Heterogeneity of neuropeptide Y immunoreactivity in patients with pheochromocytoma: influence on the diagnostic power of measuring plasma NPY using antisera with different specificities

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The nature of NPY-like immunoreactivity (NPY-LI) was investigated in plasma and tumour tissue of 17 pheochromocytoma patients by HPLC, gel filtration and isoelectric focusing using two radioimmunoassays (RIAs) directed against the C- and N-terminals of NPY respectively. The two RIAs gave similar results in pheochromocytomas: 86% of cases had higher NPY-LI concentrations than those found in normal adrenal glands and NPY-LI behaved like authentic human NPY during gel filtration and HPLC. Assessed by isoelectric focusing, NPY was found to be amidated in seven of nine tumours. Contrary to the findings obtained in tumours, the results of the two RIAs in plasma samples were not always concordant: compared to controls, elevated concentrations of NPY-LI were found in 86% of cases of pheochromocytomas using the C-terminally directed RIA and in 76% of cases using the N-terminally directed RIA. The results of HPLC and gel filtration of NPY-LI in plasma suggested that circulating C- and N-terminal NPY fragments account for the discrepancy between the results of the two RIAs. In conclusion, most pheochromocytomas contain large amounts of NPY-LI that behaved like authentic NPY by chromatographic analysis. On the contrary, circulating NPY-LI in some pheochromocytoma patients is heterogeneous with cleaved products which influence differently the power of the C- and N-terminally directed RIAs for the diagnosis of pheochromocytoma.

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Neuropeptide Y (NPY) is a 36 amino acid peptide that is co-localized with noradrenaline in perivascular sympathetic nerve fibres and in the adrenal medulla (1–4). NPY may contribute to the sympathetic control of blood pressure since it is released in response to sympathetic stimulation (5, 6) and since its effects are at least threefold: (a) direct vasoconstriction, (b) potentiation of norepinephrine-evoked vasoconstriction, (c) suppression of stimulated norepinephrine release (1, 4, 7–11).

Several studies have shown that pheochromocytomas may produce large amounts of NPY (2, 12–20), which are released into the circulation (2, 12, 17, 18, 21). Thus, NPY may contribute to the high blood pressure in pheochromocytoma patients and its measurement in plasma may help diagnose pheochromocytoma. However, no correlation between plasma NPY concentration and blood pressure has been found previously and despite the high incidence of elevated NPY levels in pheochromocytomas the incidence of elevated plasma levels seems relatively low (15, 20). This raises the possibility that NPY may be rapidly degraded and inactivated after its release.

The nature of circulating NPY-like immunoreactivity (LI) has been studied in only a few pheochromocytoma patients (2, 15, 16, 21, 22). The aim of this study was to define the nature of circulating NPY in these patients and to assess its influence on the diagnostic power of measuring plasma NPY with different assays. For this purpose, we have characterized NPY-LI in plasma and tumour tissue of 17 pheochromocytoma patients by HPLC and gel filtration, using C- and N-terminally directed NPY antisera. Since the final step in the processing of NPY is the formation of the C-terminal amide, we also investigated the amidation of the peptide in some of the tumours.

Materials and methods

Patients

Seventeen patients (11F, 6M, mean age ± SD = 51.5 ± 12.9 years) with pheochromocytoma were studied. Fourteen of them had permanent and/or paroxysmal hypertension. Three patients had abdominal pain asso-
associated with fever but no demonstrable hyper- or hypotension. Determination of urinary free catecholamine and/or metanephrine excretion allowed the diagnosis of pheochromocytoma in all cases. The diagnosis was confirmed histologically in 16 patients and was suspected in one patient who denied surgery and in whom a left adrenal mass was identified by computed tomography scanning and 131I-meta-iodobenzylguanidine scintigraphy. One patient had a malignant pheochromocytoma with liver and lymph node metastases and one patient had multiple endocrine neoplasia type IIa, including medullary thyroid carcinoma associated with neurofibromatosis.

**Plasma collection and extraction**

Repeated blood sampling was performed prior to surgery in 16 pheochromocytoma patients. Blood was also drawn during operative manipulation of the tumour in seven cases. Healthy subjects served as controls. Blood was collected in EDTA-containing tubes that were rapidly centrifuged. After separation, plasma was stored at −80°C until analysis for NPY-LI. Plasma samples of 2 to 6 ml of volume were extracted through Sep-Pak C18 cartridges (Waters, Milford, MA) which had been previously activated with acetonitrile containing 0.1% TFA (Tri Fluoro Acetic Acid) and converted to the aqueous phase with 0.1% TFA in water. After washing with 10 ml 0.1% TFA in water, NPY was eluted with 2 ml 50% acetonitrile in water containing 0.1% TFA. After drying under nitrogen, the residue was reconstituted in assay buffer. The recovery of human NPY added to hormone-free plasma ranged between 60 and 105%.

**Tissue collection and extraction**

Tumour tissue was obtained following surgery in 14 cases. Normal adrenal glands, obtained at surgery at the time of donor nephrectomy, served as control. Tissues were immediately frozen on dry ice and stored at −80°C until extraction. After weighing, the tissues were extracted by boiling for 10 min in 1.0 mol/l acetic acid (10% w/v) and homogenized. After centrifugation, the supernatant was lyophilized and stored at −20°C prior to assay.

**Radioimmunoassay**

Synthetic human NPY was labelled with 125I-iodine using the chloramine T method. Labelled NPY was purified by reverse-phase HPLC. Two antisera were used. K02B antiserum was obtained by immunizing rabbits with synthetic porcine NPY (Peninsula, Belmont, CA) conjugated with BSA through ethyl-carboxydiimide condensation. The specificity of the antiserum was examined by measuring the inhibition of [125I] NPY binding by various peptides as determined by comparing the IC50 of each peptide with that of standard human NPY (Neosystem, Strasbourg, France). Multiple dilutions (1 fmol-500 pmol/tube) of synthetic NPY fragments (gift from Dr JE Rivier and the related human pancreatic polypeptide PP) and peptide YY (PYY) (Peninsula, Belmont, CA) were measured. In addition, we examined the cross-reactivity of the antiserum with a number of peptides that have been found to be produced by pheochromocytomas, such as met-enkephalin, neurotensin, vasoactive intestinal peptide, somatostatin, ACTH (gift from Pr JA Chayvialle) and Molluscan FMRFamide (gift from Pr G Simmonet). The K02B antiserum recognizes the C-terminus of NPY since the cross-reactivity in relation to human amidated NPY is 80% with the NPY 18–36 fragment, 53% with human free-acid NPY (Peninsula, Belmont, CA) and less than 1% with the NPY 1–14 and 12–30 fragments. It shows only 1.4% cross-reactivity with human PYY and no cross-reactivity (<1%) with PP and the other peptides previously mentioned. K02B antiserum was used at a dilution of 1:100,000. The detection limit was 1.1 fmol/assay tube and the intra- and interassay coefficients of variation were below 6 and 9%, respectively. 1609/001 antiserum was purchased from UCB Bioproducts (Braine L’alleud, Belgium). As indicated by the supplier (23), it is mainly directed towards the N-terminal region of NPY, since the cross-reactivity in relation to reactivity with NPY 1–36 is 100% with NPY 1–35, 82% with NPY 1–15, 10% with NPY 16–36 and 4% with NPY 25–36. The cross-reactivity with porcine PYY and avian PP is less than 0.4% 1609/001 antiserum was used at a dilution of 1:50,000. The detection limit was 21.0 fmol/assay tube and the intra- and interassay coefficients of variation were below 8 and 11%, respectively. Assays were set up in 1 ml of 50 mmol/l sodium phosphate buffer, pH 7.5, containing 0.5% BSA, 4 mmol/l EDTA, 0.2 g/l sodium azide and 50,000 KU/l aprotinin. Standards were prepared with human NPY. Extracts and appropriate dilutions of standard NPY in 800 μl of assay buffer were incubated for 24 h at 4°C with 50 μl of antiserum; 2000 cpm of tracer in 50 μl of assay buffer were then added and the incubation was continued for a further 24 h. Free and bound tracer were separated by the addition of 50 μl of non-immune rabbit serum (final dilution = 1:2400) and 50 μl of donkey antirabbit serum (final dilution = 1:300).

**Gel filtration chromatography**

Tumour and plasma extracts were applied to a Sephadex G-50 superfine column (1.6 × 95 cm) eluted at 4°C at a constant flow of 10 ml/h with RIA buffer, fractions being collected every 10 min. The column was calibrated with BSA (void volume), cytochrome C, aprotinin (measured by spectrophotometry), NPY 18–36 (measured by RIA) and 22Na (total volume). BSA and 22Na were added to each sample to determine the elution constant (Kav) of NPY-LI.
Reverse phase HPLC

Extracts were passed through a 0.9 × 30 cm μ-Bondapak C-18 column (Waters) eluted at 1 ml/min with 0.1% TFA in distilled water and a 30 min linear gradient of 25–50% acetonitrile. One ml fractions were collected, dried under nitrogen, reconstituted in assay buffer and subjected to RIA. The system was calibrated with oxidized and non-oxidized synthetic human NPY and with the NPY 18–36 fragment. Oxidation of NPY was obtained by incubating synthetic human NPY in 3 mol/l acetic acid with 3.5% hydrogen peroxide as previously described (20). The retention time of NPY 1–36 was determined by RIA in each run.

Isoelectric focusing

Isoelectric focusing was achieved according to the method of O’Hare et al. (24). Lyophilized tumour extracts were reconstituted in 50 μl of lysis buffer prior to isoelectric focusing for the non-equilibrium pH gradient gels (25). The samples were applied to gels containing 0.25 ml each of a ampholyte pH 8–9.5 and 7–9 that had been prepared in 3 mm diameter cylindrical glass tubes (26). The gels were subjected to 350V for at least 15 h. Thereafter, they were cut into 25 slices 5 mm in length and extracted overnight at 4°C in 80% formic acid containing 10 mg/l of BSA. The acid extracts were dried under vacuum, reconstituted in RIA buffer and assayed. The system was calibrated with amidated and free acid-NPY which were included in each run.

In vitro degradation

To test the stability of NPY in plasma, 118 fmol of synthetic human NPY was added to 2 ml samples of fresh plasma obtained from controls. After homogenization, some samples were immediately extracted while the others were incubated at 37°C for 6 h before being extracted. The extracts were assayed for the measurement of C- and N-terminal NPY-LI concentration and subjected to reverse phase HPLC.

Statistics

Unless otherwise stated, data are expressed as mean ± SEM. The mean arterial pressure was calculated from the systolic and diastolic blood pressure according to the following equation: [(systolic-diastolic pressure)/3] + diastolic pressure. Comparison of the means was done with the use of the t-test for paired data. Regression analysis was made with the use of the least-square method.

Results

Measurement and characterization of NPY-LI in adrenal tissue

NPY-LI in nine human normal adrenals (cortex and medulla) was 12.6 ± 1.7 pmol/g (range: 4.7–28.3) using the C-terminally directed RIA. The high concentrations found in pheochromocytoma tumours (393.7 ± 93.7 pmol/g) were similar to that found in normal adrenals in 2 of 14 cases (24.1 and 25.7 pmol/g). NPY-LI was also measured with the N-terminally directed RIA in 13 of the tumours and in 5 normal adrenals. The NPY-LI concentrations measured with the N-terminal assay were higher than those measured with the C-terminal assay both in pheochromocytoma (706.3 ± 178.6 vs 381.4 ± 101.2 pmol/g; p < 0.002) and in normal adrenals (32.0 ± 12.5 vs 14.8 ± 4.6 pmol/g). However, the results of the two RIAs displayed a constant relationship with a N- to C-terminal NPY-LI ratio of 2.0 ± 0.2 (range 1.4–3.8) and were closely correlated (r = 0.97, p < 0.001) (Fig. 1A). The two pheochromocytoma tumours with low C-terminal NPY-LI also had low N-terminal NPY-LI concentrations.

Gel filtration of tumour extracts (N = 12) revealed that the major part of NPY-LI co-eluted with human NPY standard using the C-terminally directed RIA. Less than 10% of the amount of NPY-LI eluted as a product with a higher molecular weight. Similar elution patterns were obtained using the N-terminally directed RIA (N = 3).

During HPLC, most of the NPY-LI in tumour extracts (N = 12) measured with the C-terminal assay eluted in positions corresponding to the oxidized and non-oxidized forms of human synthetic NPY (retention times = 17 and 21 min respectively) (Fig. 2A). Only 13.6 ± 4.0% of the total amount of NPY-LI eluted as less hydrophobic material. The ratio of oxidized to non-oxidized peptide varied from one tumour to the other, but it also varied between several extractions of the same tumour, suggesting that spontaneous oxidation occurs during the extraction procedure. In contrast, the proportion of the less hydrophobic NPY-LI was unchanged after repetitive extraction of the same tumour. The N-terminal NPY-LI eluted with essentially identical HPLC profiles as the C-terminal NPY-LI (N = 8).

Measurement and characterization of NPY-LI in plasma

NPY-LI in plasma extracts of 47 control subjects was below the detection limit of the assay in half of the cases using the C-terminally directed RIA. It ranged from < 2.3 pmol/l to 20.6 pmol/l. NPY-LI concentration in 46 plasma samples obtained from 16 pheochromocytoma patients was elevated to 224.1 ± 77.6 pmol/l using the C-terminal assay. However, two patients with low NPY tumour concentration also had low plasma NPY concentration and repeated blood sampling in three patients
who had elevated NPY concentration in the tumour revealed alternately elevated and normal plasma concentrations. The overall sensitivity of plasma NPY-LI measurement using the C-terminal assay for the diagnosis of pheochromocytoma PC in this series was 80%. Operative manipulation of the tumours increased plasma NPY concentration from $255.5 \pm 79.4$ pmol/l to $2409.7 \pm 842.0$ pmol/l ($N = 7$). Data obtained from three patients in whom sequential blood sampling was performed during surgery showed that the C-terminal NPY-LI was reduced by more than 70% within 1 h of tumour removal (Fig. 3). There was no correlation between the C-terminal NPY-LI concentration and the mean blood pressure at the time of sampling ($r = 0.19$).

NPY-LI was measured with the N-terminally directed RIA in the plasma of 8 controls and in 21 plasma samples obtained from 12 pheochromocytoma patients. In controls, N-terminal NPY-LI ranged from $<5.8$ to 30.0 pmol/l. As found in tumours, there was a tendency for higher NPY-LI levels in the plasma of pheochromocytoma patients using the N-terminal assay compared to that measured with the C-terminal assay ($215.2 \pm 56.4$ vs $135.4 \pm 23.9$ pmol/l respectively; $p = 0.06$). The N- to C-terminal NPY-LI ratio was $1.5 \pm 0.2$ and the results of the two RIA correlated ($r = 0.77; p < 0.001$) (Fig. 3). However, and contrary to the findings obtained in tumours, the results obtained with the N-terminally directed RIA were not constantly higher than those obtained with the C-terminally directed RIA. The N- to C-
terminal NPY-LI ratio ranged from 0.2 to 3.1 and was below 1.0 in seven cases. Moreover, in two cases we found low NPY-LI concentrations (15.2 and 15.7 pmol/l) using the N-terminally directed RIA, in contrast to elevated concentrations using the C-terminally directed RIA (88.8 and 32.9 pmol/l respectively). The sensitivity of the C-terminally directed RIA for the diagnosis of pheochromocytomas in this subseries was 86%, while that of the N-terminally directed RIA was 76%.

The HPLC elution profile of the C-terminal NPY-LI was variable from one plasma extract to the other (N = 14) and the amount of NPY-LI that co-eluted with human NPY ranged from 34.7 to 100.0%. When present, most of the additional NPY-LI forms eluted earlier than NPY 1–36 but did not co-elute with the NPY 18–36 fragment. Comparison of the HPLC elution profiles of C-terminal NPY-LI between tumour and plasma extracts obtained from seven patients revealed large differences in three cases (Fig. 2). The elution profile of C-terminal NPY-LI in plasma samples that were drawn from the same patient on separate occasions was sometimes highly variable (Fig. 2). Comparison of the elution profiles of the N- and C-terminal NPY-LI in plasma of two pheochromocytoma patients revealed a single peak corresponding to standard NPY in one case, while a markedly different and heterogeneous pattern was seen in the other case (Fig. 4).

NPY-LI in three plasma extracts was investigated for molecular size using gel filtration. In one case, NPY-LI measured with both RIAs eluted in fractions corresponding to void volume and to standard NPY (Kav = 0.69). In two cases, 55% and 47% of the amount of NPY-LI measured with the C-terminal assay was eluted as a small molecular weight material with a Kav of 0.91. The Kav of NPY 18–36 in our system was 0.82. Low molecular weight material that accounted for 56% of the amount of NPY-LI was also identified in one of these two cases with the N-terminal assay (Fig. 5).

The total amounts of N- and C-terminal NPY-LI in plasma recovered from the gel filtration and HPLC columns were quantified and compared. The N- to C-terminal NPY-LI ratio was 2.5 and 4.1 in the two cases showing identical elution profiles and was 0.7, 0.9 and 1.1 in the three cases showing different elution patterns with the two RIAs.

In vitro degradation

Synthetic NPY appeared to be very stable in plasma, since the decrease in NPY-LI measured with both RIAs was less than 20% after a 6 h incubation period at 37°C (N = 3). The HPLC elution profiles of NPY-LI obtained before the incubation period were similar to those of the NPY standard, while they showed some heterogeneity after the incubation period. No similarity could be found between the NPY-LI elution profiles obtained after incubation of NPY with plasma and those observed in plasma extracts of pheochromocytoma patients.
constant However, C-terminal power compare NPY-LI The Discussion acid amidated tumours. 95.6% nine co-migrated acid focusing Fig. 6. Characterization of the amidation status of NPY-LI by isoelectric focusing in two pheochromocytoma tumours. Markers include the free acid form (open arrow) and the amidated form (closed arrow) of human NPY. In seven of nine cases, the largest proportion of NPY-LI co-migrated with the amidated form of NPY (Fig. 6A). In only two of nine cases was the free acid form found to predominate (Fig. 6B).

Isoelectric focusing

The amidation status of the peptide was assessed in nine tumours. In seven of these the major part of C-terminal NPY-LI (84.1 ± 6.5%) was found to co-migrate with the amidated form of NPY. However, in two cases, 79.3 and 95.6% of NPY-LI was found to co-migrate with the free acid form of NPY (Fig. 6).

Discussion

The purpose of this study was to assess the nature of NPY-LI in plasma of pheochromocytoma patients, to compare it with that of NPY-LI in pheochromocytoma tumours and to assess if it could influence the diagnostic power of measuring plasma NPY with different assays.

The NPY concentrations measured in tissues with the C-terminal and N-terminal assays were not equimolar. However, the results of the two RIAs displayed a constant relationship, were closely correlated and led to the same overall results, since 86% of pheochromocytoma tumours were found to have higher NPY-LI concentrations than in normal adrenals using both assays. Fractionation of tumour NPY-LI by gel filtration and HPLC showed that the C- and N-terminal NPY-LI had essentially identical chromatographic profiles and eluted in the position of human NPY. Taken together, these data suggest that the discrepancy between the absolute values obtained with the two antisera is due to unequal cross-reactivity with endogenous NPY 1–36. It might also be related to the extraction procedure, since, because of assay interference from extracted material, the concentration of NPY measured in tissues using RIAs has been shown to depend on the extraction methodology (27, 28).

Most of the tumours efficiently amidated the peptide. The predominant free acid form of NPY that we found in two cases may correspond to a degradation product of NPY lacking the amide structure or to a glycine-extended form of NPY. In this latter case it could reflect either a primary defect in peptidylglycine alpha-amidating monooxygenase in the tumour or a loss in enzyme activity related to a disturbed milieu (29).

Our results are in accordance with those of previous studies (2, 12, 15–20) that showed the majority of pheochromocytoma tumours to contain high amounts of NPY (84% of 97 cases) with identical chromatographic properties to synthetic NPY (2, 13–16, 20).

Contrary to the findings obtained in tumours, the relationship between the results of the two RIAs in plasma was not constant and the N- to C-terminal NPY-LI ratio varied from 0.2 to 3.1. The HPLC elution profile of C-terminal NPY-LI varied from one sample to the other and, in some cases, a complex pattern with several peaks of low hydrophobicity that accounted for the major part of NPY-LI was found. Some immunoreactive species were recognized by one antisera but not by the other. In addition, different N- and C-terminal immunoreactive molecules of smaller molecular weight were found in some plasma extracts upon gel filtration. That these additional NPY forms had been generated during the extraction procedure is highly unlikely, since repeated extraction of the same plasma sample led to identical HPLC profiles and since the extraction did not generate smaller peptides when synthetic NPY was added to fresh plasma (data not shown). Thus, our results are consistent with the presence of circulating NPY fragments in some pheochromocytoma patients. The results of RIA following HPLC and gel filtration suggest that these fragments account for the cases in which NPY-LI concentrations measured with the N-terminal assay were lower than those measured with the C-terminal assay.

The nature of NPY-LI in the circulation of pheochromocytoma patients has been assessed in a few cases only. Circulating NPY-LI was found to have identical elution behaviour to human NPY upon gel filtration and HPLC in some studies (15, 16). However, there is also evidence
for multiple immunoreactive forms in some pheochromocytoma patients (2, 22), hypertensive subjects (30) and patients with chronic renal failure (21). Similarly, C-terminal NPY fragments have been demonstrated in plasma of dogs (31). The additional forms of NPY-LI that we found in the plasma were not present in tumours. This suggests that they arise in the circulation.

No data concerning the mechanism of clearance of NPY from the circulation are available and only little can be said from our study: the HPLC elution profile was variable and none of the additional peaks co-eluted with the NPY 18–36 fragment; gel filtration results suggest the existence of circulating C-terminal fragments that are smaller than NPY 18–36. One may note the contrast between the relatively rapid disappearance of endogenous NPY-LI after its release during operative manipulation of the tumour and the apparent stability of synthetic human NPY in vitro. This suggests that NPY is degraded in tissues rather than in plasma. The elevated plasma NPY concentration found in patients with renal failure suggests that the kidney could play a part in the removal of NPY (21, 32). In this context, noteworthy is the recently reported evidence for the in vitro degradation of NPY by rat kidney cell membranes through the action of the enzyme endopeptidase-2 (33).

Peripheral administration of NPY to different species (7, 8, 10, 32), including man (34), raises blood pressure as the result of a direct vasoconstriction and/or a potentiation effect on norepinephrine-evoked vasoconstriction (4, 9, 11, 35, 36). The entire NPY molecule is required to raise blood pressure in vivo and depletions of the C- and N-terminal portions diminish or abolish the peptide vasoconstrictor effect in vitro (9, 32). Furthermore, some NPY fragments such as 17–36, 18–36 and 22–36 can induce a decrease in blood pressure (32, 37, 38). It is therefore unlikely that the small C-terminal NPY fragments that we found in plasma possess vasoconstrictor activity. Although the mechanism of hypertension in pheochromocytoma patients is complex (39), the presence of biologically inactive NPY circulating fragments may explain, at least in part, the lack of correlation between NPY levels and the arterial blood pressure that we found. The basal plasma levels of intact NPY observed in most pheochromocytoma patients may also be too low to exert any vasoconstriction per se (5, 35, 36). Lastly, an impaired amidation of NPY, as found in two of our cases, may also prevent the vasoactivity of the peptide, since the C-terminal amide is necessary for its biological activity (9, 32).

The heterogeneity of circulating NPY-LI in pheochromocytoma patients impairs the diagnostic power of NPY measurement. However, in our study the sensitivity of the C-terminally directed RIA was clearly better than that of the N-terminally directed RIA. Analysis of the literature also reveals a clear contrast between the 53.9% incidence of elevated plasma NPY levels in pheochromocytoma patients using “sandwich” IRMAs that recognize only intact NPY (16–18, 40) and the 76.1% incidence found using traditional RIAs with polyclonal antibodies that may recognize NPY fragments (2, 12, 15, 16, 21, 22). Although these studies were not carried out on the same patients, this discrepancy may be due to circulating NPY fragments, as has been shown for ACTH (41), calcitonin (42) and parathormone (43). The finding of high plasma NPY levels together with the presence of additional NPY forms upon chromatography in patients with renal failure with the use of RIAs (21, 32) but not with the use of IRMA (40) support this point.

The usefulness of NPY RIAs for diagnostic purposes is limited, since all pheochromocytoma patients of our series had elevated urinary free catecholamines and/or metanephrines but only 80% of them had elevated plasma NPY-LI concentrations. However, since the very high plasma NPY levels obtained during surgery may influence blood pressure and cardiac function (2, 18, 44) and since NPY-induced constriction of skeletal and coronary arteries is inhibited by certain Ca2+ channel blockers but not by adrenoreceptor antagonists (7, 36, 45, 46), the preoperative identification of NPY-secreting individuals among pheochromocytoma patients may still be useful for the anaesthetic management of these. For this purpose, our study suggests the preferential use of a C-terminally directed NPY RIA.

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References

8. Corder R, Lowry PJ, Wilkinson SJ, Ramage AG. Comparison of the...
23. I6090/001-antibody to neuropeptide tyrosine data sheet. UCB Bioproducts. Braine L'alleud, Belgium

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