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CO-RELEASE OF NEUROPEPTIDE Y AND CATECHOLAMINES DURING PHYSICAL EXERCISE IN MAN

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Summary: Venous plasma levels of neuropeptide Y-like immunoreactivity (with chromatographic properties of synthetic neuropeptide Y) increased in parallel with catecholamines, heart rate and blood pressure during graded physical exercise in man. The plasma levels of neuropeptide Y correlated better with the levels of noradrenaline than adrenaline, suggesting release of a neural origin. Taken together with previous results, this suggests that neuropeptide Y is released together with noradrenaline upon sympathetic activation during physiological conditions in man. Determinations of plasma neuropeptide Y may therefore be valuable in the assessment of sympathetic nerve activity.

Physical exercise in man and experimental animals is accompanied by an increase in the activity of the sympatho-adrenal system, which is manifested in elevated concentrations of noradrenaline (NA) and adrenaline (ADR) in plasma (1). This initiates a series of events including tachycardia elevated blood pressure and metabolic activation (2). Recent evidence suggests that neuropeptide Y (NPY) (3), a peptide with vasoconstrictor activity in experimental animals (4) and on isolated blood vessels from humans (5), co-exists with NA in perivascular and cardiac sympathetic nerves (6, 7). NPY-like immunoreactivity (NPY-LI) is also present in

Abbreviations: NPY, neuropeptide Y; NA, noradrenaline; ADR, adrenaline; LI, like immunoreactivity; rpHPLC, reversed phase high performance liquid chromatography.

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a subpopulation of chromaffin cells in the adrenal gland (7, 8,9), which is most likely of the ADR-containing type (8). NPY-LI is released upon electrical splenic nerve stimulation in the cat (10,11). Furthermore, part of the vasoconstrictor responses to stimulation of the sympathetic nerves in some vascular beds, such as the submandibular gland (4) and spleen (10) of the cat persists after adrenoceptor blockade, which abolishes the effects of exogenous NA. Therefore, it has been proposed that novel mediator agents, such as NPY, may be involved in the sympathetic control of blood vessels (4,10). In the present study of responses to physical exercise we have obtained evidence that NPY-LI is released together with catecholamines upon sympathetic activation during physiological conditions in man.

MATERIALS AND METHODS

Fourteen healthy male persons (aged 25-46) were fasted over night and allowed to rest in the supine position for 30 min before performing a graded cycle ergometer test. Each subject exercised at 25, 50 and 75 % of his maximal aerobic capacity (max VO2, as determined in a pretrial test) for 6 min and then at his maximal aerobic capacity until exhaustion, which was reached 3-6 min later. Blood samples (10 ml) were collected from a cubital vein in ice-chilled tubes containing EDTA to a final concentration of 10 mM via an indwelling catheter both at rest before exercise (in the supine position) and during the last 30 seconds of each 6 min period of submaximal exercise. At the maximal exercise level, blood was collected at exhaustion. After centrifugation at +4°C, plasma was collected and frozen at -80°C until assay. Systolic blood pressure and heart rate were also monitored at these intervals, using non-invasive techniques. The study was approved by the Ethics Committee at the Karolinska Institue.

The contents of NPY-LI in ethanol-extracted plasma were determined by radioimmunoassay using antiserum N1, which was raised against porcine NPY and has a similar (90 %) cross-reactivity to human NPY as porcine NPY. This antiserum, however, does not show any cross-reactivity to other structurally-related peptides, such as peptide YY or mammalian pancreatic polypeptide (11). The specificity and validity of the NPY assay have been documented elsewhere (11). Human NPY has a recovery of about 70 % in the presently observed concentration range when added to human

blood containing 10 mM EDTA at 37°C (n=5). To further characterize the NPY-LI, some ethanol extracts were subjected to reversed-phase high performance-liquid chromatography (rp HPLC), using a Nucleosil C18, 5 µm 4.6 x 300 mm column (11). Additional plasma collected before and after exhaustion at maximal exercise was also analyzed for contents of tachykinin-LI (using antiserum K1) (12) and neurotensin-LI (using antiserum 6-8206) (13). The plasma levels of NA and ADR were determined by cation exchange HPLC using electrochemical detection (14).

RESULTS AND DISCUSSION

The plasma levels of NPY-LI, NA and ADR at rest were 31 ± 2 pmol/l, 1.30 ± 0.23 and 0.24 ± 0.02 nmol/l, respectively (n = 14). The plasma levels of NPY-LI and NA, blood pressure and heart rate increased significantly already at 25 % of maximal work capacity, while the plasma levels of ADR started to increase at a relative work load of 50 % of maximal capacity (Fig. 1). At the maximal work capacity, the NPY levels in plasma were elevated 3-fold (to 88 ± 7 pmol/l), whereas the NA and ADR levels were elevated about 20-fold (to 29 ± 3 nmol/l and 5 ± 1.2 nmol/l, respectively). A marked tachycardia (heart rate increased from 63 ± 2 to 185 ± 5 beats/min) and systolic blood-pressure elevation (from 125 ± 2 mmHg to 216 ± 3 mmHg) were seen at the maximal work load (Fig. 1). The plasma NPY-LI levels correlated significantly with the plasma NA levels at exhaustion ($r = 0.60$, $p < 0.05$) (Fig. 2). However, plasma NPY-LI did not correlate significantly with plasma ADR at the maximal work load. Characterization of the NPY-LI in plasma obtained during maximal physical exercise, using rpHPLC revealed a single peak with a similar elution profile for human plasma NPY-LI as that of synthetic porcine NPY (Fig. 3).

Exercise did not influence the plasma levels of the other peptides assayed, i.e. tachykinin-LI or neurotensin-

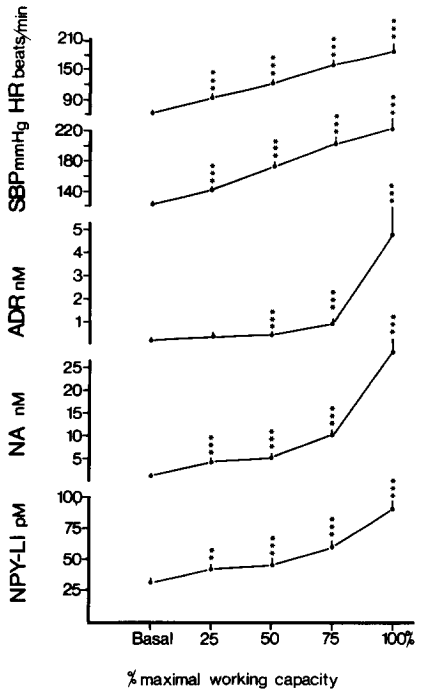
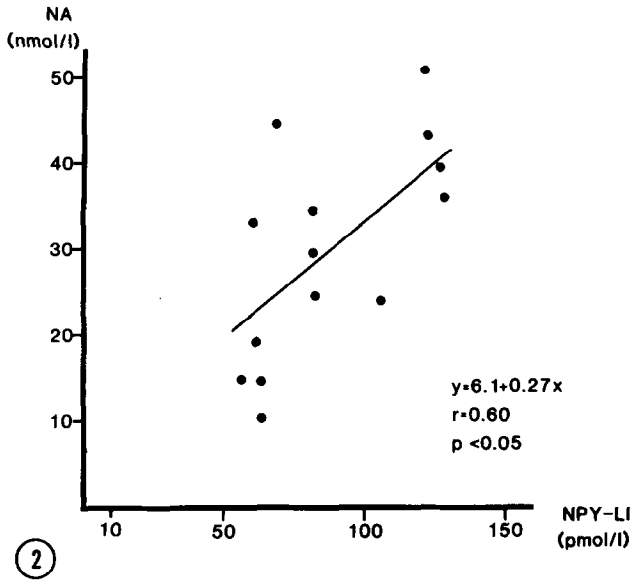


Figure 1. Effects of graded exercise for 6 min at each work level (expressed as percentage of individual, maximal work capacity) in 14 male individuals on the plasma levels of neuropeptide Y-like immunoreactivity (NPY-LI, pmol/l, pM), noradrenaline (NA, nmol/l, nM) and adrenaline (ADR, nmol/l, nM), as well as systolic blood pressure (SBP, mmHg) and heart rate (HR, beats/min). Significant differences compared to basal conditions were calculated using Kruskal-Wallis non-parametric analysis of variance with multiple comparisons (15). ** $p < 0.01$, *** $p < 0.001$.

-LI, in six experiments, suggesting that there was a selective release of NPY-LI during exercise rather than a non-specific effect on e.g. peptide kinetics in plasma.

The present results show that the plasma levels of both catecholamines and a peptide with chromatographic characteristics of NPY are increased during physiological activation of the sympathetic system by physical exercise in man. Whereas plasma NPY-LI and NA, heart rate and blood pressure increased already during mild to moderate exercise, the levels of ADR in peripheral venous plasma were markedly elevated only at higher work loads. Since the plasma levels



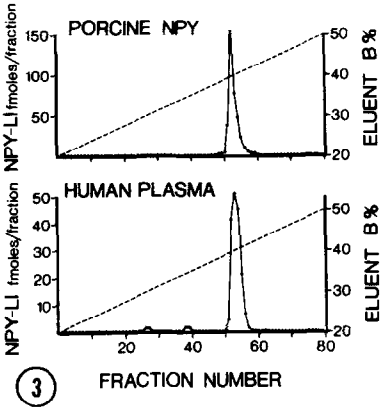


Figure 2. Relationship between plasma levels of NPY--LI (pmol/l) and NA (nmol/l) at exhaustion, which was reached after 3-6 min of maximal physical exercise in 14 male individuals.

Figure 3. Reversed-phase high performance-liquid chromatography of NPY-like immunoreactivity (LI) obtained from 5 ml of human plasma collected during physical exercise at maximal work capacity. Plasma was extracted in acid ethanol, evaporated to dryness under nitrogen gas, redissolved in 500 µl of eluent A (see below) and passed through Millipore-GS filters (0.22 um) to remove particulate matter. Samples of 100 µl were injected onto the column. The elution position of human plasma NPY-LI has been compared to that of synthetic porcine NPY determined by RIA. The NPY-LI was eluted with a 40 min linear gradient (dotted line) of 20 to 50 % eluent B. Eluent A was 0.1 % trifluoroacetic acid in water and eluent B 0.1 % trifluoroacetic acid in acetonitrile. Fractions (0.5 ml) were collected at a rate of 1 ml/min, lyophilized and subjected to radioimmunoassay for NPY-LI in the test tubes used for collection.

of other peptides, such as neurotensin-LI or tachykinin-LI, did not increase upon physical exercise, the present data are compatible with the idea that NPY is co-released with catecholamines upon sympathetic activation in man. Whether plasma NPY-LI originates preferentially from sympathetic nerves or the adrenal gland cannot be determined from the present results. The human adrenal gland, however, has a relatively low content of NPY-LI when compared to other

species (8). Furthermore, the increase in NPY-LI was more closely correlated to increases in NA than those of ADR, suggesting a neural, rather than adrenal origin of the NPY-LI appearing in plasma during exercise.

In conclusion, the plasma levels of a bioactive peptide, NPY, increase in parallel with NA during physiological activation of the sympathetic nervous system in man. This suggests a role for NPY in sympathetic neurotransmission and a possibility to monitor sympathetic activity by analyses of plasma NPY.

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