High Expression of Neuropeptide Y Receptors in Tumors of the Human Adrenal Gland and Extra-Adrenal Paraganglia

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ABSTRACT

Purpose: **Recently, a role of neuropeptide Y (NPY) in tumor biology was suggested based on the high density of NPY receptors in breast and ovarian cancers. The high frequency of NPY receptors in steroid hormone-producing ovarian sex cord-stromal tumors, together with the known influence of NPY on steroid hormone and catecholamine secretion in the rodent adrenal gland, led to the investigation of NPY receptor expression in the human adrenal gland and related tumors.**

Experimental Design: **Fifteen adrenal cortical tumors, 20 paragangliomas, 23 pheochromocytomas, 20 neuroblastomas, and 8 normal adrenal glands were investigated by *in vitro* NPY receptor autoradiography using ^{125}I-labeled peptide YY in competition experiments with receptor subtype selective analogs.**

Results: **Ninety three percent of cortical tumors express Y1, 35% of pheochromocytomas and 61% of paragangliomas express Y1 and Y2, and 90% of neuroblastomas express Y2 receptors. The NPY receptors in pheochromocytomas, paragangliomas, and neuroblastomas are often expressed concomitantly with the NPY hormone detected immunohistochemically. The adrenal cortex strongly expresses Y1, whereas no NPY receptors are found in the adrenal medulla.**

Conclusions: **These receptor data suggest a role of NPY in adrenal cortical tumors and, together with the strong NPY innervation of the cortex, a physiologic role in the adrenal gland, mediated by Y1 receptors. These NPY receptors are a potential new molecular target for the therapy of malignant tumors.**

INTRODUCTION

Peptide hormones are small, very potent molecules with regulatory functions mainly in the brain, gut, and endocrine system. These peptides are important in biology, but their receptors have become increasingly relevant clinically because they are often overexpressed in malignant tumors (1). This feature allows receptor-targeted imaging and therapy of these tumors with radiolabeled peptide hormone analogs (2). For example, gastroenteropancreatic endocrine tumors express high amounts of somatostatin receptors that can be targeted with somatostatin receptor scintigraphy for diagnostic purposes (3); in some tumors, this method represents the tool of first choice (4). Moreover, recent results from studies performing targeted radiotherapy of these tumors with radiolabeled somatostatin analogs (5) are promising.

Another such peptide hormone is neuropeptide Y (NPY). It belongs to the NPY family, together with peptide YY (PYY) and pancreatic polypeptide (PP). These peptides bind to the G protein-coupled receptors of the NPY receptor family. Of the five receptor subtypes characterized to date, four are expressed in humans, namely, Y1, Y2, Y4, and Y5 (6). NPY is a neurotransmitter in the central and peripheral nervous system and displays a wide variety of regulatory functions, for example, in feeding behavior, hypertension, and reproduction (7, 8). It is also present in nerve fibers innervating the adrenal gland and in the chromaffin cells of the adrenal medulla (9, 10). Studies performed with the rodent adrenal gland indicate that NPY plays a role in the adrenal steroid hormone and catecholamine metabolism (11–14); little is known regarding the functional role of NPY in the human adrenal gland.

Recently, it was suggested that, analogous to somatostatin receptors, NPY receptors might be of potential use in tumor management, based on the high frequency and density of NPY receptors in breast cancer (15) and in granulosa and Sertoli-Leydig cell tumors of the ovary (16). Because of this high frequency of NPY receptors in steroid hormone-producing tumors and because of the possible role of NPY in adrenal function, the aim of the present study was to investigate NPY receptor expression using *in vitro* NPY receptor autoradiography in the human adrenal gland and in tumors related to the adrenal gland and extra-adrenal paraganglia, namely, adrenal cortical tumors, pheochromocytomas, paragangliomas, and neuroblastic tumors. These tumors can also produce steroid hormones or catecholamines (17), and the malignant forms of these tumors are often afflicted with a poor prognosis (18–21), which calls for new therapeutic options.

MATERIALS AND METHODS

Tissues. Fresh frozen tissue samples were obtained from surgical specimens. These included 15 adrenal cortical tumors (9 adenomas with Conn's syndrome, 1 adenoma with Cushing's syndrome, 2 incidental adenomas, and 3 carcinomas), 20 pheochromocytomas, 23 extra-adrenal paragangliomas from various sites [retroperitoneum (1 tumor), abdomen (1 tumor), mediastinum (1 tumor), carotid body (9 tumors), base of skull (1 tumor),

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unknown (8 tumors), and 2 metastases], 20 neuroblastic tumors, and 8 normal adrenal glands consisting mainly of cortex (5 also with medulla). The initial diagnoses were reviewed and confirmed using hematoxylin and eosin-stained sections as well as immunohistochemistry for inhibin (22). The neuroblastic tumors were graded according to World Health Organization guidelines (23). The tissue was stored at −80°C. The study conformed to the ethical guidelines of the Institute of Pathology, University of Bern and was reviewed by the institutional review board.

Neuropeptide Y Receptor Autoradiography. Twenty-micrometer–thick cryostat sections were mounted on precleaned slides and stored at −20°C for several days to improve adhesion of the tissue to the slides. NPY receptor autoradiography was carried out as described previously (16). The slides were preincubated in Krebs-Ringer solution [119 mmol/L NaCl, 3.2 mmol/L KCl, 1.19 mmol/L KH_2PO_4, 1.19 mmol/L $MgSO_4$, 25 mmol/L $NaHCO_3$, 2.53 mmol/L $CaCl_2$, and 10 mmol/L D-glucose (pH 7.4)] for 60 minutes at room temperature. Afterward, they were incubated for 120 minutes at room temperature in incubation solution containing Krebs-Ringer solution, 0.1% bovine serum albumin, 0.05% bacitracin, and 10,000 cpm/100 μL of the ^{125}I-labeled radioligand human PYY (hPYY; 2000 Ci/mmol; Anawa, Wangen, Switzerland). Nonspecific binding was evaluated by incubating tissue sections with incubation solution additionally containing 25 nmol/L nonlabeled hPYY, which, at this concentration, completely and specifically displaces the radiolabeled hPYY at the receptor. To distinguish the different receptor subtypes, further such competition experiments were performed with various subtype-selective analogs. For this purpose, serial tissue sections were incubated with ^{125}I-hPYY and increasing concentrations of one of the following nonlabeled ligands: the universal ligand hPYY (Bachem, Bubendorf, Switzerland), the Y1-selective ligands [Leu^{31}, Pro^{34}]–hPYY (Bachem) or BIBP 3226 (Boehringer, Mannheim, Germany), the Y2-selective ligands hPYY-(3–36) (Bachem) or BIIE 0246 (Boehringer), the Y4-preferring ligand human PP (hPP; Bachem), or the Y5-selective ligand [Ala^{31}, Aib^{32}]-hNPY (Dr A. Beck-Sickinger, Leipzig, Germany), respectively. After incubation, the slides were washed twice for 5 minutes and then rinsed four times in ice-cold Krebs-Ringer solution. The slides were dried under a stream of cold air at 4°C and then exposed to Kodak films Biomax MR for 7 days at 4°C. The resulting signals were analyzed, and the receptor-positive cases were semiquantitatively assessed with the help of tissue standards for iodinated compounds (Amersham, Aylesbury, United Kingdom) using a computer-assisted image processing system (Analysis Imaging System, Interfocus, Mering, Germany). In all experiments, rat brain sections were used for positive control because Y1 is highly expressed in the cerebral cortex, and Y2 is highly expressed in the hippocampus.

Immunohistochemistry for Neuropeptide Y. Immunohistochemistry for NPY was performed in 11 adrenal cortical adenomas and 2 carcinomas, 12 pheochromocytomas, 13 paragangliomas, and 12 neuroblastic tumors, including NPY receptor-positive and -negative cases. The immunohistochemistry was carried out as reported previously (16). Briefly, 10-μm–thick cryostat sections were postfixed in formalin. The primary antibody was a polyclonal rabbit antibody directed against NPY (1:2,000; Progen Biotechnic GmbH, Heidelberg, Germany). The secondary antibody was a biotinylated goat antirabbit immunoglobulin. Antibody binding was visualized using the ABComplex/horseradish peroxidase (DAKO, Carpinteria, CA, USA). Staining was carried out with 3,3′-diaminobenzidine, and counterstaining was carried out with hemalum. Adrenal gland tissue served as a positive internal and external control (24, 25).

Table 1 Frequency of NPY receptor expression in the tumors of the adrenal gland and extraadrenal paraganglia as compared with the normal adrenal gland

Tissue type	Frequency (%)
Adrenal cortical tumors	14/15 (93%)
Adenomas	12/12 (100%)
Carcinomas	2/3 (67%)
Pheochromocytomas	7/20 (35%)
Paragangliomas	14/23 (61%)
Neuroblastic tumors	18/20 (90%)
Adrenal cortex	8/8 (100%)
Adrenal medulla	0/5 (0%)

RESULTS

NPY receptors are expressed in all of the investigated tumor types as well as in the normal adrenal gland. Table 1 summarizes the receptor frequencies in the tested tumors. Expression of the NPY receptor is very frequent in cortical tumors and neuroblastic tumors and moderate in the pheochromocytomas and paragangliomas.

Neuropeptide Y Receptors in Adrenal Cortical Tumors. All cortical adenomas and two of the three investigated carcinomas express NPY receptors (Table 1). The mean receptor density is high (Table 2). There is little variation in the receptor density from case to case, with a tendency for lower values in carcinomas than in adenomas. Y1 is the only receptor subtype detected (Table 2). This is illustrated in the left column of Fig. 1 with an adenoma with Conn's syndrome. Evidence of Y1 expression is seen in the complete displacement of the universal radioligand by the Y1-selective analog, but not by the Y2-selective analog. The tumor in Fig. 1 is also an example of the often heterogeneous distribution of receptors within tumor tissue (Table 2).

Neuropeptide Y Receptors in Pheochromocytomas and Paragangliomas. The NPY receptor frequencies in the pheochromocytomas and paragangliomas are 35% and 61%, respectively. As listed in Table 3, two receptor subtypes are expressed, Y1 and Y2, with predominance of the latter. A representative example is depicted in the middle column of Fig. 1. In contrast to the Y1-expressing cortical adenoma on the left, the universal radioligand is completely displaced by the Y2-selective analog but only marginally displaced by the Y1-selective analog, indicating that Y2 is the predominant subtype in this case. In most tumors, only one subtype is present, but single cases can express Y1 and Y2 simultaneously (Table 3). The mean receptor density of Y2 is higher in the paragangliomas than in the pheochromocytomas, whereas the density of Y1 is comparably low in both tumor categories. Unlike that in cortical tumors, the receptor density varies considerably from case to case, with up to 10× difference. The receptor distribution is often heterogeneous.

Table 2 Density and subtype expression of NPY receptors in adrenal cortical tumors

Tumor	Receptor density (dpm/mg) Y1	Y2
Adenoma, Conn's syndrome		
Case 1	3,265 *	0
Case 2	2,985	0
Case 3	2,156	0
Case 4	2,122	0
Case 5	1,909	0
Case 6	1,662	0
Case 7	1,613 *	0
Case 8	1,436 *	0
Case 9	945	0
Adenoma, Cushing's syndrome		
Case 10	3,492	0
Adenoma, nonfunctioning		
Case 11	3,428 *	0
Case 12	2,564 *	0
Carcinomas		
Case 13	1,248 *	0
Case 14	376 *	0
Mean density ± SE (NPY receptor-positive cases)	2,086 ± 257	0

* Heterogeneous receptor distribution.

Neuropeptide Y Receptors in Neuroblastic Tumors. The 20 investigated neuroblastic tumors comprise the entire spectrum of histologic differentiation. NPY receptors are present in 18 cases (Table 4). Y2 is the only subtype detected. The receptor density is found to be very high in selected cases; the mean value of all positive cases amounts to 913 dpm/mg. In two thirds of the cases, the receptors are homogeneously distributed in the entire tumor sample. A relationship between receptor distribution and histologic differentiation cannot be discerned. Differentiated and undifferentiated as well as stroma-rich areas can all express the receptors. Fig. 1 shows an example of a poorly differentiated neuroblastoma on the right. The NPY receptors are expressed homogeneously in the entire tumor sample. Competition experiments with receptor subtype-selective analogs provide evidence that these receptors correspond mainly to Y2.

Neuropeptide Y Receptors in the Nonneoplastic Adrenal Gland. In the normal adrenal gland, NPY receptors are consistently present in the cortex, but not in the medulla (Table 5). Table 5 shows that the individual receptor density values are variable. Only the Y1 subtype can be detected, as illustrated with the example in Fig. 2. Note that the receptors are expressed in all three histologic zones of the cortex, the zona glomerulosa, zona fasciculata, and zona reticularis. Cortical vessels also express Y1 receptors at high density.

Neuropeptide Y Receptors in Intratumoral Blood Vessels. Y1 receptors are also frequently seen in the wall of tumoral blood vessels in cortical tumors, pheochromocytomas, paragangliomas, and neuroblastic tumors. When present, the receptors are homogeneously expressed in all larger vessels of the tumor samples. In most instances, the receptor density is moderate to high.

Pharmacological Characterization of Neuropeptide Y Receptor Subtypes. To differentiate the receptor subtypes in the individual cases, the rank orders of potencies of the various subtype-selective analogs were assessed in competition experiments using increasing concentrations of these analogs. Fig. 3 shows two representative examples of such competition experiments (a Y1-expressing adrenal cortical adenoma associated with Conn's syndrome and a Y2-expressing paraganglioma). In the former, the Y1-selective analogs [Leu^{31}, Pro^{34}]–hPYY and BIPB 3226 displace the universal ligand ^{125}I-hPYY with moderate to high affinity, whereas the Y2-selective analogs hPYY (3–36) and BIIE 0246 displace it with low affinity. In contrast, in the Y2-expressing paraganglioma, there is a high affinity displacement with the Y2-selective analogs hPYY (3–36) and BIIE 0246, but low affinity or no displacement with the Y1-selective analogs [Leu^{31}, Pro^{34}]–hPYY and BIPB 3226, respectively. The synthetic nonpeptide analogs BIBP 3226 and BIIE 0246 differ from the peptide analogs [Leu^{31}, Pro^{34}]–hPYY and hPYY (3–36), respectively, by their slightly lower subtype-selective affinity but higher specificity, which corresponds well to previous reports (26, 27). The low affinity of the Y4-preferring hPP in both examples rules out the presence of significant amounts of Y4 receptors. Moreover, the Y5-selective analog [Ala^{31}, Aib^{32}]–hNPY (28) is inactive in both cases, excluding the presence of Y5. Similar rank orders of potencies are found in a Y1-expressing breast carcinoma as well as in Y1-expressing rat cerebral cortex and Y2-expressing rat hippocampus (16).

Endogeneous Neuropeptide Y Expression in Pheochromocytomas, Paragangliomas, and Neuroblastic Tumors. NPY peptide is demonstrated by immunohistochemistry in the cytoplasm of tumor cells of all investigated pheochromocytomas (Fig. 4*A*) and neuroblastic tumors (Fig. 4*B*), as well as in 5 of the 13 (69%) paragangliomas (Fig. 4*C*). The staining is weak to moderate and mostly diffuse. Simultaneous NPY receptor and NPY peptide expression is present in 10 neuroblastic tumors, 5 pheochromocytomas, and 7 paragangliomas. In the cortical tumors, no NPY can be demonstrated. Adrenal medullary chromaffin cells (Fig. 4*D*) and nerve fibers in the adrenal cortex (Fig. 4*E* and *F*), as control tissues, also stain for NPY (24, 25).

DISCUSSION

The present study shows for the first time that NPY receptors are a biological cell marker of tumors derived from the human adrenal gland and extra-adrenal paraganglia. Adrenal cortical tumors express Y1 with high frequency, pheochromocytomas and paragangliomas express Y1 and Y2 with moderate frequency, and neuroblastic tumors express Y2 with high frequency. With regard to cortical tumors, there is good matching of the NPY receptor subtype Y1 in the tumors and in their tissue of origin, the adrenal cortex.

The strong expression of Y1 in all zones of the human adrenal cortex was unknown until now. Only PP binding sites, corresponding to Y4, were described in humans in the inner cortical zone *in vitro* (11) and in the rat in the zona glomerulosa and zona fasciculata (29). Moreover, in the rat, mRNA for Y1 was seen in the capsular (13) and inner zone tissue (9). In the present study, Y1 is clearly the predominant subtype, although very small amounts of Y4 cannot be com-

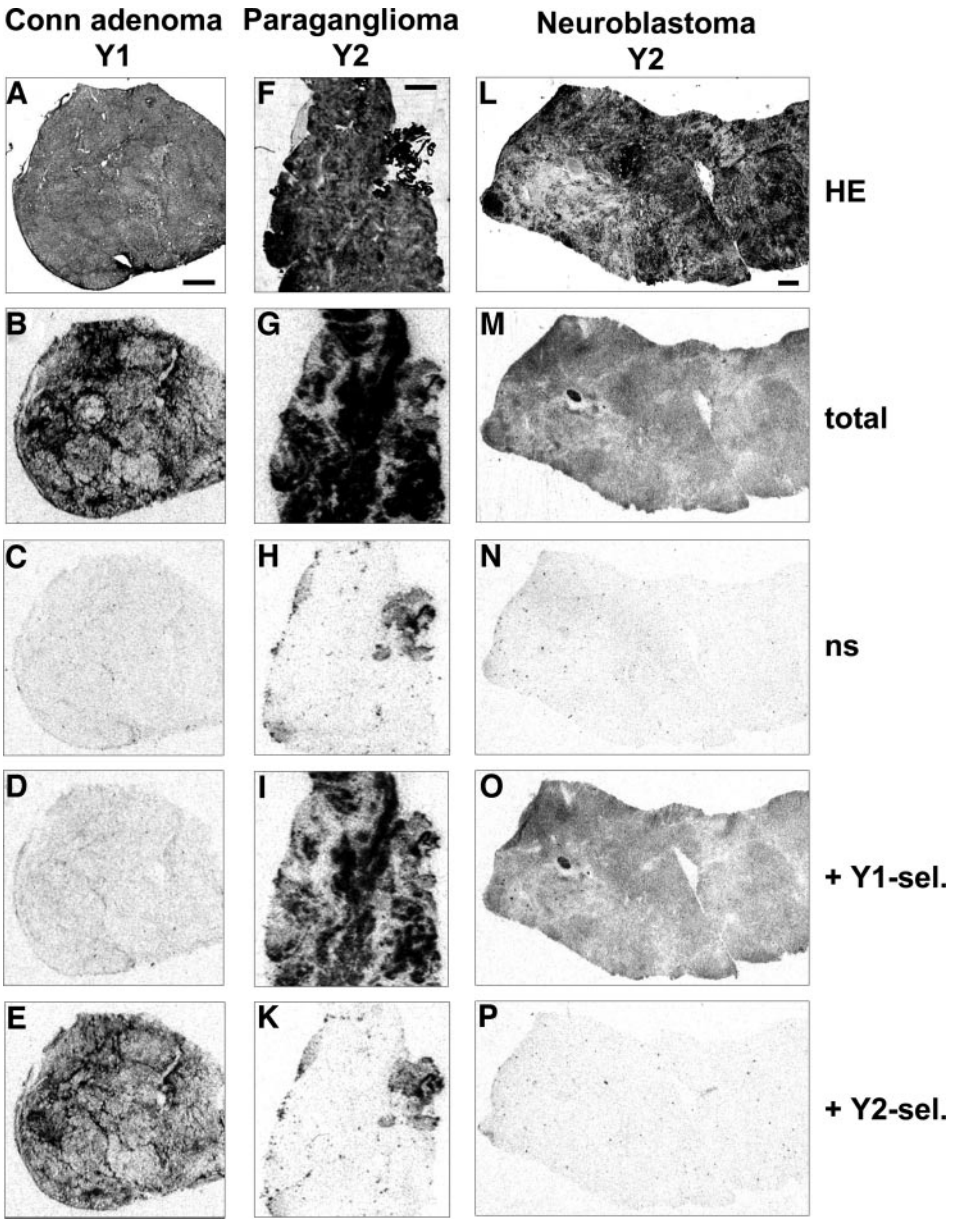

Fig. 1 NPY receptor subtypes in a cortical adenoma with Conn's syndrome (*A*−*E*), a paraganglioma (*F*−*K*), and a poorly differentiated neuroblastoma (*L*−*P*). *A*, *F*, and *L*, hematoxylin and eosin-stained sections. The adenoma and paraganglioma are solid tumors without a discernable stroma (*A* and *F*). The neuroblastoma (*L*) consists mainly of small blue cells and shows a stroma-rich area at the lower left margin, as well as a nerve. *Bars* = 1 mm. *B*, *G*, and *M*, autoradiograms showing total binding of the universal ligand ^{125}I-hPYY. *B*, heterogeneous labeling in the adenoma; *G*, very strong labeling in most parts of the paraganglioma. Homogeneous labeling of the entire neuroblastoma (*M*) and a strong signal in the nerve. *C*, *H*, and *N*, autoradiograms showing nonspecific binding (*ns*) in the presence of ^{125}I-hPYY and 25 nmol/L cold hPYY. Cold hPYY completely displaces ^{125}I-hPYY in all three tumors, indicating specific binding. Nonspecific binding is only visible in the paraganglioma at the middle right margin (*H*). *D*, *I*, and *O*, autoradiograms showing ^{125}I-hPYY binding in the presence of 25 nmol/L of the Y1-selective analog [Leu^{31}, Pro^{34}]–hPYY. There is a complete displacement of the radiolabeled universal ligand in the adenoma (*D*), whereas there is only marginal displacement in the paraganglioma (*I*) and the neuroblastoma (*O*). *E*, *K*, and *P*, autoradiograms showing ^{125}I-hPYY binding in the presence of 25 nmol/L of the Y2-selective analog hPYY (3–36). hPYY (3–36) completely displaces the universal ligand in the paraganglioma (*K*) and neuroblastoma (*P*), but only marginally in the adenoma (*E*). Therefore, the adenoma expresses mainly Y1 receptors, and the paraganglioma and neuroblastoma express predominantly Y2 receptors.

pletely excluded with the present method using displacement experiments with subtype-selective analogs. No NPY receptor expression is observed in surgically resected adrenal medulla tissue, whereas in human chromaffin cell cultures, evidence for a functional role of NPY analogs was reported (30). In the rat medulla, PP binding sites (29) and Y1 receptor mRNA (9) were observed, suggesting possible species differences in NPY receptor distribution.

Table 3 Density and subtype expression of NPY receptors in pheochromocytomas and paragangliomas

Tumor	Receptor density (dpm/mg) Y1	Y2
Pheochromocytomas		
Case 15	546	0
Case 16	118	0
Case 17	650 *	2,002 *
Case 18	0	1,172 *
Case 19	0	421 *
Case 20	0	362 *
Case 21	0	213
Mean density ± SE (NPY receptor-positive cases)	438 ± 163	834 ± 336
Paragangliomas		
Case 22, base of skull	991 *	0
Case 23, carotid body	912 *	0
Case 24, mediastinum	865 *	0
Case 25, carotid body	474	0
Case 26	330 *	1,933 *
Case 27	0	9,044
Case 28, carotid body	0	5,663
Case 29, carotid body	0	2,450 *
Case 30	0	1,910 *
Case 31	0	1,368 *
Case 32, metastasis	0	977
Case 33	0	734 *
Case 34, carotid body	0	571 *
Case 35, carotid body	0	503
Mean density ± SE (NPY receptor-positive cases)	714 ± 131	2,747 ± 795

* Heterogeneous receptor distribution.

The displacement experiments in the present study provide strong evidence that the subtypes expressed in the investigated tumors and adrenal glands correspond mainly to Y1 and Y2. Pharmacological evidence for the presence of Y1 receptors consists of a high-affinity displacement of ^{125}I-hPYY by the Y1-selective analogs [Leu^{31}, Pro^{34}]–hPYY and BIBP 3226 (26) and a low-affinity displacement by the Y2-selective analogs hPYY (3–36) and BIIE 0246 (27), respectively. Significant levels of Y4 or Y5 are unlikely to be expressed, based on the low-affinity displacement by the Y4-preferring hPP (6) and the lack of affinity of the Y5-selective analog [Ala^{31}, Aib^{32}]–hNPY (28), respectively. Conversely, pharmacological evidence of Y2 receptor expression is provided by the following rank order of potencies: ^{125}I-hPYY $\geq$ hPYY (3–36) $>$ BIIE 0246 $\gg$ [Leu^{31}, Pro^{34}]–hPYY $\geq$ hPP $>$ BIBP 3226 $=$ [Ala^{31}, Aib^{32}]–hNPY. Similar rank orders of potencies in the control tissues further confirm the results.

The presence of Y1 receptors in the adrenal cortex in such high density strongly suggests a relevant role of NPY at the parenchymal cell level on adrenal cortical function. Unfortunately, the physiologic role of NPY in the adrenal cortex has scarcely been studied. *In vitro*, NPY appears to induce glucocorticoid production in human cell preparations (11) and mainly induce aldosterone release in the rat (12–14). Taken together, the secretion data and the NPY receptor data in the adrenal cortex may suggest that NPY regulates steroid hormone synthesis and/or release in humans at all anatomic levels. Furthermore, NPY may also influence adrenal function via regulation of the adrenal blood supply (24), mediated through the NPY receptors expressed in cortical vessels. A relationship between NPY receptors and steroid hormone metabolism may not be restricted to the nonneoplastic adrenal gland but may also exist in cortical tumors that express Y1 and often produce high amounts of steroid hormones (17). In analogy, high NPY receptor expression is also present in the sex steroid hormone-producing granulosa and Sertoli-Leydig cell tumors of the ovary (16).

NPY receptors may also have functional effects in adrenal medullary and related tumors. Because NPY receptor agonists can trigger catecholamine release in human chromaffin cell cultures (30), it is conceivable that the identified tumoral NPY receptors may mediate NPY action on catecholamine secretion from pheochromocytomas, paragangliomas, and neuroblastic tumors. If a relation between NPY receptors and hormone secretory activity in these tumors exists, NPY receptor analogs might be of use in controlling hormonal symptoms in these patients.

NPY peptide was detected by immunohistochemistry in tumors originating from the adrenal medulla and extra-adrenal

Table 4 Density and subtype expression of NPY receptors in neuroblastic tumors

Tumor	Receptor density (dpm/mg) Y1	Y2
Case 36, neuroblastoma	0	5,043
Case 37, neuroblastoma	0	1,149
Case 38, neuroblastoma	0	938
Case 39, neuroblastoma	0	842 *
Case 40, neuroblastoma	0	722 *
Case 41, neuroblastoma	0	701
Case 42, neuroblastoma	0	694
Case 43, neuroblastoma	0	523 *
Case 44, neuroblastoma	0	504
Case 45, neuroblastoma	0	409
Case 46, neuroblastoma	0	366
Case 47, neuroblastoma	0	334 *
Case 48, neuroblastoma	0	91
Case 49, ganglioneuroblastoma	0	1,402
Case 50, ganglioneuroblastoma	0	1,079 *
Case 51, ganglioneuroma	0	1,120
Case 52, ganglioneuroma	0	298 *
Case 53, ganglioneuroma	0	212
Mean density ± SE (NPY receptor-positive cases)	0	913 ± 258

* Heterogeneous receptor distribution.

Table 5 Density and subtype expression of NPY receptors in the normal adrenal cortex

Case no.	Receptor density (dpm/mg) Y1	Y2
54	6,349	0
55	4,392	0
56	3,914	0
57	2,979	0
58	2,893	0
59	1,318	0
60	663	0
61	480	0
Mean density ± SE	2,874 ± 713	0

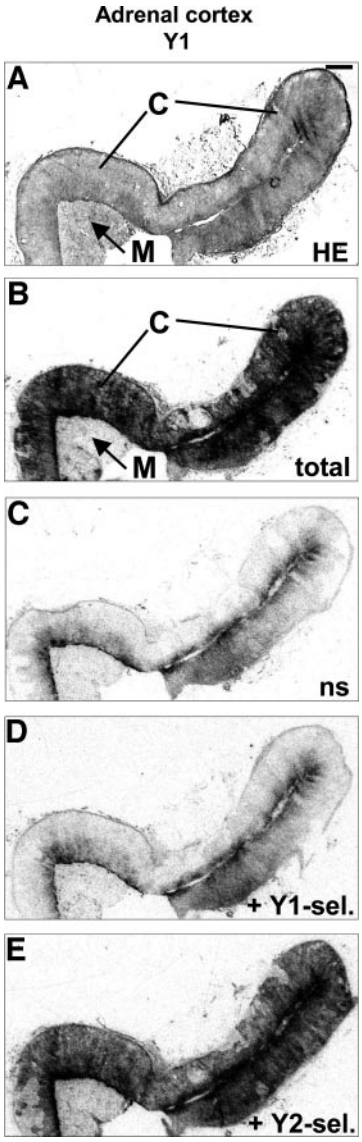

Fig. 2 NPY receptors in the normal adrenal gland (*A*−*E*). *A*, hematoxylin and eosin-stained section showing cortex (*C*) and medulla (*M*). *Bar* = 1 mm. *B*, autoradiogram showing total binding of the universal ligand ^{125}I-hPYY. There is a diffuse and strong labeling of all layers of the cortex but not the medulla. *C*, autoradiogram showing ^{125}I-hPYY binding in the presence of 25 nmol/L cold hPYY. There is a complete displacement in the cortex, indicative of specific binding. Only a small area of nonspecific binding is seen at the cortico-medullary junction. *D*, autoradiogram showing ^{125}I-hPYY binding in the presence of 25 nmol/L of the Y1-selective analog [Leu^{31}, Pro^{34}]–hPYY. There is complete displacement of ^{125}I-hPYY. *E*, autoradiogram showing ^{125}I-hPYY binding in the presence of 25 nmol/L of the Y2-selective analog hPYY (3–36). Human PYY (3–36) does not displace ^{125}I-hPYY at the receptor. This indicates that the cortex preferentially expresses Y1 receptors.

paraganglia at a frequency corresponding with that found in the literature (25, 31–33). The concomitant presence of NPY hormone detected by immunohistochemistry and NPY receptors measured by receptor autoradiography provides a molecular basis for a possible autocrine feedback of NPY on these tumors. Of interest, in neuroblastomas, it has been shown that NPY is processed to the Y2-selective NPY (3–36) (34). We show here that Y2 is the predominant receptor subtype in these tumors, which may represent the basis for an autocrine effect of NPY (3–36). A possible consequence of such an autocrine feedback could be growth inhibition because NPY was shown to reduce growth of the neuroblastoma cell line SK-N-MC (15). *In vivo*, this may especially be the case for early-stage tumors because it was demonstrated that cleavage of the inactive pro-NPY to the active NPY occurs significantly less often in advanced-stage tumors and metastases (34). If such a mechanism of NPY exists, administration of NPY may prove to be useful in controlling tumor growth.

The clinical significance of the presence of NPY receptors does not lie only in a putative long-term application of cold

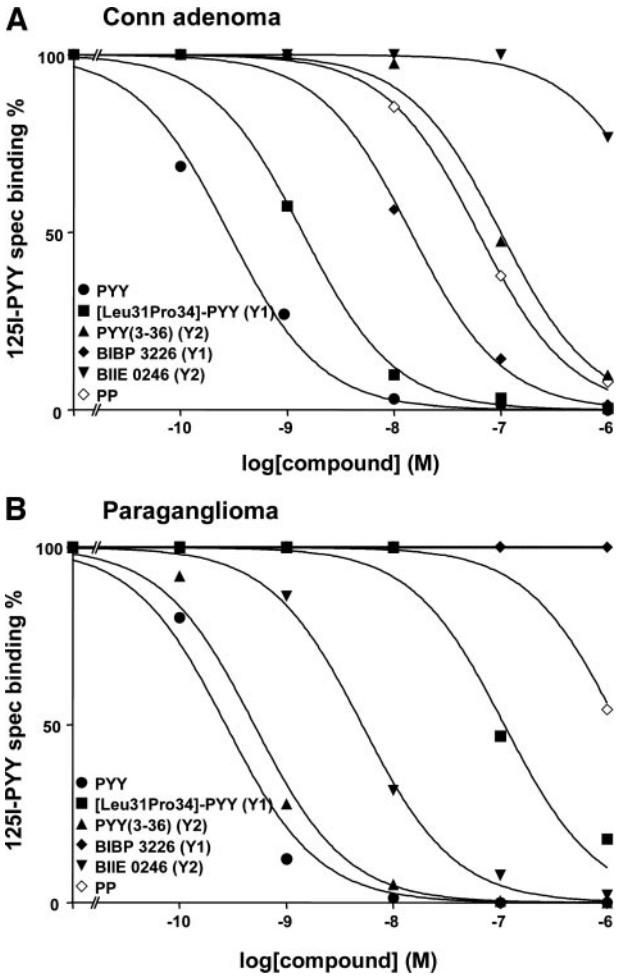

Fig. 3 Competition experiments in a Y1-expressing adrenal cortical adenoma with Conn's syndrome (*A*) and a Y2-expressing paraganglioma (*B*). *A*, high-affinity displacement of ^{125}I-hPYY by hPYY, [Leu^{31}, Pro^{34}]–hPYY, and BIBP 3226 is shown; low-affinity displacement by hPYY-(3–36) and BIIE 0246 is characteristic of Y1 receptors. Human PP has a very low affinity. *B*, high-affinity displacement of ^{125}I-hPYY by hPYY, hPYY (3–36), and BIIE 0246 is shown; low-affinity displacement by [Leu^{31}, Pro^{34}]–hPYY and BIBP 3226 is characteristic of Y2 receptors. Human PP is inactive.

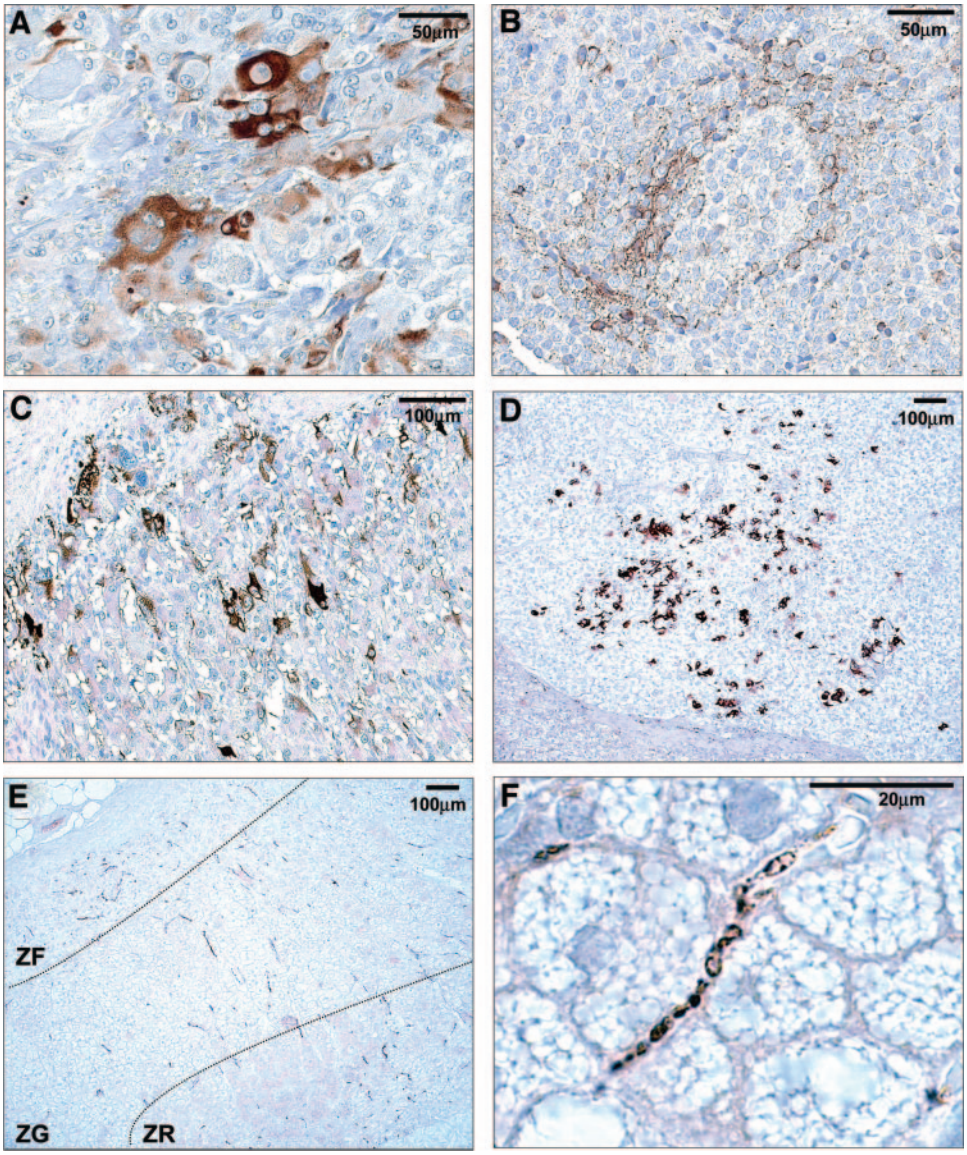

Fig. 4 Immunohistochemistry for NPY performed on formalin-fixed paraffin-embedded sections of a pheochromocytoma (*A*), a neuroblastoma (*B*), a paraganglioma (*C*), and a nonneoplastic adrenal gland (*D*–*F*). Clusters of cells of the pheochromocytoma (*A*) display a strong cytoplasmic staining, which is also observed in the paraganglioma (*B*) and, in lower intensity, in the neuroblastoma (*C*). Similarly, in the adrenal medulla (*D*), the chromaffin cells show a strong cytoplasmic reactivity. Adrenal cortical cells do not stain for NPY, but the cortex (*E*) is traversed from the capsule to the medulla by many NPY reactive nerve fibers (*ZG*, zona glomerulosa; *ZF*, zona fasciculata; *ZR*, zona reticularis). Higher magnification (*F*) demonstrates the presence of NPY in neural varicosities adjcacent to blood vessels.

peptide analogs to control hormonal secretion, as mentioned above. More importantly, these receptors may represent potential targets for scintigraphy and targeted radiotherapy of malignant tumors (2). NPY receptors have been previously suggested to represent tumor targets based on their high expression in breast and ovarian cancer (15, 16, 35). The present data on adrenal and related tumors may further expand the indication range for NPY receptor targeting. In addition, intratumoral vessels expressing NPY receptors are also potential targets. Certainly, new and/or additional treatment modalities are desirable for adrenal and adrenal-related tumors because their therapy is an unsolved problem. Overall, adrenal cortical carcinomas are highly lethal, even after curative resection (36). Adjuvant treatment of malignant residual or metastatic pheochromocytomas and paragangliomas is afflicted with only partial response and relapses (18–20), and therapy of high-risk neuroblastomas is associated with a high rate of drug-resistant residual disease and severe long-term morbidity (21). A NPY analog coupled to an anthracycline, which may be suitable for receptor-targeted chemotherapy, has already been developed (37). Systemic side effects of the peptide itself in such therapies can be expected to be minimal because the therapeutic doses are usually very small (1). Moreover, no side effects originating from the central nervous system should be expected, although the majority of NPY receptors are located in the brain: indeed, most peptide hormones, including NPY analogs, do not cross the blood–brain barrier (1). Finally, because neuroblastomas express not only NPY receptors but also somatostatin receptors with high frequency (38), a combined NPY receptor and somatostatin receptor targeting could even be introduced.

In conclusion, NPY receptors are highly expressed in adrenal cortical tumors, pheochromocytomas, paragangliomas, and neuroblastic tumors, as well as in all layers of the adrenal cortex. Promising preliminary data using functional autoradiography (39)

suggest that these receptors may be functional, as shown by [Leu^{31}, Pro^{34}]–NPY-induced [^{35}S]GTPγS binding in Y1-expressing adrenal cortical tumors and NPY (3–36)–induced [^{35}S]GTPγS binding in Y2-expressing neuroblastomas.[1] These receptors may therefore be functional and represent the molecular basis for a role of NPY on the adrenal cortex and adrenal cortical tumors, as well as a potential new target for the diagnosis and therapy of these tumors.

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