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ARTICLE

Region-Specific Neuropeptide Y Overflows at Rest and During Sympathetic Activation in Humans

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ABSTRACT: Neuropeptide Y coexists with norepinephrine in sympathetic nerves and is coreleased into the circulation on sympathetic activation. Little is known about the regional release of neuropeptide Y in humans under normal conditions or in pathophysiological situations of sympathetic activation or denervation. We measured plasma neuropeptide Y–like immunoreactivity and norepinephrine concentrations in samples taken from the brachial artery; coronary sinus; and internal jugular, antecubital, or hepatic veins in volunteers aged 20 to 64 years. Regional neuropeptide Y overflow at rest was calculated from venoarterial plasma concentration differences and plasma flow, and norepinephrine spillover was determined by [^{3}H]norepinephrine infusion techniques. Cardiac release of neuropeptide Y and norepinephrine was examined in response to various stressors as well as in clinical models of sympathetic activation, cardiac failure, and denervation after cardiac transplantation. In healthy volunteers, cardiac, forearm, and jugular venous sample neuropeptide Y concentrations were similar to arterial levels. Hepatic vein plasma neuropeptide Y was greater than arterial both at rest (119±5% of arterial, n=7) and after a meal (132±12%, n=7), with neuropeptide Y overflows of 6±2 and 11±2 pmol/min, respectively. In contrast, hepatomesenteric norepinephrine spillover was not significantly increased by feeding. Although coronary sinus plasma norepinephrine concentrations increased significantly with the cardiac sympathetic activation accompanying mental arithmetic, coffee drinking, isotonic exercise, and bicycle exercise, only the latter powerful sympathetic stimulus increased neuropeptide Y overflow. Cardiac failure was associated with increased resting release of both norepinephrine and neuropeptide Y from the heart, whereas postcardiac transplant norepinephrine spillover from the heart was reduced. The net overflow of neuropeptide Y to plasma observed at rest across the hepatic circulation, but not the cardiac, forearm, or cerebral circulations, indicates that the gut, the liver, or both make a major contribution to systemic plasma neuropeptide Y levels in humans. Sympathetic activation by exercise produced a modest increase in cardiac neuropeptide Y overflow but to only approximately 25% of the resting input from the gut and without a change in arterial neuropeptide Y concentration. Plasma neuropeptide Y measurements are less sensitive than those of plasma norepinephrine concentrations as an index for quantifying sympathetic neural responses regulating the systemic circulation.

Key Words: exercise ▪ heart ▪ neuropeptide Y ▪ norepinephrine

Neuropeptide Y (NPY) is a 36–amino acid peptide originally described in porcine brain[1] and subsequently shown to be widely distributed across the species in the central and peripheral nervous systems, where it coexists with catecholamines.[2] In addition to direct pressor actions, NPY can potentiate the effect of adrenergic stimulation. Corelease of NPY and norepinephrine has been demonstrated on sympathetic activation in vivo or on electrical stimulation of sympathetic nerves in a variety of mammals (for review, see Reference 3).

In humans, NPY circulates in plasma and has been shown to be released into the circulation on sympathetic activation by a number of stimuli, including exercise,[4 5] the cold pressor test,[4 6] head-up tilt,[7] and cigarette smoking.[8] We and others[4 5] have described a closer correlation between plasma NPY and norepinephrine concentrations than epinephrine under physiological and pathological conditions, suggesting a largely neuronal source for plasma NPY. Furthermore, an adrenal stimulus, hypoglycemia, had no effect on plasma NPY and norepinephrine in humans,[9] suggesting that any contribution from the adrenal gland is minor. Hemorrhage studies performed in adrenalectomized and denervated rats also supported a neuronal origin for plasma NPY.[10]

Although arterial and antecubital venous plasma NPY measurements have been widely used for quantification of sympathetic neural cardiovascular responses in both clinical and experimental research, few studies have investigated the regional sources of inputs to the circulating NPY pool. This study examined whether region-specific release of NPY-like immunoreactivity (NPY-LI) could be demonstrated at rest across several vascular beds, including those of the heart, brain, forearm, and gut and liver. The effect of a variety of stressors on the release of NPY-LI across the heart was also examined. Furthermore, cardiac release of NPY-LI at rest was investigated under two pathological conditions: in patients with cardiac failure, who provided a clinical model of chronic sympathetic activation,[11 12] and in cardiac transplantation patients, in whom there is sympathetic denervation of the heart.[13]

METHODS

Simultaneous regional measurements were made of NPY-LI overflow to plasma and of norepinephrine spillover determined by isotope dilution[14] for determination of the regional inputs of NPY to plasma and evaluation of the sensitivity of NPY-LI overflow as a measure of sympathetic nervous activity. The measurements were made in healthy subjects at rest, with sampling from the venous drainage of the forearm, brain, heart, and gut and liver. Measurements were also made in healthy subjects during the application of various stimuli that activate the sympathetic nerves of the heart, with sampling from the coronary sinus and before and after ingestion of a liquid meal and with hepatic vein sampling for study of the effect of feeding on hepatomesenteric release of norepinephrine and NPY-LI. Additional measurements were made with coronary sinus sampling in patients with cardiac failure and in those with recent cardiac transplantation.

Study Subjects

Forty-eight subjects who were participating in established research protocols in the Human Autonomic Function Laboratory and Alfred and Baker Medical Unit formed the basis of this report. Subjects were drawn from studies examining regional sympathetic nervous function in healthy volunteers and in disease states in which radiotracer-derived measurements of norepinephrine plasma kinetics were performed. The participants were 29 healthy volunteers (aged 20 to 64 years), 13 patients with chronic congestive heart failure without significant noncardiac illness (aged 26 to 68), and 6 patients (aged 37 to 61) with cardiac transplantation performed within the previous 18 months.

The healthy volunteers were recruited from the general community by advertisement and found to be in good health. Women were excluded because of an institutional ethics committee stipulation that radiotracers not be used in clinical research involving healthy women of childbearing age. A comprehensive clinical evaluation was performed in all experimental subjects by a specialist physician; testing included a chest radiograph, bicycle ergometry with exercise electrocardiography, hematology, and multipanel serum biochemistry testing. None of the recruited volunteers had a history of neurological or other disease known to affect autonomic nervous system function. All were unmedicated. None was obese; mean body mass index was 22.7 kg/m^2. No subject had a history of hypertension, and at the time of clinical evaluation and entry into the research project, recorded clinic blood pressure was in every case less than 150/90 mm Hg. Only nonsmokers were recruited for the research study.

The heart failure patients were recruited from the medical outpatient clinics of the Alfred Hospital and the cardiovascular clinics of the Baker Medical Research Institute. All patients were severely symptomatic, with breathlessness at rest or during minimal exertion, and were in New York Heart Association functional class III or IV. Left ventricular ejection fraction assessed by radionuclide ventriculography was 19±1% (mean±SE). The heart failure patients studied were undergoing assessment for suitability as candidates for cardiac transplantation. They were under treatment with angiotensin-converting enzyme inhibitors (12 patients), diuretics (12 patients), digoxin (10 patients), warfarin (10 patients), and antiarrhythmic drugs (2 patients). In view of the severity of their heart failure, medication was not discontinued for the research testing. Of the 13 heart failure patients, 8 had ischemic cardiomyopathy and 5 had idiopathic dilated cardiomyopathy.

The cardiac transplantation patients were under the care of the Heart/Lung Replacement Service of the Alfred Hospital, Melbourne. All had received their transplanted heart less than 18 months (average, 48 weeks) before the time of study, and their condition was clinically stable. Mean age was 49±3 years. Five of the six orthotopic cardiac transplant recipients had developed posttransplantation hypertension, for which they were under treatment. Current immunosuppressant therapy was prednisolone (mean dose, 11 mg/d) and azathioprine (mean dose, 90 mg/d), which were continued on the day of the study, and cyclosporine (3.5 to 7.4 mg/kg per day), which was discontinued on the morning of the study. Antihypertensive medications (differing combinations of angiotensin-converting enzyme inhibitors, diuretics, β-adrenoceptor blockers, and slow channel calcium antagonists) were discontinued for 72 hours before the study.

All experimental subjects gave written informed consent for their participation in the study, which was approved by the Alfred Hospital Ethics Review Committee.

General Procedures

Subjects were studied when they were supine in the morning after they had eaten a standardized light breakfast that excluded tea and coffee. This was done to minimize the risk of a vasovagal reaction occurring during placement of the vascular catheters. The healthy subjects given the test meal were studied after they had fasted overnight. For plasma NPY-LI and norepinephrine sampling, a brachial or radial arterial cannula and central venous catheter were inserted percutaneously under local anesthesia.

Healthy Subjects: Measurements at Rest The central venous catheter, a 7F coronary sinus thermodilution catheter (Webster Laboratories, type CCS-7 U-90B), was introduced through an antecubital venous sheath and placed with fluoroscopic control in the coronary sinus, the right hepatic vein, or an internal jugular vein. The catheter position was verified with 2 mL radiopaque contrast medium (Omnipaque, Winthrop Pharmaceuticals). Arterial blood samples were obtained from the brachial artery, simultaneous with venous sampling, with the use of a percutaneously placed 21-gauge cannula. In the healthy subjects, plasma samples were collected at rest from the individual sites for measurement of regional catecholamine kinetics as follows: heart (n=21), hepatomesenteric circulation (n=7), and internal jugular vein (n=14). Additional sampling was performed from an antecubital vein in four subjects. Radiolabeled norepinephrine (levo-[7-^{3}H]norepinephrine, New England Nuclear) was infused throughout at a rate of 0.5 to 1.0 μCi/min, allowing a minimum of 45 minutes before blood sampling, by which time steady-state concentrations are reached in plasma.[14]

Healthy Subjects: Measurements With Laboratory Stressors and Test Meal Plasma sampling for NPY-LI and norepinephrine measurements was performed from the brachial or radial artery and the coronary sinus after 10 minutes of mental challenge (difficult mental arithmetic) in 6 subjects, isometric exercise (handgrip at 30% of maximal power) in 7 subjects, aerobic exercise (supine cycling at 60% of maximal work capacity) in 7 subjects, and coffee drinking in 4 subjects, according to our previously published protocols.[14 15] No more than three stimuli were applied in any particular test; we used fluoroscopy to confirm that the catheter position in the coronary sinus did not change during application of the stressors. Further details of the application of the stressors follow below. In an additional study of 7 healthy subjects, we also performed arterial and hepatic venous sampling, before and 60 to 90 minutes after ingestion of a high-calorie mixed liquid meal, to study the effect of feeding on hepatomesenteric release of norepinephrine and NPY-LI.

Transplantation and Heart Failure Patients Plasma sampling was performed simultaneously from the coronary sinus and brachial or radial artery with the patients at rest. The coronary sinus catheter was placed with fluoroscopic control in the coronary sinus such as to include the middle cardiac vein drainage.

Application of Laboratory Stimuli

Stimuli that activate the sympathetic nervous system were applied in the cardiac catheterization laboratory. Mental stress (difficult mental arithmetic), isometric exercise (sustained handgrip), dynamic exercise (supine cycling), and coffee drinking challenges were used, according to the following experimental methods.[14 15]

Mental Stress We used a cognitive challenge with forced mental arithmetic to simulate mental stress. Each subject was expected to add or subtract single- or two-digit numbers from a three-digit number in a serial fashion as rapidly as possible for 10 minutes. The task was supervised

by a staff member at the Baker Medical Research Institute who was previously unknown to each subject but common to all subjects. The staff member changed the magnitude of the addition or subtraction or the starting number (within the limits of constructed tables) as frequently as desired to maintain the complexity of the challenge, given the subjects' differing abilities in mental arithmetic.

Isometric Exercise Isometric exercise was examined with a handgrip dynamometer (Harpenden Hand Grip, British Indicators). Subjects were requested to sustain 10 minutes of isometric handgrip at 30% of maximal grip strength using their dominant hand. Despite constant encouragement, most subjects were unable to maintain this level beyond 7 to 8 minutes and were therefore permitted to reduce the effort to 25% for the last 2 to 3 minutes.

Dynamic Exercise Cycling was performed with an electrostatically braked bicycle, with subjects lying supine in the cardiac catheter laboratory and exercising for 10 minutes at 60% of their previously determined maximal work capacity.

Coffee Drinking Measurements were made after subjects had drunk a standardized cup (200 mL, 55°C) of black coffee containing 1.5 g instant coffee (Nescafé, Blend 43 nondecaffeinated instant coffee, Nestlé) over a 5-minute period. Plasma was sampled 30 minutes later.

Each subject performed from one to three stress tests, with adequate time being allowed between the stressors for blood pressure and heart rate to return to baseline.[15] The order of testing was randomized, with the exception that dynamic exercise and coffee drinking were always tested as the last procedure. Arterial pressure and heart rate were monitored continuously throughout the application of the stimuli. Arterial and coronary sinus blood was sampled for subsequent NPY-LI and plasma norepinephrine assay, and coronary sinus blood flow was measured by thermodilution during the final 2 minutes of testing in each case.

Response to Test Meal

Response to a test meal was measured in seven healthy, lean men (aged 18 to 49 years). The meal was in the form of a liquid oral supplement ("Ensure Plus," Ross Laboratories), the energy content of which was 53.3% carbohydrate, 32.0% fat, and 14.7% protein, with an energy density of 6.3 kJ/mL. The energy content of the meal, which was ingested over 10 minutes, was calculated as 41.8 kJ/kg fat-free mass. Arterial and right hepatic vein blood was sampled before the meal and 60 to 90 minutes postprandially for measurement of plasma NPY-LI and endogenous and tritium-labeled norepinephrine. Hepatic plasma flow was measured concurrently.

NPY-LI Measurements

NPY-LI concentration was determined by radioimmunoassay[4] of plasma samples (50 µL) and NPY standards (2 to 600 fmol per tube, Auspep) with the use of a rabbit NPY antibody and ^{125}I-NPY labeled with Bolton Hunter reagent (Amersham). Bound and free radioactivities were separated by incubation with sheep anti-rabbit second antibody (Silenus). Interassay and intra-assay coefficients of variation were 13% and 6%, respectively; the assay sensitivity was 0.5 fmol. Chromatographic characterization of the NPY-LI detected in human plasma was carried out by high-performance liquid chromatography, with 0.1% trifluoroacetic acid as solvent A and a linear acetonitrile gradient, 20% to 60%, in 0.1% trifluoroacetic acid as solvent B at a flow rate of 1 mL/min. Fractions (1 mL) were collected, lyophilized, and assayed for NPY-LI; the assay revealed a single peak of immunoreactivity that coeluted with NPY standard (Fig 1).

Net NPY-LI overflow was calculated as the product of plasma flow and the venoarterial difference in NPY-LI concentration (picomoles per liter).

Norepinephrine Spillover Measurements

Regional release of norepinephrine from individual organs during constant-rate infusion of tritiated norepinephrine was calculated from the equation[14 15] Regional Norepinephrine Spillover=$[(NE_V-NE_A)+NE_A(E)]\times PF$, where NE_V and NE_A are regional venous and arterial plasma norepinephrine concentrations, respectively; E is the fractional extraction of tritiated norepinephrine across each organ; and PF is the regional plasma flow. Regional plasma flows were measured by clearance or thermodilution techniques, as described previously.[11 12 16] Coronary sinus and internal jugular plasma flows were determined by thermodilution, with adjustment for arterial hematocrit. Hepatomesenteric plasma flow was determined from the plasma clearance and hepatic extraction of indocyanine green.[14 15 16]

We used measurements of the regional spillover of norepinephrine to plasma to estimate organ-specific sympathetic activity. Rather than the rate of norepinephrine release from sympathetic nerve varicosities, which cannot be measured clinically, the norepinephrine spillover measurement gives the rate at which released norepinephrine enters plasma. In humans, this is approximately 10% to 20% of the norepinephrine synthesis rate.[17] Blood samples (5 mL) for norepinephrine assay were transferred immediately to ice-cold tubes containing EGTA and reduced glutathione, centrifuged at 4°C, and then stored at −70°C before assay (always within 2 months). Plasma concentrations of endogenous and radiolabeled norepinephrine were determined by high-performance liquid chromatography with electrochemical detection, as described previously.[18] Timed collection of the eluate leaving the detection cell with the use of a fraction collector permitted separation of tritium-labeled norepinephrine for counting by liquid scintillation spectroscopy. Tritiated norepinephrine made a negligible contribution to plasma concentrations (<2%), so no adjustment of the endogenous assay value was needed.[14]

Statistical Analysis

Results are expressed as mean±SE. Within-subject data were analyzed by paired Student's *t* test; comparisons between cardiac failure or transplant patients and healthy subjects were made by the unpaired *t* test. Differences were considered significant at a value of $P<.05$.

RESULTS

Resting Plasma NPY-LI in Healthy Subjects

To determine whether detectable overflow of NPY occurred at rest, we measured venoarterial NPY-LI concentrations across the cardiac, brain, and hepatomesenteric circulations. Resting plasma NPY-LI concentrations are shown in Fig 2 (top). Resting hepatic vein plasma NPY-LI concentrations were significantly greater than arterial levels (119±5% of arterial values, n=7, $P<.05$). Detectable overflow of NPY-LI to the plasma pool, representing 6±2 pmol/min, was observed from the gut and liver. For the heart and brain, similar NPY-LI concentrations were observed in arterial and venous samples, with no significant NPY-LI overflow to plasma from either at rest (Fig 2). Forearm venous and arterial plasma NPY-

LI concentrations were also similar, with venous concentrations representing 104% of arterial levels, indicating that there was no NPY-LI overflow across the forearm at rest.

Regional Spillover of Norepinephrine at Rest in Healthy Subjects

In contrast to NPY-LI overflow, norepinephrine spillover to plasma was evident at rest in the cardiac, hepatomesenteric, and cerebral circulations. Norepinephrine spillover from the heart in healthy men was 67±11 pmol/min (n=21), into the hepatomesenteric circulation was 378±109 pmol/min (n=7), and from the brain was 174±62 pmol/min (n=14).

Activation of Cardiac Sympathetic Outflow by Stressors

Table 1 shows the effects of mental stress, coffee drinking, isometric exercise, and dynamic bicycle exercise on blood pressure and heart rate. Significant increases were observed with all stressors, with dynamic exercise causing the greatest increase in heart rate. The effects of sympathetic activation by the four stimuli on arterial and coronary sinus plasma NPY-LI concentrations in healthy subjects are shown in Fig 3. Only dynamic exercise caused a significant elevation in coronary sinus plasma NPY-LI concentrations (Fig 3); arterial NPY-LI concentrations were not increased. Bicycle exercise thus resulted in a net modest increase in NPY overflow to 1.3±0.4 pmol/min (n=7, $P<.05$, Fig 3). In contrast, cardiac norepinephrine spillover was significantly increased by all stimuli, the magnitudes of the increases ranging from 2.7 times resting for coffee drinking and isometric exercise to 20 times for dynamic exercise (Fig 3).

Cardiac Failure and Cardiac Transplantation

Table 2 compares plasma NPY-LI and norepinephrine data in healthy subjects and in patients with cardiac failure and cardiac transplantation. No detectable NPY-LI overflow was observed from the heart at rest in healthy subjects, whereas norepinephrine spillover was readily detected at rest (67±11 pmol/min, Table 2). In cardiac failure patients, norepinephrine spillover was significantly increased, being fivefold higher than in healthy subjects, whereas a more modest increase in NPY-LI overflow was evident (Table 2), consistent with activation of cardiac sympathetic outflow.

In patients who had undergone recent cardiac transplantation, norepinephrine spillover was markedly reduced relative to healthy subjects ($P<.01$, Table 2). The fractional transcardiac extraction of plasma tritiated norepinephrine in this group of patients was markedly depressed, consistent with denervation.[13] Although in these patients NPY-LI extraction of 0.5 pmol/min, representing approximately 10% of net extraction, was evident across the transplanted heart, this did not reach statistical significance.

Hepatomesenteric NPY-LI and Norepinephrine Concentrations in Response to Feeding

Ingestion of the high-calorie mixed liquid meal resulted in significant increases in both arterial and hepatic vein NPY-LI concentrations (Fig 4, n=7, $P<.05$). NPY-LI overflow almost doubled after feeding (Fig 4, n=7, $P<.05$). In contrast, norepinephrine spillover was not altered significantly in response to the meal (Fig 4).

DISCUSSION

Measurement of resting arterial and venous plasma NPY-LI concentrations across a number of vascular beds in healthy men revealed that NPY overflow was detectable only across the hepatomesenteric circulation. No significant NPY overflow was observed across the brain, heart, or forearm at rest. This is the first study describing significant NPY overflow from any region at rest in humans, and our finding suggests that the liver, the gut, or both contribute importantly to resting plasma NPY levels. On the other hand, significant norepinephrine spillover was detected across all vascular beds studied, as has been well documented previously.[14] Norepinephrine spillover from the brain, although perhaps surprising in view of a partial interchange block provided by the brain-blood barrier, has been previously well documented.[19]

It should be emphasized that for norepinephrine, much as for NPY, there may be no net overflow of norepinephrine in healthy subjects in some vascular beds.[14] Use of a radiotracer technique with tritiated norepinephrine, by application of the principle of isotope dilution, allows demonstration of outward flux of the neurotransmitter ("spillover"), which in terms of net release is balanced in part by norepinephrine uptake from plasma.[14 15] It is possible that were we to have studied NPY release with isotope dilution, by infusing radiolabeled NPY, we may have increased the sensitivity of the method for detecting neuronal NPY release by taking account of possible NPY uptake from plasma by individual organs. Given that NPY has a much slower rate of removal from plasma than norepinephrine, with half-times of 20[20] and 2[14] minutes, respectively, this error is probably small. It should be noted, however, that in patients with recent cardiac transplantation, in whom no NPY release was possible because of the existing sympathetic denervation,[13] some net extraction of plasma NPY (approximately 10%) was evident.

Previous studies from laboratories including our own have in some circumstances been able to demonstrate NPY release into the human circulation in response to sympathetic activation.[4 5 6 7 21 22 23 24] In the present study investigating cardiac release of NPY-LI and norepinephrine in response to four different sympathetic stressors, NPY-LI was released from the heart to plasma only in response to the powerful sympathetic nervous stimulation provided by dynamic exercise. In contrast, increased norepinephrine spillover to plasma was present, to varying degrees, during the application of all the stressors tested. The spillover of norepinephrine on a molar basis was some three orders of magnitude greater than that of NPY-LI overflow. This result, showing much greater sensitivity of norepinephrine than NPY-LI plasma concentrations to change with sympathetic nervous stimulation, possibly relates to the vesicular storage pattern of NPY in sympathetic terminals[25] and to preferential neuronal release of NPY at high frequencies of sympathetic nerve discharge.[26] Clinical studies have demonstrated substantial increases in plasma norepinephrine concentration with sympathetic nervous stimulation but relatively small increases in plasma NPY with the cold pressor test,[6] cigarette smoking,[8] and upright posture.[4 7 27] No increase in plasma NPY-LI was observed in response to mental stress[28] or isometric exercise,[7] whereas dynamic exercise resulted in greater increases in plasma NPY,[4 5] all in line with the present finding. The failure to detect release of NPY to plasma in a given situation, of course, does not rule out neural release of NPY and cardiovascular actions by the peptide on postsynaptic receptors. The diffusibility of NPY differs from that of norepinephrine, so the major sympathetic neurotransmitter enters plasma more readily.

Patients with cardiac failure provide a clinical model of chronic sympathetic nervous activation, with this sympathetic stimulation affecting all neural outflows but preferentially those of the heart.[11] Heart failure patients demonstrated exaggerated release of both norepinephrine and NPY from the heart at rest, to a level, for both, comparable to that seen in healthy men during dynamic exercise, indicative of a substantial increase in cardiac sympathetic nerve firing rates.[12]

In patients with recent cardiac transplantation, such as those studied here less than 18 months after they had received their transplanted heart and before any sympathetic reinnervation occurs, there is unequivocal evidence of total sympathetic denervation.[13] As expected, these patients showed zero cardiac norepinephrine spillover, consistent with sympathectomy. In addition, there was approximately 10% net NPY-LI extraction from plasma in passage through the transplanted heart, indicating that for innervated organs, the NPY flux is most probably bidirectional, with a balancing of small inward and outward fluxes under most conditions.

Although it is clear that the human heart is able to release NPY with powerful sympathetic stimulation in healthy subjects and with long-term sympathetic activation in cardiac failure, evidence from this and an earlier study[29] indicates that under normal conditions, the heart does not make a major contribution to systemic NPY concentrations. Little is known regarding the possible regional sources of circulating NPY nor indeed how NPY is eliminated, although plasma NPY is known to be influenced by renal function.[6 30] An earlier study reported a postprandial increase in plasma NPY in conscious dogs,[31] and generalized sympathetic activation in this species caused NPY release from the liver but not the gut.[32] Data from the present study provide for the first time strong evidence that under resting conditions, the gut and liver make a major contribution to circulating NPY concentrations in humans.

NPY is present in the human gastrointestinal tract, with a dense innervation of all regions of the liver described by the use of immunohistochemistry[33] and descending concentrations in the stomach, duodenum, ascending colon, descending colon, and ileum.[34] Many intestinal NPY-containing fibers are intrinsic and thus may not release NPY in response to sympathetic stimulation. We found that feeding caused almost a doubling of NPY-LI overflow, with little alteration in hepatomesenteric norepinephrine spillover. The observed release of NPY-LI, independent of a detectable increase in hepatomesenteric norepinephrine spillover with feeding, suggests that NPY-containing nerve fibers in the gut may be capable of releasing NPY independent of norepinephrine release. In this regard, it may be pertinent that the previously described[35] increase in portal NPY induced by feeding in dogs was blocked by vagal cooling. It should be noted, however, that norepinephrine release by the gut can escape detection with hepatic vein sampling because of almost complete subsequent extraction by the liver.[36] The physiological significance of increased postprandial NPY-LI overflow remains to be elucidated. Patients with dopamine-β-hydroxylase deficiency, who cannot synthesize norepinephrine but whose neural release of NPY is unimpaired, do not suffer postprandial hypotension.[37 38] In contrast, patients with autonomic failure caused by degeneration of sympathetic nerves lack reflex neural postprandial vasoconstriction.[37 38] It is possible, therefore, that NPY released postprandially has a vasoconstrictor action in the human gut.

Plasma NPY measurements have been widely used for quantification of sympathetic cardiovascular responses in both clinical and experimental research. The particular justification provided for measuring the plasma concentration of NPY rather than that of norepinephrine is that unlike norepinephrine, NPY is not subject to neuronal reuptake after release. Accordingly, interpretation of plasma concentrations during sympathetic stimulation becomes more straightforward. However, it has been observed that NPY measurements provide a less sensitive measure of sympathetic nervous system response than norepinephrine measurements.[39] This has been attributed to the fact that NPY is preferentially released from sympathetic nerves at high rates of nerve firing. We now find an additional explanation for the insensitivity of plasma NPY measurements in the quantification of sympathoneural cardiovascular responses. In humans, major sources of NPY-LI in plasma are the gut and liver, and feeding is a more powerful stimulus for NPY release to plasma than sympathetic nervous responses involved in cardiovascular control, even with the powerful sympathetic activation provided by stimuli such as dynamic exercise.

In summary, measurements of NPY-LI release provide a less sensitive indicator of sympathetic nervous system response than measurements of norepinephrine release, a finding partly attributable to the gut and liver being the major sources of NPY in plasma. Feeding was found to be a more powerful stimulus for NPY release to plasma than the powerful sympathetic activation provided by stimuli such as dynamic exercise. Cardiac failure was associated with increased norepinephrine and NPY release from the heart at rest, whereas after cardiac transplantation, norepinephrine spillover from the heart was reduced.

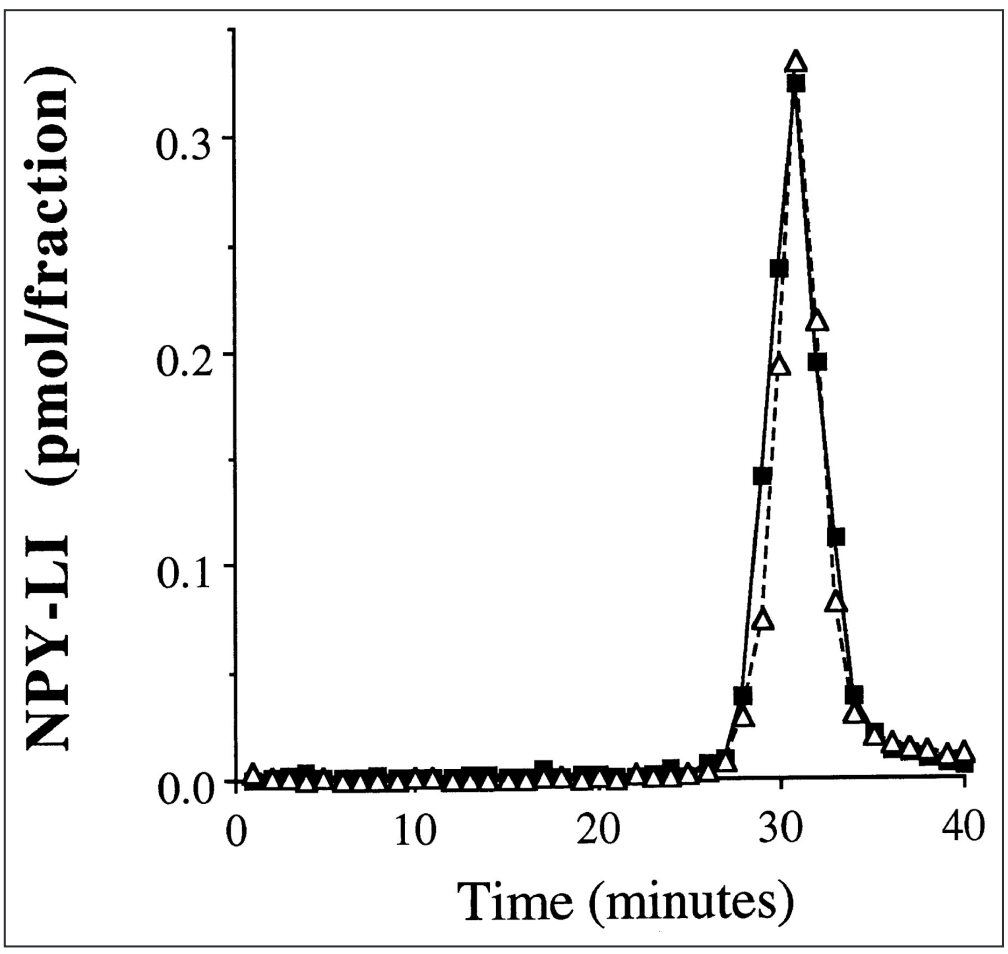

Figure 1. Reversed-phase high-performance liquid chromatography of human plasma (▪) and synthetic neuropeptide Y standard (△). Fractions (1 mL) were collected, lyophilized, and assayed for neuropeptide Y–like immunoreactivity (NPY-LI). Solvent A was 0.1% trifluoroacetic acid; solvent B was 20% to 60% acetonitrile in 0.1% trifluoroacetic acid.

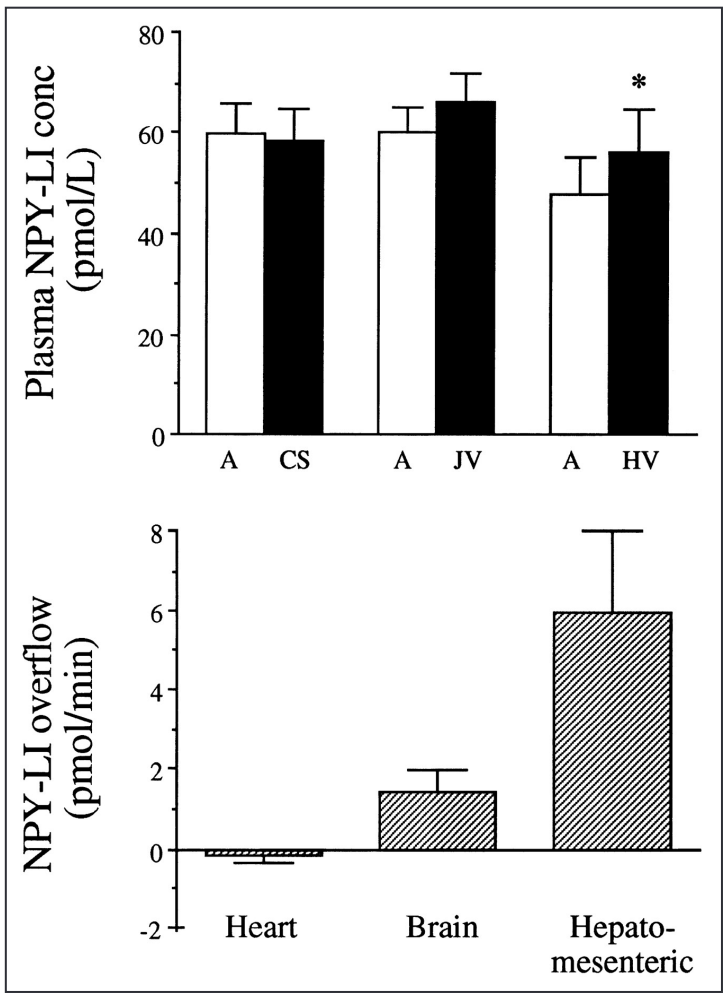

Figure 2. Resting arterial (A) and venous plasma concentrations of neuropeptide Y–like immunoreactivity (NPY-LI) across various vascular beds. Venous samples were taken from the coronary sinus (CS, n=21), jugular vein (JV, n=14), and hepatic vein (HV, n=7). NPY-LI overflow was calculated as the product of the venoarterial concentration difference and plasma flow. *P<.05, compared with arterial value, paired t test.

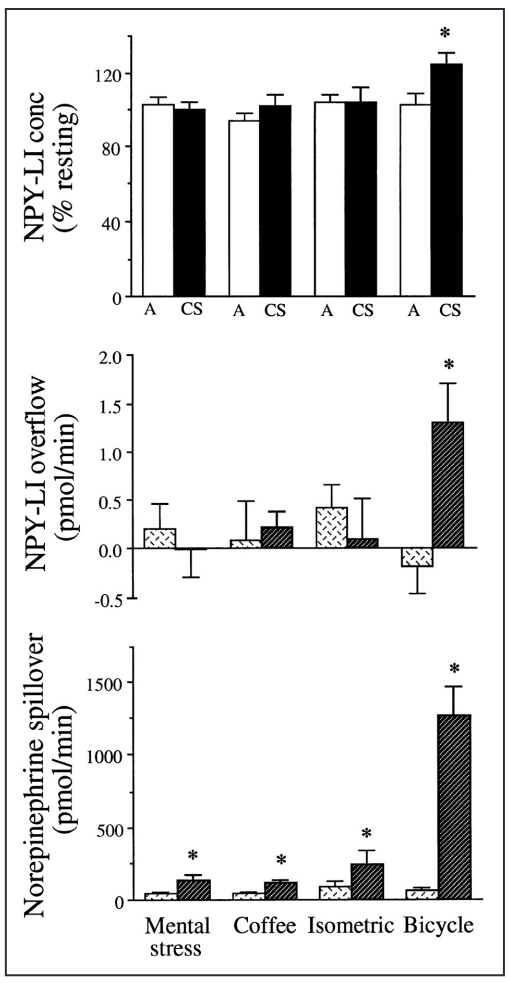

Figure 3. Effects of various sympathetic stressors on neuropeptide Y–like immunoreactivity (NPY-LI) concentrations (top), NPY-LI overflow (middle), and norepinephrine spillover (bottom). Arterial (A, open bars) and coronary sinus (CS, solid bars) NPY-LI concentrations are expressed as a percentage of each subject's resting value; n=6 (mental stress), n=4 (coffee), n=7 (isometric and dynamic bicycle exercise). *P<.05, compared with arterial value, paired t test. NPY-LI overflow and norepinephrine spillover are shown at rest (stippled bar) and after each stressor (striped bar) as described in the text; *P<.05, compared with resting value, paired t test.

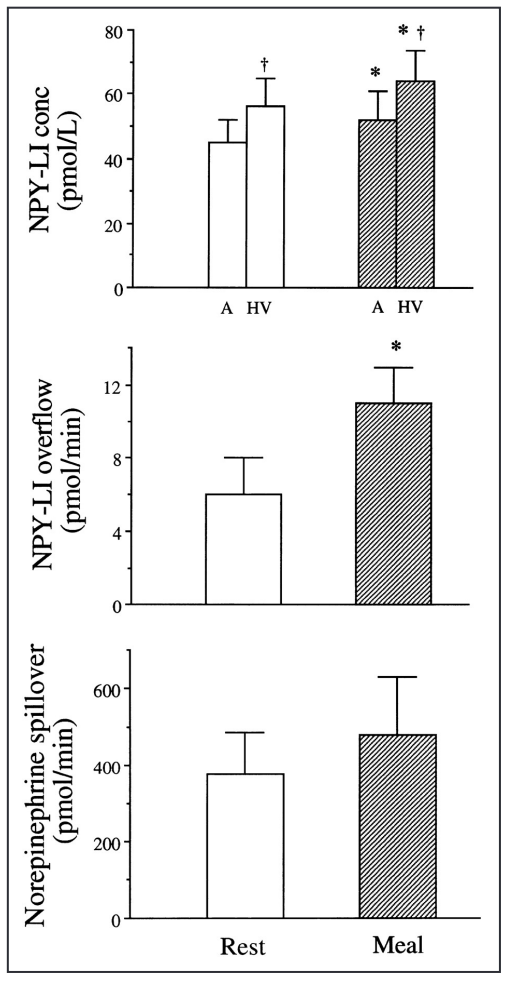

Figure 4. Arterial (A) and hepatic vein (HV) plasma neuropeptide Y–like immunoreactivity (NPY-LI) concentrations (top), NPY-LI overflow (middle), and norepinephrine spillover (bottom) in seven subjects at rest (open bars) and 60 to 90 minutes after a meal (striped bars). *P<.05, compared with resting value, paired t test; †P<.05, compared with arterial value, paired t test.

Table 1. Increases in Systolic and Diastolic Pressures and Heart Rate in Response to Stressors in Healthy Volunteers (Table view)

Stressor	n	Systolic Pressure, mm Hg	Diastolic Pressure, mm Hg	Heart Rate, bpm
Mental stress	6	20±2	16±1	16±2
Coffee	4	12±2	9±2	9±2
Isometric exercise	7	32±5	16±2	18±2
Bicycle exercise	8	37±4	2±2	74±8

Results are mean and standard error of the difference. All increases are significant at a value of P<.01 except diastolic pressure during bicycle exercise, P=NS.

Table 2. Plasma NPY-LI and Norepinephrine Concentrations and Transcardiac NPY-LI and Norepinephrine Kinetics in Healthy Subjects and in Situations of Sympathetic Activation and Denervation: Cardiac Failure and Cardiac Transplantation (Table view)

Parameter	Healthy Subjects (n=21)	Cardiac Failure (n=13)	Cardiac Transplant (n=6)
Arterial NPY-LI, pmol/L	59.6±6.3	45.6±6.9	52.0±8.7
Coronary sinus NPY-LI, pmol/L	58.1±6.7	48.9±7.2*	47.0±8.3†
NPY-LI overflow, pmol/min	−0.150±0.180	0.548±0.229‡	−0.516±0.116
Arterial norepinephrine, pmol/L	1137±85	2897±240†	1734±177
Coronary sinus norepinephrine, pmol/L	940±103	4000±507†	1666±177
Coronary sinus plasma flow, mL/min	103±12	145±22	116±20
Fractional [^{3}H]norepinephrine extraction	0.70±0.02	0.54±0.04§	0.10±0.02§
[^{3}H]Norepinephrine spillover, pmol/min	67±11	380±75‡	12±4§

NPY-LI indicates neuropeptide Y–like immunoreactivity.
*P<.05, †P<.01, compared with arterial concentration (paired t test); ‡P<.05, §P<.01 compared with healthy subjects (unpaired t test).

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