeptide Y levels. The aim of this study was to investigate whether increased neuropeptide Y release altered vascular neuropeptide Y responses in the dorsal hand veins in patients with chronic heart failure.**
Methods and results: **Neuropeptide Y responsiveness was studied in vivo with use of a hand vein tonometry technique in 14 patients with chronic heart failure and left ventricular ejection fraction (LVEF) values <20%, 16 patients with LVEF values from 20% to 35%, and 16 age-similar healthy control subjects. Plasma norepinephrine and neuropeptide Y levels were significantly elevated in patients with chronic heart failure and LVEF values <20% compared with control subjects ($P < .01$). Plasma neuropeptide Y but not norepinephrine levels were significantly elevated in patients with chronic heart failure and LVEF values from 20% to 35% compared with control subjects ($P < .01$). Increasing doses of neuropeptide Y (25 to 2000 pmol/min) were infused into a dorsal hand vein of each subject. Dose-dependent venoconstriction to neuropeptide Y was observed in all subjects studied. The neuropeptide Y dose-response curve in patients with LVEF values from 20% to 35% was significantly shifted to the left compared with patients with LVEF values <20% and control subjects ($P < .01$), whereas no significant difference was observed between the control subjects and the patients with LVEF values <20%. No significant difference in neuropeptide Y dose responses was observed between patients with chronic heart failure with plasma neuropeptide Y levels above the median and patients with chronic heart failure with plasma neuropeptide Y levels below the median.**
Conclusions: **In vivo venous neuropeptide Y receptor responsiveness is increased in patients with chronic heart failure and LVEF values from 20% to 35%. This increased neuropeptide Y responsiveness may contribute to venoconstriction at this stage of heart failure. (Clin Pharmacol Ther 2000;67:292-8.)**

Qingping Feng, MD, PhD, Michelle L. Lambert, MSc, Ian D. Callow, MSc, and J. Malcolm O. Arnold, MD, FACC *London, Ontario, Canada*

The sympathetic nervous system is important in the regulation of cardiovascular function. Tatemoto et al[1] have described neuropeptide Y as a major cotransmitter in the sympathetic nervous system.[1] Neuropeptide Y consists of 36 amino acids, with tyrosine as N-terminal and tyrosine amide as C-terminal residues.[1] Neuropeptide Y is widely distributed throughout the central and peripheral nervous systems of many species, including humans.[2] In the central nervous system, neuropeptide Y–containing nerve cell bodies and fibers are numerous in many parts of the brain and spinal cord.[3,4] In the peripheral nervous system, neuropeptide Y is mainly stored along with norepinephrine in sympathetic gan-

Table I. Clinical characteristics of patients with chronic heart failure and age-similar healthy control subjects

	Healthy control subjects	*Patients with chronic heart failure*	
		LVEF from 20% to 35%	*LVEF <20%*
n	16	16	14
Age (y)	56 ± 3	62 ± 3	63 ± 3
Sex			
Men	10	14	10
Women	6	2	4
New York Heart Association class	—	2.9 ± 0.1	3.5 ± 0.2*
Left ventricular ejection fraction (%)	—	31.4 ± 3.1	15.1 ± 0.7*
Plasma norepinephrine (pmol/L)	1308 ± 198	2088 ± 387	3604 ± 504†
Plasma neuropeptide Y (pmol/L)	131 ± 3	146 ± 4†	148 ± 5†
Mean arterial pressure (mm Hg)	87 ± 2	91 ± 3	88 ± 2
Heart rate (beats/min)	59 ± 1	70 ± 3‡	81 ± 4†
Basal hand vein diameter (mm)	1.09 ± 0.14	1.01 ± 0.10	1.01 ± 0.15

Data are mean values ± SEM.
*$P < .01$ versus left ventricular ejection fraction from 20% to 35%.
†$P < .01$; ‡$P < .05$ versus healthy control subjects.

glia and in tissues that receive dense sympathetic innervation, such as the vas deferens, heart atrium, blood vessels, and spleen.[2] However, the role of neuropeptide Y in the control of cardiovascular function in healthy and disease states is still not clear.[5]

Chronic heart failure is associated with increased sympathetic nervous activity, which results in increased release of sympathetic neurotransmitters such as norepinephrine.[6,7] Our recent studies have shown that plasma levels of neuropeptide Y are increased in patients with chronic heart failure.[8] However, it is not known whether increased neuropeptide Y release alters vascular neuropeptide Y receptor–mediated responses in chronic heart failure in humans. We hypothesized that vascular neuropeptide Y receptor–mediated responses would be decreased in patients with severe chronic heart failure as a result of increased sympathetic stimulation. We therefore designed this study to investigate vascular neuropeptide Y responses in vivo in patients with chronic heart failure and left ventricular ejection fractions (LVEF) above or below 20%. The study was performed with use of the technique of hand vein tonometry, which allows the construction of dose-response curves to agonists with little or no systemic effect to stimulate cardiovascular reflexes.

METHODS

The study was reviewed and approved by the University Review Board for Health Sciences Research involving human subjects, and all subjects gave written informed consent. Intravenous neuropeptide Y was manufactured under Good Manufacturing Practices (Peninsula Laboratories, Belmont, Calif).

Patients with chronic heart failure. We studied 30 patients (24 men and six women) with clinical symptoms (New York Heart Association functional class II to IV) and physical signs of stable chronic heart failure attributable to either coronary heart disease (17 patients) or nonischemic dilated cardiomyopathy (13 patients) (Table I). No patient had unstable angina or a history of recent myocardial infarction (within 3 months of study). None had evidence of valvular heart disease or hypertrophic cardiomyopathy by echocardiography. In all patients, LVEF was assessed by radionuclide angiogram or echocardiogram. Sixteen patients had LVEF values equal to or greater than 20% but less than 35%, and 14 patients had LVEF values less than 20%. Fifteen patients with LVEF values from 20% to 35% were taking angiotensin-converting enzyme inhibitors, 11 patients were taking digoxin (mean daily dose of 0.19 ± 0.02 mg), and eight patients were taking furosemide (mean daily dose of 65.0 ± 16.4 mg). Thirteen patients with LVEF values <20% were taking angiotensin-converting enzyme inhibitors, 13 patients were taking digoxin (mean daily dose of 0.26 ± 0.03 mg), 13 patients were taking furosemide (mean daily dose of 72.3 ± 17.2 mg), and one patient was taking hydrochlorothiazide (12.5 mg once a day). To minimize acute vasodilation or other acute drug effects on the study morning but to maintain the stability of the clinical condition of patients, medications were temporarily stopped as follows: Patients originally taking a long-acting angiotensin-converting enzyme inhibitor (ie, lisinopril or enalapril) were switched to the short-acting angiotensin-converting enzyme inhibitor captopril (at a comparable dose) 1 week before

the study, and captopril was stopped for 24 hours before the study. Long-acting nitrates, digoxin, diuretics, and all other medications were not given on the study morning. At the completion of the study, all medications were restored immediately. No patients had been taking calcium channel antagonists or β-blockers within 1 month of the study.

Healthy control subjects. Sixteen nonsmoking subjects (10 men and six women) with no evidence of heart disease as assessed by medical history, physical examination, and electrocardiogram acted as age-similar (56 ± 3 years) healthy control subjects. None of the control subjects had histories of hypertension or vascular disease or were taking any vasoactive medications.

Hand vein tonometry. Patients rested quietly in a temperature-controlled room (22°C to 24°C) and in a recumbent position with forearm and hand comfortably elevated above heart level. A venous occlusion cuff was placed around the upper arm and a short (1.9 cm) 25-gauge needle (Butterfly-Abbocath) was inserted into a dorsal superficial hand vein with a long straight section and no visible tributaries. Normal saline solution (0.9%) was infused at 0.4 mL/min. Subsequently, a linear variable differential transformer, an electromechanical device consisting of primary and secondary coils with a lightweight movable ferromagnetic core, was vertically placed over the summit of that vein, 10 mm proximal to the tip of the needle. Hand vein distention was measured as the difference between the position of the core before inflation of the venous occlusion cuff (baseline) and the height of the plateau during cuff inflation (45 mm Hg for 2 minutes).[9-12]

Neuropeptide Y was initially diluted to 250 to 500 μg/10 mL with 0.5% albumin saline solution then passed through a low protein binding filter (Millex GV, 0.22 μm, Millipore, Bedford, Mass) for sterilization before being further diluted to the required dose in glass bottles and infused into a dorsal hand vein through a glass syringe by a Harvard infusion pump (Harvard Apparatus Inc, South Natick, Mass).[13]

Study design. After 30 minutes of supine rest, at least two recordings of hand vein distention during saline infusion were obtained to ensure a stable baseline and the mean was taken as the control distention. Graded local infusions (0.4 mL/min) of neuropeptide Y (GMP, Peninsula Laboratories) diluted in 0.5% albumin saline solution (25, 50, 100, 200, 500, 1000, and 2000 pmol/min) were given (5 minutes at each dose level) with the cuff inflated at the third minute and deflated at the fifth minute of each 5-minute interval. Venoconstriction was expressed as the percentage of change from control distention. Mean arterial pressure and heart rate were monitored in the contralateral arm by a semiautomated blood pressure recorder (Dinamap 846SX, Critikon Inc, Tampa, Fla) throughout the experiment.

Measurement of plasma catecholamines. Thirty minutes after needle insertion into an antecubital vein, 5 mL blood were drawn into a cooled syringe and transferred to an ice-cold centrifuge tube that contained glutathione. The blood sample was centrifuged (3000*g* for 10 minutes at 4°C) for separation of plasma. The catecholamines were extracted from plasma according to the method of Anton and Sayre[14] and assessed by reversed-phase HPLC with electrochemical detection.[15] The detection limit of norepinephrine was 25 pg/mL. The intraassay and interassay coefficients of variation were 3% and 8%, respectively.

Radioimmunoassay of plasma neuropeptide Y. A second 5-mL blood sample obtained as above was transferred to an ice-cold tube that contained EDTA (1 mg/mL) and centrifuged (3000*g* for 10 minutes at 4°C). The plasma was removed and stored at –80°C for later analysis of neuropeptide Y. Plasma neuropeptide Y levels were analyzed by radioimmunoassay according to a previously described method.[16] A rabbit antiserum raised against synthetic porcine neuropeptide Y conjugated to bovine serum albumin with carbodiimide was used. The antiserum cross-reacted with human neuropeptide Y to 100% but not with C-terminal fragments of neuropeptide Y. Antiserum, 200 μL (diluted 1:40,000), was incubated first with 100 μL of blood samples or standard (synthetic human neuropeptide Y, Peninsula Laboratories) and with 200 μL (about 2500 cpm) of the HPLC purified tracer for 24 hours. Bound and free ^{125}I–neuropeptide Y samples were separated with dextran-coated charcoal. Each sample was assayed in duplicate and corrected for nonspecific binding. The detection limit was 11.7 pmol/L. Intraassay variation was 6.5% and interassay variation was 7%.[16]

Data analysis. Neuropeptide Y dose-response curves (semilogarithmic) were constructed by use of the InPlot program (version 4.0, GraphPad Software, San Diego, Calif). All values are expressed as mean values ± SE. Differences among groups were compared by two-way ANOVA, and post hoc analysis was done on significant data with the unpaired Student *t* test. A two-tailed *P* value <.05 was considered to be statistically significant.
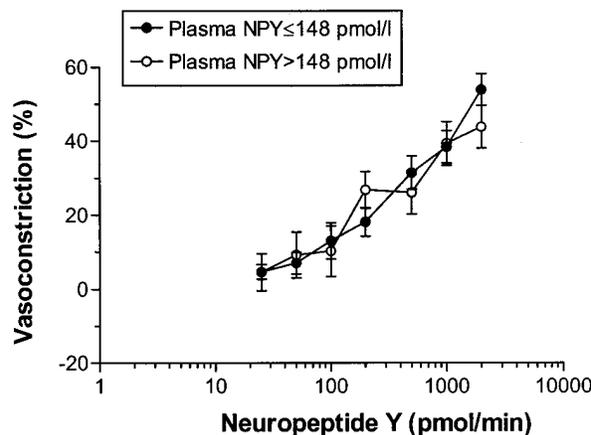
RESULTS

Both groups of patients with chronic heart failure had a significant increase of baseline heart rate compared with control subjects ($P < .01$), although mean arterial pressure was not significantly different from that of control subjects (Table I). Plasma norepinephrine and
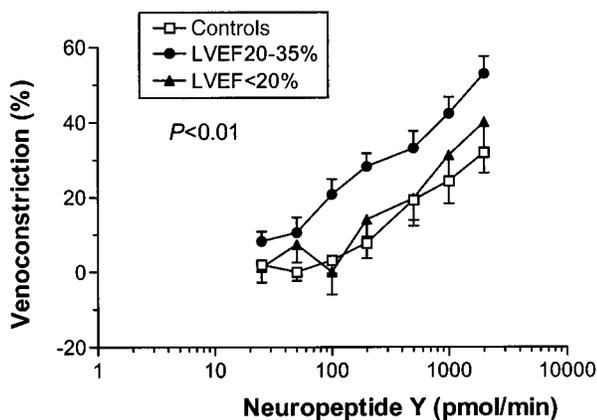
Fig 1. Response to neuropeptide Y (NPY) in the dorsal hand veins in patients with chronic heart failure and left ventricular ejection fraction (LVEF) values from 20% to 35%, LVEF values <20%, and healthy control subjects. *P* < .01 for LVEF values from 20% to 35% versus healthy control subjects.

Fig 2. Response to neuropeptide Y (NPY) in the dorsal hand veins in patients with chronic heart failure and plasma NPY levels above the median value (148 pmol/L) and those with plasma NPY levels below the median value. There was no significant difference between the dose-response curves for the two groups of patients.

neuropeptide Y levels were significantly elevated in patients with chronic heart failure and LVEF values <20% compared with control subjects (*P* < .01). Plasma neuropeptide Y levels were also significantly elevated in patients with chronic heart failure and LVEF values from 20% to 35% compared with control subjects (*P* < .01), but plasma norepinephrine levels were not significantly altered in this group of patients compared with control subjects (Table I).

Basal vein diameter at 45 mm Hg venous occlusion pressure during 0.9% saline infusion was not different among control subjects and patients with chronic heart failure (Table I). Graded infusions of neuropeptide Y induced dose-dependent venoconstriction in all subjects studied. The average dose-response curve for neuropeptide Y in patients with LVEF values from 20% to 35% was significantly shifted to the left compared with that of age-similar control subjects (*P* < .01; Fig 1). However, the dose-response curve for neuropeptide Y in patients with LVEF values <20% was not significantly altered compared with control subjects. Peak venoconstrictions by graded neuropeptide Y infusions (percentage change from saline control) in patients with LVEF values from 20% to 35% (52.8% ± 4.4%) were significantly increased compared with control subjects (32.5% ± 5.5%) (*P* < .05). However, peak venoconstrictions in patients with LVEF values <20% (42.5% ± 6.7%) were not statistically different from those of control subjects. The average dose-response curve for neuropeptide Y in patients with chronic heart failure

and plasma neuropeptide Y levels above the median value (148 pmol/L) was not significantly altered compared with that of patients with chronic heart failure and plasma neuropeptide Y levels below 148 pmol/L (Fig 2). There was no significant difference between neuropeptide Y responses between patients with chronic heart failure who were taking (n = 20) and not taking (n = 10) aspirin (mean daily dose of 325 mg) as a daily drug therapy.

No correlation was found between basal vein diameter and plasma norepinephrine or neuropeptide Y, between basal vein diameter and peak neuropeptide Y venoconstriction, or between peak neuropeptide Y venoconstriction and plasma norepinephrine or neuropeptide Y. Although not statistically significant, plasma neuropeptide Y levels tended to increase with plasma norepinephrine levels (*r* = 0.34; *P* = .09). No significant changes in arterial pressure or heart rate were observed in any group during the experiment.

DISCUSSION

In this study, we have demonstrated in vivo for the first time that venous neuropeptide Y receptor–mediated responses are increased in patients with chronic heart failure and LVEF values from 20% to 35% compared with age-similar healthy control subjects. Despite elevated plasma neuropeptide Y levels, venous neuropeptide Y receptor responsiveness in patients with LVEF values <20% was not decreased compared with age-similar healthy control subjects.

With increased neuropeptide Y release, one would expect a decreased neuropeptide Y response. We observed an increased neuropeptide Y response in some patients with elevated plasma neuropeptide Y levels, which was unexpected. This novel finding in patients with chronic heart failure and LVEF values from 20% to 35% may suggest that increased neuropeptide Y responsiveness is an important contributor to increased vasoconstriction in these patients.

Neuropeptide Y is a regulatory peptide coreleased with norepinephrine from sympathetic nerve endings during times of increased sympathetic nerve activity.[2,17-19] Stimulation of postjunctional Y1 and Y2 receptors induces potent and long-lasting constriction of both arteries and veins in human forearm, suggesting that neuropeptide Y may be of importance in the sympathetic vascular control in humans.[13,20] In patients with chronic heart failure, plasma neuropeptide Y levels are increased[21] and are associated with increases in plasma norepinephrine,[8] indicating strong sympathetic activation. However, the functional changes of vascular neuropeptide Y receptors in patients with chronic heart failure have not been described.

Patients with chronic heart failure in this study all had depressed left ventricular function with LVEF values ranging from 11% to 35% and had stable symptoms. In patients with chronic heart failure and LVEF values <20%, plasma norepinephrine, neuropeptide Y levels, and basal heart rate were significantly increased, indicating strong sympathetic activation. In patients with LVEF values from 20% to 35%, although plasma norepinephrine levels were not increased compared with control subjects, plasma neuropeptide Y levels and basal heart rate were significantly increased compared with age-similar healthy control subjects, suggesting some degree of sympathetic activation. With chronic sympathetic activation, one would expect that postjunctional receptors may undergo down-regulation or desensitization as an autoregulatory mechanism of receptor function, which has been shown for β-adrenergic receptors in hearts removed at the time of transplantation for severe chronic heart failure.[22] Although we did not show altered neuropeptide Y responsiveness in our patients with LVEF values <20% in this study, we clearly demonstrated that vascular neuropeptide Y receptor responsiveness in the dorsal hand veins was significantly increased in patients with LVEF values from 20% to 35% compared with control subjects. This increased neuropeptide Y response is more likely to be related to the sympathetic response because the response to nonadrenergic, nonsympathetic vasoconstrictor prostaglandin $F_2\alpha$ is not altered in these patients (our unpublished data, 1997).

The mechanism of this increased neuropeptide Y receptor responsiveness in patients with LVEF values from 20% to 35% is not known, but it is not likely that it is caused by receptor up-regulation because sympathetic activity and neuropeptide Y release are not decreased in these patients.[7,8]

Evidence from experimental models of early stages of chronic heart failure have also shown similar functional changes in vascular α-adrenergic receptors. In a pacing-induced chronic heart failure model in dogs, increased α_1-adrenergic receptor responsiveness in vitro has been reported in isolated pedal arteries 3 to 4 weeks after rapid pacing.[23] Increased vascular responsiveness to norepinephrine has been observed in the thoracic aortas of rats with myocardial infarction 1 week after coronary artery ligation and was endothelium dependent.[24] Recent studies have shown that endothelium-dependent relaxation is decreased in patients with chronic heart failure.[25] Neuropeptide Y receptors are present in the vascular endothelium and stimulation of these neuropeptide Y receptors releases endothelium-derived vasodilators and induces vasorelaxation.[26] The decreased endothelial function in chronic heart failure may result in a decreased release of endothelium-derived vasodilators by neuropeptide Y receptor stimulation, which may cause an increased vascular neuropeptide Y receptor response in patients with chronic heart failure and LVEF values from 20% to 35% compared with control subjects with intact functional endothelium. In this study, when the patients with chronic heart failure were divided into two groups according to their plasma neuropeptide Y levels, there was no significant difference in the responses between the two groups (above and below the median plasma neuropeptide Y value of 148 pmol/L), suggesting that plasma neuropeptide Y levels do not predict venous responsiveness. This finding may further support the notion that it is not a functional change in the neuropeptide Y receptor or coupling mechanisms that is causing the increased responsiveness in patients with chronic heart failure but rather that neuropeptide Y responsiveness may be altered as a result of decreased endothelial vasodilator release in chronic heart failure. The exact mechanism of the increased neuropeptide Y responsiveness in patients with LVEF values from 20% to 35% requires further investigation.

Because endothelial dysfunction would also be present in patients with more severe chronic heart failure and LVEF values <20%, the above dysfunction would argue for these curves also being shifted to the left of control subjects. This was not observed but could have occurred if patients with severe chronic heart failure also

had down-regulation of neuropeptide Y receptors in addition to endothelial dysfunction with loss of modulating vasodilator release. Indeed, our recent studies have shown a decreased vascular neuropeptide Y response in rats with severe heart failure after myocardial infarction.[27] However, in this study, plasma neuropeptide Y levels were not greater in patients with LVEF values <20% compared with values from 20% to 35%, and there was no difference between the neuropeptide Y dose-response curves for patients with plasma neuropeptide Y levels below or above the median. It is not known whether plasma neuropeptide Y levels accurately reflect neuropeptide Y receptor exposure and function. In addition, the extent of interaction between elevated norepinephrine levels and neuropeptide Y receptor function is not known.

In conclusion, the demonstrated changes in patients with mild to moderate chronic heart failure and LVEF values from 20% to 35% may produce a beneficial effect by maintaining venous preload in the failing heart. Alternatively, the changes could exert a detrimental effect of increased peripheral vasoconstriction, which increases workload on an already diseased heart. In either scenario, the current findings suggest an in vivo role for neuropeptide Y vascular responsiveness in chronic heart failure in humans.

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