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Research Article

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Production, Characterization, and Expression of Neuropeptide Y by Human Pheochromocytoma

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Abstract

Neuropeptide Y (NPY) levels are increased in plasma and tumors of patients with pheochromocytoma. The present study was designed to evaluate plasma and tissue NPY levels simultaneously as well as to study its release and expression in patients with either adrenal or extraadrenal pheochromocytomas.

Plasma NPY levels were higher ($P < 0.01$) in patients with adrenal tumors than in matched normal subjects and patients with extraadrenal tumors. NPY levels were also higher ($P < 0.05$) in adrenal than in extraadrenal tumors. Bioactive NPY(1–36) was the predominant form in plasma and tumors of patients with adrenal pheochromocytomas. In contrast, patients with extraadrenal pheochromocytomas had an abundance of NPY fragments. NPY mRNA was abundant in 11 of 13 adrenal tumors but in only 1 of 6 extraadrenal tumors. Moreover, NPY was coreleased with NE with manipulation of adrenal but not extraadrenal tumors.

These findings indicate that increased NPY gene expression in adrenal pheochromocytomas accounts for the greater biosynthesis and storage of NPY in these tumors and that increased release of NPY results in elevated plasma NPY. Factors regulating NPY gene expression in pheochromocytoma and the role of NPY in the clinical manifestations of the disease remain to be elucidated. (*J. Clin. Invest.* 1995. 96:2503–2509.) Key words: Neuropeptide Y(1–36) • pheochromocytoma • adrenal • extraadrenal • mRNA

Introduction

Neuropeptide Y (NPY)[1] is a tyrosine-rich peptide distributed throughout the central and peripheral nervous systems. It is synthesized as a 97 amino acid precursor and is posttranslationally processed to the mature 36 amino acid form (NPY1–36) in sympathetic nerves and adrenal medulla, where it is costored and coreleased with NE (1–6).

NPY has potent direct and indirect cardiovascular effects. It increases coronary and peripheral vascular resistance independently of α-adrenergic receptor mechanisms by interacting with vascular G-protein–coupled NPY receptors (7, 8). In some vascular beds, NPY has no direct vasoconstrictor effects but potentiates NE-induced vasoconstriction (9, 10). NPY also inhibits the release of acetylcholine and norepinephrine from sympathetic nerve terminals (11, 12) and markedly inhibits the effects of parasympathetic stimulation on atrial myocardium and on the sinus and atrioventricular nodes (13, 14).

In addition to mediating or modulating the effects of the sympathetic nervous system, NPY is synthesized and released from tumors of neural origin, including the chromaffin cell tumors, pheochromocytomas. Plasma and tumor NPY levels have been reported to be increased in some patients with pheochromocytoma, particularly those with adrenal tumors (15–18). NPY gene expression has also been evaluated in pheochromocytoma tissue and has been suggested to be low in malignant tumors (19). However, because pheochromocytomas are rare, the number of subjects evaluated in these reports has been small. Our study was designed to evaluate plasma and tissue NPY levels simultaneously, as well as to study its release and expression in a cohort of patients with pheochromocytoma.

Methods

Patient population and sample collection. The study population consisted of 20 patients with proven pheochromocytoma and 20 age- and sex-matched controls. 12 patients had adrenal tumors (Table 1). All tumors were surgically removed, and the diagnosis was confirmed by detailed histopathological evaluation of the tumor tissue. Malignant tumors were defined as those tumors with known metastases or tumors that were locally or focally invasive. One normal adrenal gland was obtained at the time of total nephrectomy for renal carcinoma and separated into medulla and cortex by gross dissection. Tissue specimens were snap frozen in liquid nitrogen and stored at −70°C until peptide and RNA extractions were done.

Blood samples were collected into chilled tubes containing heparin (20 U/ml blood) and aprotinin (50 KIU/ml blood) for the measurement of NPY. For catecholamine determinations, blood was collected into chilled tubes containing heparin (20 U/ml blood). Blood samples were collected before, during, and after surgery, and during an outpatient follow-up evaluation. Blood collected at the time of surgery was sampled before incision, before tumor manipulation, during tumor manipulation, after resection of the tumor, and after closing of the surgical incision. Blood was also sampled similarly in one patient with an adrenal cortical

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1. *Abbreviations used in this paper:* IR, immunoreactive; NPY, neuropeptide Y.

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Table I. Clinical Characteristics of 20 Patients with Pheochromocytoma

Patient No./tumor location	Age	Sex	Blood pressure	
			Presurgery	Postsurgery
Adrenal tumors				
1[a]	37	F	146/84*	120/90
2[a]	37	F	220/112	100/80
3[a]	40	F	170/110	116/184
4[b]	44	F	140/82	115/70
5[a]	42	M	120/68*	112/72
6[a]	43	M	160/120	130/90
7[a]	33	M	146/98*	110/80
8[a]	59	M	160/98*	132/74
9[a]	36	F	200/112	132/94
10[a]	49	M	116/74*	138/20
11[a,c]	37	F	160/100	142/90*
12[a,c]	52	M	180/125	160/100
Extraadrenal tumors				
13[a]	15	F	175/125	112/58
14[b]	17	F	150/115	110/60
15[b]	34	M	120/82	118/86
16[a]	24	F	210/110	108/92
17[a]	18	M	143/102*	115/70
18[d,c]	27	M	170/86	150/98
19[a,c]	49	M	152/100	126/84
20[a,c]	65	M	138/82*	130/80

[a] Sporadic case; [b] familial; [c] malignant (metastases to lungs and bone); [d] von Hippel Lindau Syndrome. * Patients receiving antihypertensive medication.

adenoma. Blood samples were centrifuged at 4°C, and the plasma was stored at −70°C until analysis.

Neuropeptide Y characterization and quantification. Plasma NPY was extracted using Sep-Pak cartridges (Waters Associates, Millipore Corp., Milford, MA) and an acetonitrile solvent system, as previously described (20), and used directly to determine total immunoreactive NPY (IR-NPY) levels. In a subset of patients, the extract was fractionated by reverse-phase, HPLC, and the NPY immunoreactivity of the eluted fractions was quantified by the method of Senanayake et al. (20). Frozen tissue samples were weighed and immediately boiled in 0.5 M acetic acid for 15 min to extract NPY (21). NPY(1–36) and its peptide fragments were separated on an LKB 2150 gradient system equipped with a Vydac C18 column (4.6 × 150 mm; The Separations Group, Hesperia, CA). The column was equilibrated with 60% mobile phase A (0.13% aqueous heptafluorobutyric acid) and 40% mobile phase B (80% acetonitrile/aqueous 0.13% heptafluorobutyric acid) at flow rate of 1 ml/min. Waters C18 Sep-Pak processed plasma was dissolved in 40% mobile phase B, 60% phase A and applied to the column. NPY and its fragments were eluted at a flow rate of 1 ml/min with a 24-min three step gradient. The gradient consisted of 40% eluant B for 1 min, increased linearly to 47% over 1 min, and held at 47% for 22 min. Fractions (0.2 ml) were collected at 12 s intervals and evaporated to dryness in a Speed Vac concentrator (Savant Instruments, Inc., Hicksville, NY). The dried extracts were reconstituted in the assay buffer and routinely quantified by RIA using AB I. AB II was reserved to confirm the authenticity of NPY(1–36). Simultaneous assays were done using two antisera: AB I (RAS 7172; Peninsula Laboratories, Inc., Belmont, CA), and AB II (NPY [2–8], a gift from Professor Marvin R. Brown, Departments of Medicine and Surgery, University of California, San Diego) (22). The lower detection limit of the assay using AB I was 10 pg/ml. The intra- and interassay coefficients of variation were 10% and 14%, respectively. The lower detection limit of the assay using AB II was 8 pg/ml. The intra- and inter-assay coefficients of variation were 9% and 12%, respectively. Both antisera showed 100% cross-reactivity with human NPY(1–36) and its oxidized form. AB I, which detects a range of NPY-like peptides, was used for routine analysis. It cross-reacts with COOH-terminal NPY fragments [NPY-(20–36), -(22–36), -(26–36)], human peptide YY, and human pancreatic polypeptide. AB II was used to confirm the authenticity of the mature peptide and its oxidized form. It cross-reacts with human NPY free acid. The final dilution of AB I was 1:36,000, and the final dilution of AB II was 1:240,000. NPY contains methionine, an amino acid prone to oxidation. The conversion of the nonpolar side chain of methionine to a polar methionine sulfoxide alters its chromatographic mobility and, more importantly, may determine its biological potency. It was important to include the oxidized form with the investigation of NPY and its fragments because tissue specific NPY variants might be influenced by changes in the physiological state of the organism and be functionally distinct thus providing a basis for the diversity of actions attributed to NPY.

Plasma catecholamines. Plasma catecholamine concentrations were measured by radioenzymatic assay (23). The sensitivity of the assay is 15 pg/ml for both norepinephrine and epinephrine. Intra- and interassay coefficients of variation were 2.4% and 3.7%, respectively, for norepinephrine, and 6.0% and 6.0% for epinephrine.

Preparation of mRNA and Northern blot analysis. RNA was prepared by the method of Chirgwin et al. (24). Frozen tissue was homogenized in 4 M guanidium isothiocyanate and then layered onto a cesium chloride cushion for overnight ultracentrifugation at 150,000 *g*. After careful aspiration of the supernatant and removal of most of the upper portion of the centrifuge tube, the RNA pellets were harvested by resuspension in RNase-free water, precipitated with ethanol, and then resuspended in RNase-free water. The RNA was then either applied directly to formaldehyde-agarose gels, or first fractionated with oligo-dT cellulose to select poly A^+ RNA. After electrophoresis, the RNA was transferred by capillary action to nylon membranes (Gene Screen Plus; DuPont Co., Wilmington, DE). The filters were air dried and prehybridized at 42°C for 4 h in a buffer containing 10 mg/ml BSA, 20 mM Na phosphate, pH 7.2, 15% formamide, 1 mM EDTA, and 7% SDS; human NPY cDNA (supplied by Dr. Carolyn Minth, University of Michigan, Ann Arbor, MI); ^{32}P-labeled by nick translation (25) was then added. After overnight hybridization, the filters were washed briefly at room temperature in 0.5× SSC (1× SSC = 150 mM NaCl, 15 mM sodium citrate), 0.1% SDS, and again in the same buffer at 65°C to remove unhybridized probes. The blots were then exposed to XAR 5 x-ray film (Eastman Kodak Co., Rochester, NY) at −70°C for 1–4 d.

Statistical analysis. Descriptive statistics are expressed as the range and median. Comparisons of non-normally distributed data were made with the Wilcoxon rank-sum test. Comparisons between the groups were assessed by the Kruskal-Wallis test. Fisher's exact test was used to evaluate the difference in NPY mRNA abundance in adrenal and extraadrenal pheochromocytomas. Values were considered significant when $P < 0.05$. In the case of undetectable plasma NPY levels, a value equivalent to the least detectable concentration of the assay was assigned for statistical calculations.

Results

Plasma catecholamines and IR-NPY levels. Plasma NE was significantly increased in all patients (Fig. 1). In contrast, plasma epinephrine was within the normal range in seven patients (three adrenal, four extraadrenal). As a group, the patients with pheochromocytoma tumors had higher IR-NPY levels than did normal subjects. This increase was predominantly the result of markedly higher levels ($P < 0.05$) in patients with adrenal

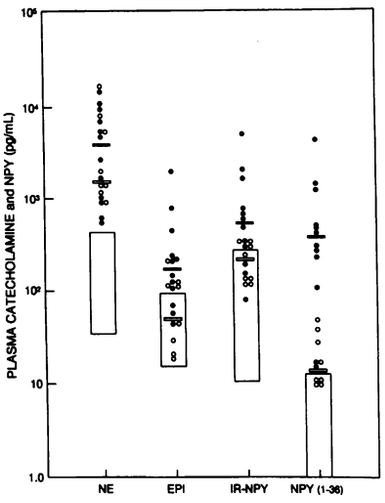

Figure 1. Levels of plasma norepinephrine (*NE*), epinephrine (*EPI*), immunoreactive neuropeptide Y (*IR-NPY*), and NPY(1–36) (determined by RIA coupled to reverse phase–HPLC) in patients with an adrenal (•) or extraadrenal (○) pheochromocytoma. The rectangles in each lane indicate the normal range. (NE 126–402, 218; EPI 24–78, 42; IR-NPY 10–271, 174; NPY(1–36) < 10–12, 10; range and median for the respective peptides). The median for each group is indicated by the transverse bars (*solid bar:* adrenal pheochromocytomas, *hollow bar:* extraadrenal pheochromocytomas).

tumors (median 539 pg/ml, range 75–4,780 pg/ml) than those with extraadrenal tumors (median 203 pg/ml, range 10–378 pg/ml). Moreover, the difference between the patients with adrenal and extraadrenal tumors was even greater for plasma NPY(1–36) levels (adrenal pheochromocytoma: median 275 pg/ml, range 12–4,000 pg/ml); extraadrenal tumors (median 20 pg/ml, range 10–48 pg/ml); normal subjects (median 10 pg/ml, range < 10–12 pg/ml; $P < 0.05$ for the adrenals versus either the extraadrenals or normal). The IR-NPY levels in patients with extraadrenal tumors were not significantly different from those in normal subjects (median 174 pg/ml, range < 10–271 pg/ml).

Tumor total IR-NPY levels. Tumor IR-NPY levels were at least 1,000 times higher than the total plasma IR-NPY levels, which is consistent with synthesis and storage of NPY by these chromaffin tumors (Fig. 2). However, consistent with the plasma NPY levels, the levels of tumor tissue IR-NPY or NPY(1–36) were significantly higher ($P < 0.05$) in the adrenal

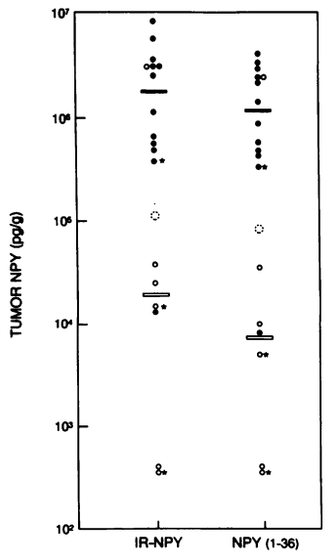

Figure 2. Levels of immunoreactive NPY (*IR-NPY*) and NPY(1–36) in adrenal (•) and extraadrenal (○) pheochromocytomas. The broken circles indicate the levels found in a single normal adrenal gland. The median for each group is indicated by the transverse bars (*solid bar:* adrenal pheochromocytoma, *hollow bar:* extraadrenal pheochromocytomas). Malignant tumors are denoted by asterisks.

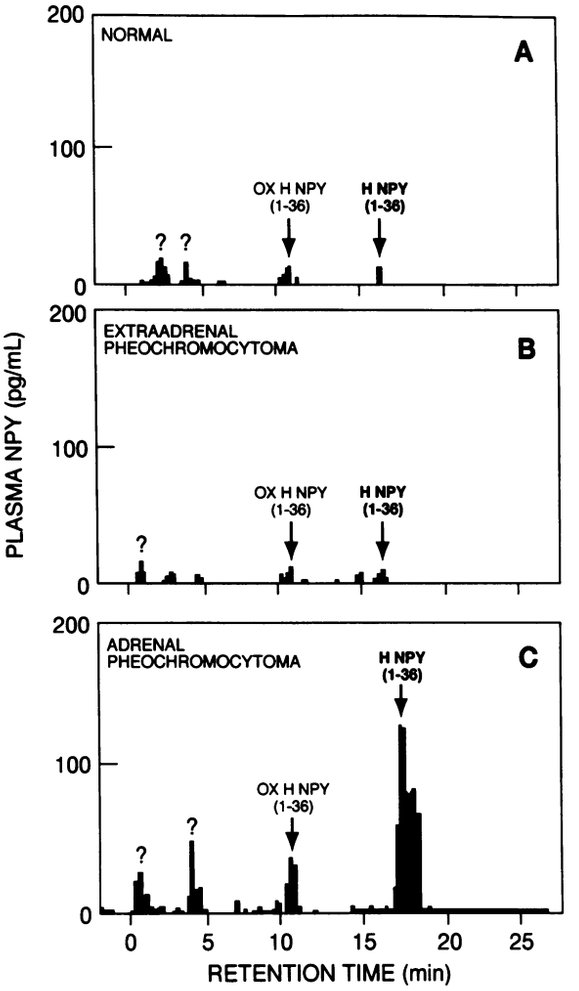

Figure 3. High performance, reverse-phase chromatographic separation of neuropeptide Y and its immunoreactive fragments in plasma from normal subjects (*A*), and patients with an extraadrenal pheochromocytoma (*B*), or an adrenal pheochromocytoma (*C*). Unidentified peptide fragments (*?*) and oxidized NPY (*OX H NPY*) constitute the predominant component of IR-NPY in normal and extraadrenal plasma. In contrast, NPY(1–36) [*H NPY (1–36)*] is the major component in the plasma of patients with adrenal pheochromocytoma.

tumors (medians 2,490 and 1,410 ng/g, ranges 13–8,200 and 8–4,000 ng/g for total IR-NPY and NPY(1–36), respectively) as compared to the extraadrenal tumors (medians 20 and 8 ng/g, ranges < 0.4–3,140 and < 0.4–230 ng/g, respectively) (Fig. 2). Moreover, the median tumor tissue IR-NPY and NPY(1–36) levels were similar to those in a single normal adrenal gland (110 and 84 ng/g, respectively). Tumor tissue IR-NPY and NPY(1–36) levels were high in one malignant adrenal tumor but not in two malignant extraadrenal tumors.

IR-NPY levels in fractionated plasma and tissue samples. Examples of the plasma IR-NPY peptide profiles observed after HPLC fractionation in normal subjects and in patients with adrenal or extraadrenal pheochromocytomas are shown in Fig. 3. In plasma from normal subjects, unidentified, less hydrophobic, IR-forms constituted 80% to 90% of the IR-NPY (Fig. 3 *A*). In the plasma from patients with extraadrenal tumors, the peptide profiles were similar to those observed for normal sub-

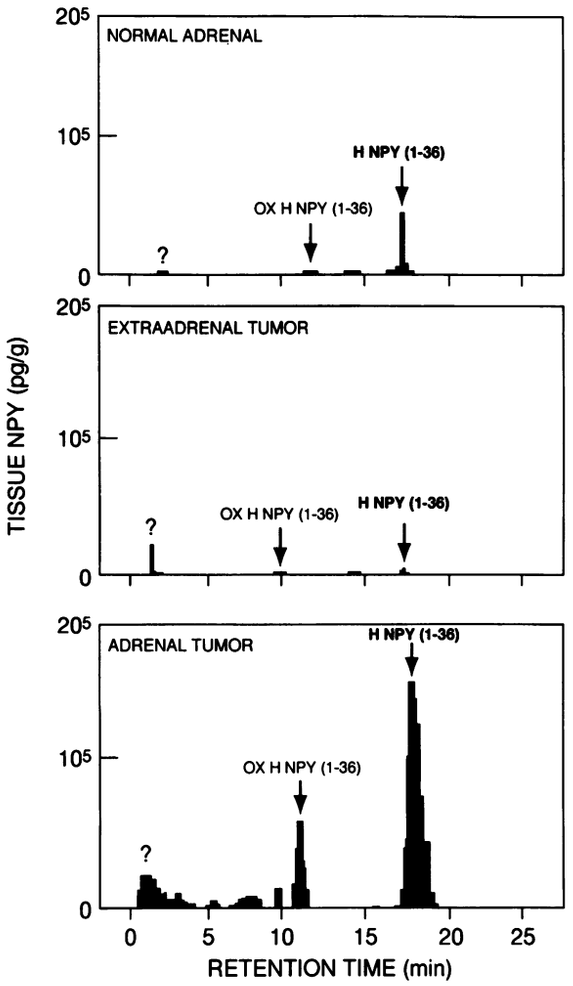

Figure 4. High performance, reverse phase chromatographic separation of NPY and its immunoreactive fragments in tissues from a normal adrenal (*A*), extraadrenal pheochromocytoma tumor (*B*), and an adrenal pheochromocytoma tumor (*C*). The levels of NPY were 1,000-fold higher in the tissues when compared to its corresponding plasma levels. *OX H NPY(1–36)* oxidized NPY.

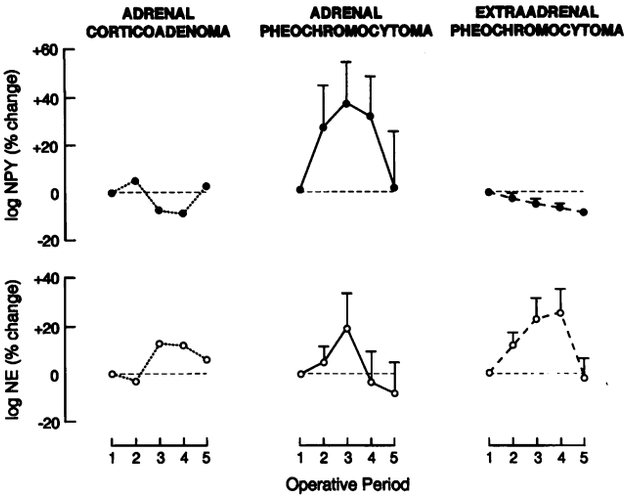

Figure 5. Plasma NPY and NE levels during surgery in patients with an adrenal (n = 5) or extraadrenal pheochromocytoma (n = 3) or an adrenal corticoadenoma (n = 1). Blood for the plasma levels was sampled during the following operative periods: (*1*) preincision (baseline), (*2*) pretumor manipulation, (*3*) during tumor manipulation, (*4*) after resection, and (*5*) after closing the surgical incision. Levels are the means±SEM.

jects; NPY(1–36) was present but was not the predominant species (Fig. 3 *B*). By contrast, NPY(1–36) was the predominant molecular weight form in plasma from patients with adrenal tumors (Fig. 3 *C*). Similar peptide profiles were observed for patients with malignant and benign tumors.

Fractionation of tumor-tissue extracts revealed that the mature peptide, NPY(1–36), was the predominant form (> 70%) in all adrenal tumors. In the extraadrenal tumors, however, the contribution of NPY(1–36) to the total immunoreactivity was more variable and ranged from 30 to 70%. In both types of tumor, at least three other less hydrophobic species of NPY were present in the tumor extracts, one of which was the oxidized form of NPY(1–36). Examples of the tumor IR-NPY peptide profiles observed after HPLC fractionation in a normal adrenal and in patients with adrenal or extraadrenal pheochromocytomas are shown in Figure 4, *A–C*.

A high correlation was observed for the peptide levels determined using AB I and AB II (total IR-NPY, r = 0.97, P = 0.0001; NPY(1–36), r = 0.91, P = 0.001; oxidized NPY(1–36), r = 0.8, P = 0.013), confirming the data obtained with AB I.

Release of NPY and catecholamines from pheochromocytomas. During surgery and with manipulation of the tumors, NE, and IR-NPY levels increased ($P < 0.05$) concordantly from preincision mean values of 7,420 and 319 pg/ml, to maximum values of 26,000 and 1,130 pg/ml, respectively, in the five patients with adrenal tumors (Fig. 5). By contrast, in the three patients with extraadrenal tumors, IR-NPY levels decreased during surgery, despite significant increases in NE from 4,410 to 44,400 pg/ml ($P < 0.01$). In a single patient with an adrenal cortical adenoma, the plasma NE and IR-NPY levels were similar before incision (310 and 37 pg/ml, respectively) and during tumor manipulation (638 and 28 pg/ml) (Fig. 5).

Effects of surgery. With surgical removal of the tumors, plasma NPY(1–36) levels (Fig. 6), as well as plasma IR-NPY and NE levels (data not shown) decreased toward the normal range in all but one patient with an adrenal tumor who had metastases to the lungs and bone. In keeping with these changes in plasma NPY levels, after surgery the peptide profiles reverted to those observed with fractionation of normal plasma. Moreover, in postoperative studies, the plasma IR-NPY (263 pg/ml), NPY(1–36) (10 pg/ml), and NE (245 pg/ml) levels have continued to be in the normal range, except in the patient with metastatic disease whose IR-NPY and NPY(1–36) levels remained elevated (6,390 and 4,900 pg/ml).

Expression of NPY mRNA in tumor tissue. Steady state NPY gene expression was evaluated by Northern blot analysis in nine patients with pheochromocytoma. In seven of eight adrenal tumors, the abundance of NPY mRNA was greater than in the one extraadrenal tumor (Fig. 7). Because of the low levels of NPY mRNA in the extraadrenal tumor, these studies were repeated in a further subset of 10 tumors using poly $(A)^+$ RNA. In this subset, NPY mRNA was abundant in four of five adrenal tumors, but in only one of five extraadrenal tumors (Fig. 7).

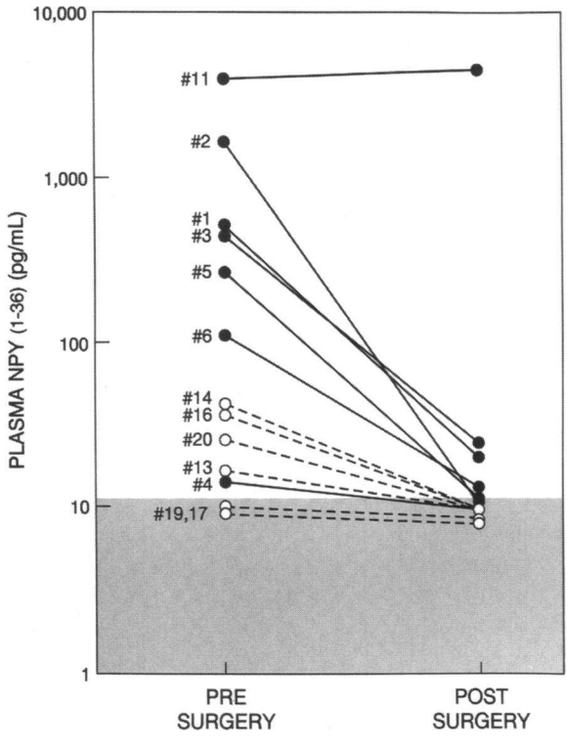

Figure 6. Plasma NPY(1–36) levels before (*PRESURGERY*) and after (*POSTSURGERY*) removal of a pheochromocytoma. NPY(1–36) levels decreased into the normal range 3–4 d after tumor removal in patients with adrenal (● —— ●; $n = 7$) and extraadrenal (○ – – – ○; $n = 6$) tumors. In patient #11 with metastatic disease, NPY(1–36) remained elevated after the removal of the primary tumor.

Taken together, the difference in the abundance of NPY mRNA in 13 adrenal and 6 extraadrenal tumors is significant ($\chi^2 = 7.6$, $P < 0.01$). Moreover, in the single normal adrenal gland evaluated, the NPY mRNA level was low and similar to those observed in the extraadrenal tumors (Fig. 7).

Discussion

In these studies we demonstrate that adrenal pheochromocytomas synthesize and release higher amounts of NPY than extraadrenal pheochromocytomas. The amounts of NPY mRNA are higher in adrenal tumors than those in extraadrenal tumors and normal adrenal glands. These findings indicate that NPY gene expression is increased in adrenal pheochromocytomas and accounts for the high plasma IR-NPY in these patients.

Several findings confirm the adrenal origin of the high plasma NPY levels in patients with adrenal pheochromocytomas. First, plasma NPY levels, and in particular plasma NPY(1–36) levels, are significantly higher in patients with adrenal pheochromocytomas than in those with extraadrenal tumors (Fig. 2). Second, tumor tissue NPY levels, both total IR-NPY and NPY(1–36), are significantly higher in adrenal tumors. Moreover, these higher tissue NPY levels are associated with increased steady state levels of NPY mRNA. Third, NPY is coordinately released with NE in patients with adrenal tumors but not in those with extraadrenal pheochromocytomas. Because plasma NPY levels decreased after removal of the adrenal tumors, and the plasma NPY peptide profile reverted to that ob-

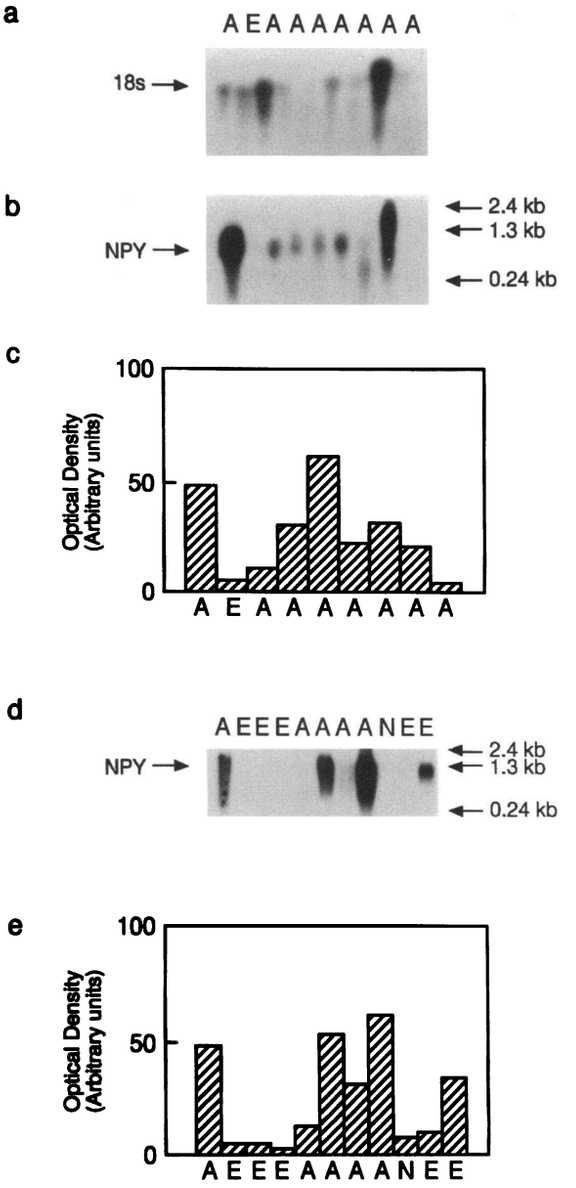

Figure 7. Northern blot analysis of total cellular RNA (*a–c*) prepared from pheochromocytoma tumors. Each lane contained 30 μg of RNA and was hybridized sequentially with probes for 18S rRNA (*a*) and NPY (*b*). The autoradiograms (*a* and *b*) were scanned using a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA), and the results of NPY hybridization relative to 18S are shown by the bar graphs (*c*). Northern blot analysis of polyA$^+$ RNA prepared from pheochromocytoma tissue and from a normal adrenal. Each lane contained ~ 2.8 μg of mRNA and was hybridized with a probe for NPY. Bar graph (*e*) illustrates the results of PhosphorImager analysis of polyA$^+$ RNA detected in (*d*) normalized to the amount of mRNA loaded. *A*, adrenal pheochromocytoma; *E*, extraadrenal pheochromocytoma; *N*, normal adrenal. The migration positions of molecular weight standards are indicated on the right (*b* and *d*).

served in normals, it is likely that the higher NPY mRNA levels in adrenal tumors are caused by increased NPY gene expression and that this increase leads to increased NPY biosynthesis, storage, and release from adrenal, but not from extraadrenal, pheochromocytomas. The studies of Higuchi et al. (26) have also

suggested that high levels of expression could be responsible for marked overproduction of NPY by adrenal pheochromocytomas.

Why should NPY biosynthesis be increased only in adrenal pheochromocytomas? Unfortunately, the factors regulating NPY gene expression are not well understood, and most of our insights in this regard come from studies of the rat pheochromocytoma cell line (PC12 cells) (27–30). For example, a variety of agents, including forskolin, phorbol esters, dexamethasone, and nerve growth factor, influence the steady state levels of NPY mRNA in PC 12 cells. These findings suggest that NPY gene expression in these cells is regulated by the synergistic actions of glucocorticoids, cAMP elevation, and protein kinase C activation. Unlike human pheochromocytoma cells, however, PC12 cells do not synthesize epinephrine (28). Also, nerve growth factor is an absolute requirement for neurite growth from PC12 cells but not from human pheochromocytoma cells, and it increases PC12 cells NPY mRNA levels but does not alter cellular NPY concentrations in human adrenal pheochromocytoma cells (29). Finally, dexamethasone treatment decreases the content of NPY in human pheochromocytoma cells but not in PC12 cells (29). These observations indicate varying degrees of specialization of human and rat pheochromocytoma cells and suggest that studies of the molecular and cellular mechanisms of NPY biosynthesis, storage, and release in pheochromocytomas must be confined to studies of human cells. Unfortunately, continuously growing human pheochromocytoma cell lines are not currently available.

It is tempting to speculate that the juxtaposition of the adrenal cortex to adrenal tumors, but not to extraadrenal tumors, is in some way responsible for the enhanced NPY biosynthesis observed in adrenal tumors. However, because dexamethasone decreases NPY content in human pheochromocytoma cells (29), it is likely that glucocorticoids are not the trophic factor responsible for increased NPY biosynthesis. Thus, alternative trophic factors may be epinephrine or ascorbic acid. Epinephrine can be synthesized and secreted by adrenal but not by extraadrenal tumors, and increasing intracellular cAMP levels may stimulate both the synthesis and secretion of NPY. Ascorbic acid, on the other hand, is abundant in the adrenal medulla and is a cofactor for both dopamine-β-hydroxylase and peptidylglycine-hydroxylating monooxygenase which are key enzymes in catecholamine and NPY biosynthesis, respectively.

Recently, Grouzmann and co-workers (31) demonstrated that type I angiotensin II receptors are transcribed and translated in functional proteins in a human adrenal pheochromocytoma. In a perifused system of primary cell cultures angiotensin II increased intracellular calcium and induced a dose-dependent secretion of NE and NPY. These results were interpreted to mean that NPY secretion in pheochromocytoma is regulated by angiotensin II. This observation requires further assessment in a larger number of tumors.

The lack of NPY mRNA has been suggested to be a marker of malignancy. Only three malignant tumors were available for the study of NPY mRNA in our patient population, limiting our evaluation of NPY as a marker of malignant disease, as suggested by Helman et al. (19). These malignant tumors (one adrenal and two extraadrenal) were analyzed for peptide content. The adrenal tumor had at least 15 times the concentration of IR-NPY observed in the extraadrenal tumors. The only extraadrenal tumor with appreciable NPY message levels was in the proximity of the urinary bladder. This tumor contained 15 ng/g of IR-NPY, and the mature peptide [NPY(1–36)] accounted for 30% of the total immunoreactivity. The lack of concordance between the message and peptide levels in this tumor may be the result of the tumor arising in nonadrenergic NPY-containing fibers emanating from the neurons in the pelvic ganglia, similar to those observed in the rat (32, 33).

Situations in which NPY mRNA is not translated to yield NPY have been reported for rat heart and spleen (34, 35). NPY in the heart is considered to occur predominantly in the sympathetic nerve terminals. Cardiac sympathetic ablation has no effect on the concentrations of mRNA, despite the loss of all measurable immunoreactivity. One extraadrenal tumor had peptide levels far in excess of the low message levels detected; this discordance between plasma NPY values and mRNA is unexplained.

The significantly elevated concentration of IR-NPY in all of the adrenal tumors must be a consequence of efficient in vivo posttranslational processing of the nascent protein. Efficient maturational processing of NPY propeptide has been observed in human pheochromocytoma and neuroblastoma tumors (18) and in insulin-producing tumors (36). Consistently high levels of the mature peptide in adrenal pheochromocytomas suggest that an increased biosynthesis of the peptide predominates in these tumors.

The finding that adrenal pheochromocytomas synthesize and store the mature, physiologically-active peptide [NPY(1–36)] has potentially important clinical implications. First, increased plasma NPY levels might distinguish adrenal from extraadrenal pheochromocytoma. However, the availability of accurate and sensitive noninvasive imaging techniques makes it less useful than currently proposed (37). Second, the peptide causes vasoconstriction, especially of small arterioles, both by itself and by potentiating the effect of catecholamines (9). Thus, NPY released from pheochromocytoma may be partly responsible for some of the cardiovascular symptoms of these patients. Indeed, Lundberg and co-workers (17) have reported that preoperative α-blockade with 200 mg of phenoxybenzamine daily did not prevent the hypertensive response induced by surgical manipulation of an adrenal pheochromocytoma when both plasma norepinephrine and NPY levels were markedly increased. The future availability of clinically applicable NPY receptor antagonists would clarify this issue and may prove to be therapeutically useful in patients with adrenal pheochromocytoma. Third, binding of NPY to its receptors results in the activation of intracellular secondary messenger systems (38). Since the tumors produce bioactive NPY, it is possible that autocrine stimulation of the cancer cells occurs. This autocrine-stimulatory mechanism may be related to the growth of these tumors and to their well known resistance to chemotherapeutic and radiotherapeutic modalities. Although receptors for NPY have not yet been described in human pheochromocytoma cells, Sheikh et al. (39, 40) have found receptors on human neuroblastoma cell lines for NPY. Clearly, these possibilities remain to be investigated.

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