Paradoxical decrease in circulating neuropeptide Y-like immunoreactivity during mild orthostatic stress in subjects with and without congestive heart failure

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Neuropeptide Y (NPY) is thought to be co-released with catecholamines in response to major cardiovascular stresses, but its relation to the release of catecholamines in response to minor stresses has been less well described. We therefore studied the response of plasma NPY-like immunoreactivity (NPY-Li) levels to standing (10 min) in eight normal subjects and 11 patients with congestive heart failure, and to short-term (6 h) vasodilator therapy in 13 patients with congestive heart failure. In both normal and heart failure patients, NPY-Li decreased (296 ± 73 to 233 ± 63 pg ml⁻¹ and 652 ± 36 to 516 ± 25 pg ml⁻¹ (P < 0.01) respectively) in response to standing, whereas catecholamines increased in both groups (norepinephrine 203 ± 73 to 507 ± 165 pg ml⁻¹ and 493 ± 197 to 813 ± 336 pg ml⁻¹ (P < 0.001) respectively and epinephrine 23 ± 12 to 38 ± 12 pg ml⁻¹ and 46 ± 19 to 62 ± 28 pg ml⁻¹ (P < 0.001) respectively). Both basal circulating NPY-Li and catecholamine levels were markedly increased in congestive heart failure patients, but catecholamines and NPY-Li did not correlate with each other. After 6 h of nitroglycerin infusion, mean arterial pressure was decreased, but circulating neurohumoral levels remained unchanged and NPY-Li levels decreased (653 ± 37 to 517 ± 26 pg ml⁻¹, P < 0.01). It is concluded that basal circulating NPY-Li and catecholamine levels are increased in congestive heart failure and that this neurohormone could play a concomitant role in the increase in peripheral resistance in these patients. However, changes in circulating NPY-Li levels correlate poorly with changes in circulating catecholamines in response to minor cardiovascular stress in normal and heart failure patients. These results suggest a differential release of these two sympathetic neurotransmitters.

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide that has been found to be distributed in the central and peripheral nervous systems of all species investigated, including man[1,2]. NPY is a potent vasoconstrictor, that acts not only directly but also indirectly by potentiating the effects of other vasoconstrictor neurohormones and by inhibiting the vasodilatory effects of adenylyl cyclase stimulation[1,2].

Although NPY is co-stored with norepinephrine in peripheral nerve endings, it is not stored in the same vesicles and, once released there is no reuptake, NPY being resupplied to the nerve endings by axonal transport[3,4]. Because of this, nerve terminals could be depleted of NPY during periods of intense and prolonged NPY release[8,9]. As a general rule, the release of NPY-Li immunoreactivity (NPY-Li) has been found to parallel that of norepinephrine, both increasing during periods of major stress such as haemorrhage, congestive heart failure, exercise and septic shock with the proportion of noradrenaline to NPY-Li release being approximately 10:1[2,5,7,10-18]. However, in situations of less pronounced adrenergic stimuli, such as orthostatic stress and handgrip, despite a consistent increase in circulating catecholamines, the increase in NPY-Li has been found to be small and variable[12,15,16-20], suggesting that the release of NPY-Li and other catecholamines is not invariably linked.

In this study, various stimuli for NPY-Li release and its relation to the release of other catecholamines were examined. NPY-Li and catecholamine activation during mild orthostatic stress, lying to standing, were examined in normal patients and in patients with congestive heart failure patients, known to have basal neurohumoral activation and a blunted response to the postural stress of lying to standing[21]. In addition, the response to NPY-Li, and other neurohormones, to the haemodynamic changes caused by the vasodilator nitroglycerin were evaluated. Changes in the variables measured were then correlated to one another. We found that in both normal and heart failure patients, under the experimental conditions studied, NPY-Li could decrease in spite of an increase or no change in circulating catecholamines.

Patients and methods

Eight normal subjects (age 36 ± 7 years) with no history of cardiac or coronary disease, with a normal ECG and physical examination were used as controls for protocol A.
Thirteen patients, all male but one, (age range 43 to 72 years; mean 61 ± 12 years) with chronic stable New York Heart Association (NYHA) class III or IV congestive heart failure were also studied. Eleven of these 13 patients were chosen for protocols A and B and two had only protocol B because they did not meet the stability requirements for protocol A. Each patient had had a clinical history of heart failure for at least 6 months. The cause of heart failure was ischaemic in all patients, but no patient had had an acute myocardial infarction or unstable angina in the previous 6 months. The left ventricular ejection fraction, determined by equilibrium gated radionuclide ventriculography, averaged 17 ± 11%. All were receiving digitalis and diuretics (furosemide: mean of 160 mg. day⁻¹), and nine received long-term therapy with vasodilatators: captopril in seven, and nitrates in two. Written informed consent was obtained from every patient. All study procedures were approved by the Ethics Committee of l’Hôpital du Sacré-Coeur de Montréal.

PROTOCOL A — ORTHOSTATIC STRESS (LYING-STANDING)

Eleven patients with congestive heart failure and the eight control patients were chosen for this portion of the study. All patients with congestive heart failure had been stable without medication change for at least one month and had stopped all medications for at least 24 h. In none of these patients was there subjective or objective evidence that withholding their medication for 24 h led to a change in their clinical condition. After an overnight fast, patients were admitted to the Hôpital du Sacré-Coeur research unit and an indwelling catheter was placed in a forearm vein 1 h prior to starting the study and maintained patent with a slow infusion of 5% dextrose in water.

Once patients had been lying in the supine position for at least 20 min, a venous blood sample was drawn via the indwelling catheter for measurement of plasma norepinephrine, epinephrine, dopamine and NPY-Li. Simultaneously, heart rate was recorded from an electrocardiographic tracing and arterial pressure was measured by a mercury sphygmomanometer and stethoscope. Patients were then asked to stand and, except for norepinephrine, epinephrine and dopamine which were not via the indwelling catheter for measurement of plasma NPY-Li in our laboratory are 284 ± 15 pg. ml⁻¹. Limits of the assay in the conditions used were 10 pg. ml⁻¹.

PROTOCOL B — NITROGLYCERIN INFUSION

Only the 13 patients with congestive heart failure were chosen for this portion of the study. At least one month had elapsed between protocols A and B. Patients were admitted and stabilized in hospital for a minimum of 5 days on a 2 g. day⁻¹ sodium diet, with a fluid intake restricted to a maximum of 150 ml. day⁻¹. All patients were at bedrest. All vasodilators were discontinued a minimum of 5 days prior to the study, and patients were maintained on digoxin and diuretics. Once the patients were considered optimally controlled on digoxin and diuretics, and their weight varied less than 0.5 kg over 3 consecutive days, they were entered into the study.

The evening prior to the study, a 7F triple-lumen, flow-directed, balloon-tipped thermodilution catheter (Edwards Laboratories, Santa Anna, CA) was inserted transcutaneously through the right internal jugular vein by the Seldinger technique and advanced to a pulmonary ‘wedge’ position according to pressure tracings. Pulmonary arterial and pulmonary capillary wedge pressures were monitored using a Gould-Statham P2311d transducer and were recorded on an Electronics for Medicine VR-6 Photographic Recorder. Cardiac output was measured in triplicate by means of the computerized thermodilution technique by injecting 10 ml of iced 5% dextrose in water. Systemic arterial blood pressure was measured through a radial canula. All derived haemodynamic variables were calculated as previously described.

The next morning, while patients were still fasting and prior to any medication, baseline haemodynamic measurements were done and blood was drawn (from the arterial canula) for measurements of plasma neurohumoral levels (plasma renin activity, aldosterone, arginine vasopressin, atrial natriuretic peptide, norepinephrine, epinephrine, and NPY-Li) and plasma electrolytes. Patients had been resting supine for at least 30 min. Nitroglycerin was then started at 0.25 μg. kg⁻¹.min⁻¹ (0.8 mg.m⁻¹ of 5% dextrose in water) and increased by 0.25 μg. kg⁻¹.min⁻¹ increments every 5 min to a maximal dose of 1.5 μg. kg⁻¹.min⁻¹. The target dose was reached within the first hour in all patients. Haemodynamic variables and plasma neurohumoral levels were measured again 1 and 6 h after beginning the infusion.

Plasma NPY-Li extraction

The plasma NPY-Like immunoreactivity (NPY-Li) extraction method used in our laboratory was adapted from the one previously described by Theodorsson-Norheim et al. 500 μl of plasma were agitated with 1 ml of an acid-ethanol mixture (1:5 ml of concentrated hydrochloric acid added to 1000 ml of absolute ethanol), evaporated and redissolved in 220 μl of a buffer containing 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Triton-X, 0.01% Na₂SO₄ and 1.5% aprotinin prior to the assay. The recovery from this extraction was about 85%. Plasma levels of NPY-Li were measured in duplicate using a radioimmunoassay adapted from the technique devised by Peninsula Laboratories (RAS No. 7172, CA, U.S.A.). The antiserum was purchased from Peninsula Laboratories (RAS No. 7172 <0.1% cross-reactivity with structurally related peptides) and the standard curves, ranging from 5 to 1280 pg, were prepared using synthetic human NPY (Sigma Chemicals, St. Louis, U.S.A.). The radiolabelled tracer used was¹²⁵I-NPY prepared from synthetic porcine NPY by Amersham (Ontario, Canada). The detection limits of the assay in the conditions used were 10 pg. ml⁻¹ for plasma. The coefficients for intra- and inter-assay variation were respectively 3 and 10%. Normal values for plasma NPY-Li in our laboratory are 284 ± 15 pg. ml⁻¹.

OTHER METHODS AND TECHNIQUES

Arginine vasopressin, plasma renin activity and aldosterone were measured according to the methods previously developed and reported by our laboratory.
Atrial natriuretic peptide was measured by radioimmunoassay according to the technique of Wilson et al. Arterial norepinephrine, epinephrine and dopamine levels were measured according to the radioenzymatic assay of Peuler and Johnson. The normal values in our laboratory are: plasma renin activity < 2.5 ng . ml⁻¹ . h⁻¹ for normal sodium diets, plasma aldosterone < 25 ng . dl⁻¹ for normal sodium diets, plasma vasopressin 0.5 pg . ml⁻¹ to 2.5 pg . ml⁻¹ for a plasma sodium between 135 mEq . l⁻¹ and 145 mEq . l⁻¹. Normal values for atrial natriuretic peptide are < 30 pg . ml⁻¹. Normal values for arterial norepinephrine are 170 ± 40 pg . ml⁻¹, for epinephrine are 35 ± 8 pg . ml⁻¹ and for dopamine are 20 ± 8 pg . ml⁻¹.

STATISTICAL ANALYSIS

For each group, sequential measurements of haemodynamic parameters and neurohormones were analysed by a repeated measure ANOVA followed, when a significance was found, by the multiple comparison test of Newman–Keuls. Differences between the two groups were compared by Student’s t-test. The relationship between the two parameters was assessed by linear regression analysis. Statistical significance was accepted for P < 0.05. Values are all mean ± SD.

Results

Control patients had normal resting blood pressures and neurohumoral levels. Patients with heart failure had moderately severe left ventricular dysfunction and neurohumoral activation. Plasma norepinephrine was 664 ± 357 pg . ml⁻¹, plasma epinephrine was 81 ± 39 pg . ml⁻¹ and plasma NPY-Li was 653 ± 37 pg . ml⁻¹.

PROTOCOL A

In normal control patients, heart rate and mean arterial pressure tended to increase after 3 min of standing (Fig. 1). After 10 min, mean arterial pressure remained constant, however, heart rate decreased despite a significant increase in plasma norepinephrine, epinephrine and dopamine. Also, despite the increase in circulating catecholamines, there was a significant and progressive decrease in plasma NPY-Li levels (Fig. 1).

Patients with congestive heart failure had a response to standing similar to that already described for these patients (Fig. 1). Heart rate and mean arterial pressure did not vary, but plasma norepinephrine and epinephrine increased significantly (Fig. 1). As was the case with normal control patients, NPY-Li decreased progressively despite an increase in plasma catecholamines.

PROTOCOL B

Patients with congestive heart failure had moderately severe ventricular dysfunction. Stroke work index was decreased (17.8 ± 5.7 g . m . m⁻²) and both pulmonary capillary wedge pressure (28.2 ± 6.8 mmHg) and right atrial pressure (9.7 ± 6.7 mmHg) were increased. These patients also had generalized basal neurohumoral activation as reflected by increased plasma norepinephrine (664 ± 48 pg . ml⁻¹), epinephrine (81 ± 11 pg . ml⁻¹), renin activity (6.0 ± 1.0 ng . ml⁻¹ . h⁻¹), arginine vasopressin (6.3 ± 1.0 pg . ml⁻¹), aldosterone (76 ± 14 ng . dl⁻¹), and atrial natriuretic peptide (200 ± 93 pg . ml⁻¹). Plasma NPY-Li was also increased (652 ± 36 pg . ml⁻¹). Plasma sodium was normal (139 ± 2 mEq . l⁻¹). There was no significant correlation between NPY-Li levels and any of the haemodynamic or neurohumoral variables measured.

The infusion of nitroglycerin caused mean arterial pressure to decrease, particularly during the first hour.
Neuropeptide Y release during orthostasis

Figure 2: Haemodynamic and neurohumoral response of patients with chronic congestive heart failure (n=13) to a 6-h infusion of nitroglycerin. Despite a transient increase in plasma epinephrine and no change in plasma norepinephrine, plasma neuropeptide Y-like immunoreactivity (NPY-Li) decreased progressively. Values are mean ± SD. ** = P<0.01 versus baseline.

Figure 3: Despite apparent discrepancies in the change in plasma neuropeptide Y-like immunoreactivity (NPY-Li) and plasma catecholamines during a 6-h infusion of nitroglycerin, a certain correlation existed between changes from baseline (Δ) NPY-Li and both norepinephrine and epinephrine.

Discussion

This study indicates that although NPY-Li and norepinephrine are co-localized in nerve terminals, they are not necessarily released by the same stimuli. NPY-Li and catecholamines can be co-released in situations of severe...
adrenergic stress, but their release can be independent and even paradoxical in more complex situations or situations of less pronounced adrenergic stimuli. From this and other studies, it would appear that NPY-Li plays a relatively minor role in the maintenance of cardiovascular homeostasis in situations of minor stress and that its release is reserved for situations of severe stress. In normal patients, the orthostatic stress of going from lying to standing caused NPY-Li to decrease in spite of causing a significant increase in all other measured catecholamines. In previous reports, the relationship between the release of NPY-Li and catecholamines has varied according to the degree of adrenergic stimulation. In situations of greater stress, such as exercise and septic or haemorrhagic shock, both NPY-Li and circulating catecholamines increased while in situations of lesser stress, such as handgrip and orthostatic tilt, NPY-Li increased only slightly or not at all despite large increases in circulating catecholamines.

Results from these previous studies have been interpreted as indicating that circulating NPY-Li and catecholamine levels are a reflection of sympathetic tone and that their release is controlled by similar mechanisms. This study indicates that the relationship between NPY-Li, adrenergic tone and circulating catecholamines is much more complex than previously thought and, that in certain situations of mild adrenergic stress, such as going from lying to standing, NPY-Li can paradoxically decrease in spite of an increase in circulating catecholamines.

In patients with congestive heart failure, as in the paper by Maisel et al., both NPY and catecholamine levels were increased but no good correlation existed between circulating NPY-Li and catecholamines. In this study, we also attempted to find a correlation between NPY-Li and other neurohumoral and haemodynamic variables, but no good correlation was found. As occurred in normal subjects, the stress of standing lowered NPY-Li levels despite an increase in circulating catecholamines. Similarly, during a nitroglycerin infusion, where mean arterial pressure decreased and stroke volume index tended to increase, the changes in circulating NPY-Li and catecholamine levels differed; NPY-Li decreasing and plasma catecholamine levels remaining unchanged. However, despite these differences, a direct correlation between changes in plasma NPY-Li and changes in both plasma epinephrine and norepinephrine levels occurred, suggesting that at least some of the factors determining NPY-Li and catecholamine release are related. When the point with the best correlation is removed, the relationship between changes in plasma epinephrine and NPY-Li becomes insignificant, a finding not surprising as most NPY is thought to be released from nerve terminals. However, removing this point did not significantly alter the relationship between norepinephrine and NPY-Li, both of these neurohormones being released largely from nerve terminals.

Previous studies have shown that even under circumstances where both NPY-Li and norepinephrine are released, the pattern of release of these two neurohormones can differ. Briand et al. have shown that the threshold for the release of NPY-Li during electrical stimulation of nerves was much higher than that for norepinephrine. In addition, they demonstrated that the increase in circulating NPY-Li levels caused by nerve stimulation was only a fraction of that of norepinephrine. If those results can be taken as a reflection of a higher threshold for the physiological release of NPY-Li, then the lack of increase of NPY-Li in this study, despite an increase in catecholamines, may reflect inadequate physiological stimuli for NPY release. A higher threshold for release of NPY, as compared to norepinephrine, would also explain why Watson et al. did not find an increase in NPY-Li during endotoxic shock in dogs, despite an increase in circulating catecholamines. Why NPY-Li actually decreased in spite of an increase in circulating catecholamines is not directly addressed in this study.

However, the findings of Dahlov et al., which show that during mild stress, norepinephrine may act on a presynaptic adrenoreceptor to reduce neuronal NPY-Li release, provides a plausible answer. In any case, the previously described 10:1 relation between noradrenaline and NPY-Li release was clearly not found in the setting of the present study, indicating that the relationship between the release of the two is dynamic rather than set at a given level.

The timing of the release of NPY as compared to norepinephrine has been found to differ, norepinephrine being released much sooner after adrenergic stimulation than NPY. Rudehill et al. have shown that that increase in plasma levels of NPY-Li is delayed a full 30 min after the induction of haemorrhagic hypovolaemia while epinephrine and norepinephrine plasma levels increased nearly immediately. This raises the possibility that in this study, the decrease in NPY-Li levels that we measured during lying to standing was only transitory and that had we measured NPY-Li levels for a longer period of time, we would have eventually documented an increase in NPY levels. However, delayed release of NPY-Li cannot explain our results in patients infused with nitroglycerin and, whether delayed release would have occurred or not in patients during standing, this study clearly indicates that the relationship between the release of NPY-Li and norepinephrine or epinephrine is much more complex than initially thought, and may even be paradoxical in some situations.

During the nitroglycerin infusion, NPY-Li decreased despite a decrease in mean arterial pressure and no change in catecholamines. These results differ from those in normal patients where a decrease in blood pressure caused by nitroprusside causes an increase in both norepinephrine and NPY-Li. In heart failure, the lack of change in catecholamines (with vasodilators) has been attributed to the beneficial effects of improved ventricular function compensating for a decrease in mean arterial pressure. It is thus tempting to speculate that improved ventricular function led to the decrease in NPY-Li levels documented during the nitroglycerin infusion. However, as NPY-Li also decreased during standing, a situation where cardiac index is known to decrease, such a simple mechanism cannot be the whole explanation.
Neuropeptide Y release during orthostasis

Conclusion
This study indicates that despite some synergistic effects and co-localization in nerve terminals, the relationship between the release of NPY-Li and norepinephrine in humans is complex. NPY-Li decreasing, not changing or increasing in various situations where norepinephrine increases. This appears to be the case in normal as well as patients with congestive heart failure. When taken together, this and other studies suggest that NPY-Li plays a relatively minor role in the minute-to-minute maintenance of cardiovascular homeostasis, its role being one of a second line of defence that comes into play in situations of greater or more prolonged stress. More work is needed to better establish the relationship and interplay between the release of NPY-Li and other catecholamines in response to various physiological and pathophysiological stimuli.

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