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Neuropeptide Y receptors in renal cell carcinomas and nephroblastomas

Meike Körner, Beatrice Waser and Jean Claude Reubi*

Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Bern, Bern, Switzerland

Numerous peptide receptors are overexpressed in human cancer, permitting *in vivo* tumor targeting. Among such receptors, those for the neurotransmitter neuropeptide Y (NPY) are overexpressed in various tumors. Since NPY can play a role in the kidney, NPY receptor expression and/or endogenous production of peptides of the NPY family (NPY, PYY, PP) were evaluated in 40 renal cell carcinomas (RCCs) and 18 nephroblastomas. NPY receptor protein expression was investigated by *in vitro* autoradiography using ^{125}I-labeled PYY in competition with NPY receptor subtype-selective analogs. NPY, PYY and PP production was assessed immunohistochemically. Fifty-six percent of RCCs expressed the Y1 receptor subtype in moderate density, and 80% of nephroblastomas expressed Y1 and Y2 subtypes in moderate to high density. Y1 was also highly expressed in intratumoral blood vessels. In selected cases, NPY was observed in nerve fibers in close association with intratumoral blood vessels and in the vicinity of tumor cells, while no PYY or PP was detected immunohistochemically in these sites. NPY receptors on renal tumor cells and tumor blood vessels may therefore be the molecular targets of endogenous NPY released by intratumoral nerve fibers. With regard to clinical applications, NPY receptors may act as *in vivo* targets for receptor-directed therapy of RCCs and nephroblastomas for which alternative therapeutic approaches are still required.

Key words: NPY receptor; renal cell carcinoma; nephroblastoma; receptor autoradiography

Peptide hormone receptors are overexpressed in a wide variety of human tumors.[1] Like other cell surface molecules, these receptors become increasingly important for clinical applications. In particular, they allow receptor-targeted tumor imaging and therapy with corresponding peptide hormone analogs. For instance, scintigraphy using the somatostatin analog Octreoscan is highly sensitive at detecting gastroenteropancreatic neuroendocrine tumors which express somatostatin receptors in high amounts.[2,3] Moreover, targeted radiotherapy of these tumors with ^{90}Y- or ^{177}Lu-labeled somatostatin analogs is also promising.[4,5]

Another peptide hormone receptor family with a potential future role in this field is the NPY receptor family. It belongs to the G protein–coupled receptor superfamily and comprises various subtypes. Subtypes Y1, Y2, Y4 and Y5 are expressed in humans.[6] They are present mainly in the central and peripheral nervous systems as well as other tissues, such as the cardiovascular system. Their physiologic ligands are the neurotransmitter NPY and the 2 hormones PYY and PP. Among many other sites, NPY has been localized to perivascular nerves in the human kidney.[7]

NPY receptors may also play a role in human neoplasia. High incidence rates of subtypes Y1 and Y2 were found in tumors related to steroid hormone metabolism (adrenal cortical tumors, ovarian sex cord-stromal tumors, breast carcinomas),[8,9] in neuroendocrine tumors (pheochromocytomas and paragangliomas)[10] and in embryonal tumors (neuroblastomas).[10] It is expected that radiolabeled or drug-coupled NPY analogs may be used for *in vivo* targeting of these tumors.[11,12]

Presently, renal cancer is the seventh most frequent malignancy in adults,[13] with rising incidence.[14] The prognosis of RCC is favorable if complete surgical excision is feasible, but poor response to adjuvant therapies in advanced disease results in significant overall tumor death rates.[15,16] In contrast, today most children suffering from nephroblastoma can be cured, but the current multimodal therapy strategies are afflicted with severe late side effects.[17–19] Therefore, novel treatment options for both RCC and nephroblastoma are the subject of ongoing research.[14,15,20] Our aim in the present study was to investigate NPY receptor expression in these 2 most common adult and childhood kidney cancers as well as in the nonneoplastic kidney using NPY receptor autoradiography.

Material and Methods

Tissues

For NPY receptor autoradiography, fresh frozen tumor tissue samples were obtained from surgical nephrectomy specimens. These included 24 RCCs (21 clear cell carcinomas; 2 papillary carcinomas, type 2; 1 chromophobe carcinoma, eosinophilic variant) and 10 nephroblastomas (6 triphasic, 4 blastemal and stromal). Nonneoplastic parenchyma of kidneys resected for neoplasia was assessed in 7 cases. Tissue was stored at –80°C.

For NPY, PYY and PP immunohistochemistry, formalin-fixed, paraffin-embedded material of another 16 RCCs (7 clear cell carcinomas; 4 papillary carcinomas, types 1 and 2; 5 chromophobe carcinomas) and 8 nephroblastomas (3 triphasic, 2 blastemal and stromal, 2 epithelial and stromal, 1 blastemal) was used.

Tumor typing was performed according to the WHO guidelines.[21] Grading of RCC was based on the 4-tiered Fuhrman system.[22] Clinical data on patient age, sex and presence of metastatic disease were reported earlier as these tumor samples were used previously for the investigation of somatostatin receptors.[23]

The study conformed to the ethical guidelines of the Institute of Pathology, University of Bern, and was reviewed by the institutional review board.

Receptor autoradiography

Cryostat sections (20 μm thick) were mounted on precleaned slides and stored at –20°C for several days to improve adhesion of the tissue to slides. NPY receptor autoradiography was carried out as described previously.[9] Slides were preincubated in Krebs-Ringer solution (NaCl 119 mM, KCl 3.2 mM, KH_2PO_4 1.19 mM, $MgSO_4$ 1.19 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2.53 mM, D-glucose 10 mM; pH 7.4) for 60 min at room temperature. Afterward, they were incubated for 120 min at room temperature in the incubation solution containing Krebs-Ringer solution, 0.1% BSA, 0.05% bacitracin and 10,000 cpm/100 μl of the ^{125}I-labeled radioligand hPYY (2,000 Ci/mmol; Anawa, Wangen, Switzerland). Nonspecific binding was evaluated by incubating tissue sections with the incubation solution containing additionally 25 nM of nonlabeled hPYY, which, at this concentration, displaces completely and specifically the radiolabeled hPYY at the receptor. To distinguish the

Abbreviations: DAB, 3,3′-diaminobenzidine; hPP, human pancreatic polypeptide; hPYY, human peptide YY; HRP, horseradish peroxidase; NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY; RCC, renal cell carcinoma.

***Correspondence to:** Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Bern, Murtenstrasse 31, P.O. Box 62, CH-3010 Bern, Switzerland. Fax: +41-31-632-89-99.
E-mail: reubi@pathology.unibe.ch

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TABLE I – CLINICOPATHOLOGIC AND RECEPTOR DATA OF 24 PATIENTS WITH RCC

Case	Age (years)	Sex	Carcinoma subtype	Grade	Metastases	Y1 receptor density in tumor cells (dpm/mg tissue)	Y1 in tumor vessels
1	73	F	Clear cell	I	No	306	+
2	55	M	Clear cell	I	No	0	+
3	50	F	Clear cell	I	Unknown	0	+
4			Clear cell	I	Unknown	0	+
5	66	F	Clear cell	II	No	1,202[1]	+
6	44	F	Clear cell	II	No	973[1]	+
7	53	M	Clear cell	II	No	957[1]	
8	66	F	Clear cell	II	No	890[1]	+
9	71	F	Clear cell	II	No	584[1]	+
10	47	M	Clear cell	II	No	506[1]	+
11	73	M	Clear cell	II	No	212[1]	+
12	66	M	Clear cell	II	Unknown	650[1]	+
13	29	F	Clear cell	II	Unknown	338[1]	+
14	65	F	Clear cell, sarcomatoid focus	II	Yes	609[1]	+
15	59	M	Clear cell	II	Yes	0	+
16	67	M	Clear cell	III	No	1,170[1]	+
17	81	M	Clear cell	III	No	654	+
18	75	F	Clear cell	III	No	0	+
19	51	F	Clear cell	III	Yes	475[1]	
20	53	F	Clear cell	III	Yes	0	
21	66	M	Clear cell	III	Yes	0	
22	59	M	Papillary	II	No	0	+
23	38	M	Papillary	III	Yes	0	
24	75	F	Chromophobe	IV	No	0	+
Mean receptor density ± SEM (NPY receptor-positive cases)						680 ± 84	

[1]Heterogeneous receptor distribution (value represents mean density in the entire tumor sample).

different NPY receptor subtypes, 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 and BIBP 3226 (Boehringer-Ingelheim, Biberach an der Riss, Germany), the Y2-selective ligands hPYY(3–36) and BIIE 0246 (Boehringer-Ingelheim), the Y4-preferring ligand hPP (Bachem) and the Y5-selective ligand [Ala^{31}, Aib^{32}]-hNPY (Dr. A. Beck-Sickinger, Leipzig, Germany). After incubation, slides were washed twice for 5 min and then rinsed 4 times in ice-cold Krebs-Ringer solution. Slides were dried under a stream of cold air at 4°C and then exposed to Kodak (Rochester, NY) film (Biomax MR) for 7 days at 4°C. The resulting signals were analyzed, and receptor-positive cases were semiquantitatively assessed using tissue standards for iodinated compounds (Amersham, Aylesbury, UK) and a computer-assisted image-processing system (Analysis Imaging System; Interfocus, Mering, Germany). In all experiments, rat brain sections were used as the positive control because Y1 is highly expressed in the cerebral cortex and Y2 in the hippocampus.[24]

Immunohistochemistry

Immunohistochemistry for NPY was performed on frozen tumor samples of 10 RCCs and all 10 nephroblastomas, which had also been investigated by receptor autoradiography. In addition, formalin-fixed, paraffin-embedded material of another 16 RCCs and 8 nephroblastomas was assessed for the presence of NPY and, in a subset of these cases, for the presence of PYY and PP. The procedure was carried out as reported previously.[10] Cryostat sections (10 μm thick) were postfixed in formalin. Paraffin-embedded tissue sections (4 μm thick) were pretreated with trypsin for NPY immunohistochemistry, for 5 min in a pressure cooker for PYY immunohistochemistry and in the microwave for PP immunohistochemistry. Primary antibodies were polyclonal rabbit antibodies directed against NPY (1:2,000, Progen Biotechnic, Heidelberg, Germany), PYY (1:600, Progen) and PP (1:500; Novocastra, Newcastle-upon-Tyne, UK). The secondary antibody was a biotinylated goat antirabbit immunoglobulin. Antibody binding was visualized using the ABComplex/HRP (Dako, Zug, Switzerland). Staining was carried out with DAB and counterstaining, with hemalum. Nerve fibers in the adjacent renal parenchyma served as the positive internal control[7] and adrenal medulla, as the positive external control for NPY immunohistochemistry.[25] Colonic mucosa was used as a positive control for PYY and PP immunohistochemistry.[26]

Results

NPY receptor expression in RCC

NPY receptors were expressed in 14 of 24 (56%) investigated RCCs. Table I summarizes the results classified by tumor type, tumor grade and presence of metastases. Receptors were observed in 67% of clear cell-type RCCs, including one with focal sarcomatoid differentiation, but in none of the 3 chromophobe and papillary RCCs. Mean receptor density was moderate, with considerable variation from case to case. Regarding tumor grade, nearly all grade 2 and half of the grade 3 carcinomas expressed receptors in moderate density. In comparison, the receptor incidence and density in grade 1 carcinomas was low. The study included 5 metastasized clear cell carcinomas. NPY receptors were present in 2 of them. Table I further shows that NPY receptors were expressed irrespective of patient age or sex.

Y1 was the only NPY receptor subtype expressed, as illustrated in the upper row of Figure 1. Pharmacologic evidence of Y1 expression consists of complete displacement of the universal ligand by the Y1-, but not by the Y2-, selective analog. The example in Figure 1 shows a predominantly homogeneous receptor distribution in the whole tumor sample. However, in most cases, the receptor distribution was heterogeneous within the tumor tissue, as indicated in Table I.

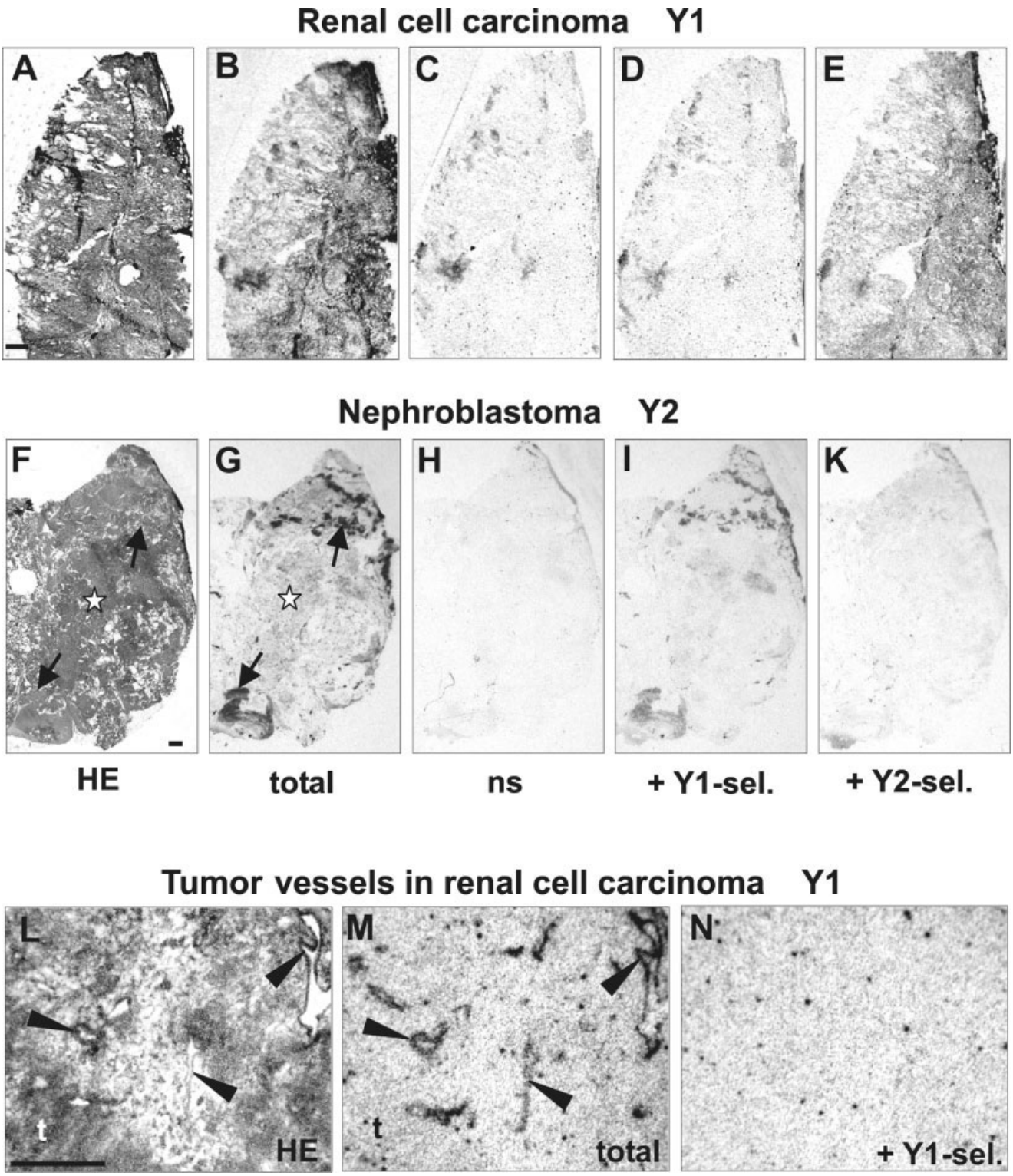

Figure 1 – NPY receptor expression and subtypes in RCC (*a–e*) and nephroblastoma (*f–k*). *(a,f)* Hematoxylin and eosin (HE)–stained sections show a clear cell RCC (*a*) and a nephroblastoma (*f*) consisting predominantly of blastema, (asterisk) with focal tubular differentiation (arrow). Scale bars = 1 mm. (*b,g*) Autoradiograms showing total binding of the universal ligand ^{125}I-hPYY. In the RCC (*b*), there is moderate diffuse labeling of the entire tumor tissue. In the nephroblastoma (*g*), there is a strong signal present focally (arrows) and weak diffuse labeling of the rest of the tumor tissue (asterisk). Higher magnification of the corresponding hematoxylin and eosin–stained section reveals that in areas of high receptor expression tubular differentiation is present, whereas the weak signal in the rest of the tissue corresponds to the blastemal component. (*c,h*) Autoradiograms showing ^{125}I-hPYY binding in the presence of 25 nM cold hPYY (nonspecific binding, ns). In both tumor samples, cold hPYY completely displaces ^{125}I-hPYY. (*d,i*) Autoradiograms showing ^{125}I-hPYY binding in the presence of 25 nM of the nonradiolabeled Y1-selective analog [Leu^{31}, Pro^{34}]-hPYY. In the RCC (*d*), there is complete displacement of ^{125}I-hPYY, whereas in the nephroblastoma (*i*), there is only marginal displacement. (*e,k*) Autoradiograms showing ^{125}I-hPYY binding in the presence of 25 nM of the nonradiolabeled Y2-selective analog hPYY(3–36). In the RCC (*e*), ^{125}I-hPYY is only partly displaced. Conversely, in the nephroblastoma (*k*), there is complete displacement. Therefore, the RCC expresses predominantly Y1 and the nephroblastoma expresses predominantly Y2. (*l–n*) NPY receptor expression in the tumor vessels of RCC. (*l*) Hematoxylin and eosin–stained section shows a clear cell RCC with numerous mid-sized blood vessels (arrowheads) in the fibrotic tumor center and an area of tumor cells (t) in the lower left corner. Scale bar = 1 mm. (*m*) Autoradiogram showing total binding of the universal ligand ^{125}I-hPYY. Vessel walls are labeled with predominantly strong intensity (arrowheads). (*n*) Autoradiogram showing ^{125}I-hPYY binding in the presence of 25 nM of the nonradiolabeled Y1-selective analog [Leu^{31}, Pro^{34}]-hPYY. Complete displacement of the universal ligand ^{125}I-hPYY by the Y1-selective analog indicates the presence of Y1 receptors in tumor vessels.

TABLE II – HISTOLOGIC AND RECEPTOR DATA OF 10 NEPHROBLASTOMA CASES

Case	Histologic differentiation	Receptor density (dpm/mg) Mean (maximum)[1]		Tissue distribution of receptors
		Y1	Y2	
25	Triphasic	280 (907)	549 (2,597)	Predominantly stromal
26	Triphasic	0	906 (1,272)	Predominantly blastemal
27	Triphasic	1,041 (3,387)	540 (1,371)	Predominantly stromal and glomerular differentiation
28	Triphasic	0	383	Predominantly glomerular differentiation, tumor vessels
29	Triphasic	503 (1,713)	484 (2,803)	Predominantly tubular differentiation
30	Biphasic (blastemal, stromal)	511 (1,588)	0	Predominantly stromal
31	Biphasic (blastemal, stromal)	1,130 (2,068)	0	Predominantly blastemal
32	Biphasic (blastemal, stromal)	857 (3,130)	0	Predominantly stromal, tumor vessels
33	Triphasic	0	0	
34	Biphasic (blastemal, stromal)	0	0	
Mean receptor density ± SEM (NPY receptor-positive cases)		720 ± 138	572 ± 89	

[1]Mean value represents the receptor density measured in the entire tumor sample. Maximum value (in parentheses) represents the receptor density in the area with highest density.

NPY receptor expression in nephroblastomas

The NPY receptor incidence in nephroblastomas was 80%. The characteristics are summarized in Table II. NPY receptors were observed in all 3 tissue components: blastemal, mesenchymal and epithelial differentiation. Within a given tumor sample, receptors were often expressed predominantly in one tissue type in very high density, with weaker diffuse expression in the rest of the tissue. The middle row of Figure 1 and Table II show how high the receptor density was in specific tumor areas.

In contrast to RCCs, nephroblastomas expressed both Y1 and Y2 subtypes in comparable incidence and density, in some cases even simultaneously (Table II). Y2 expression in a nephroblastoma is illustrated in Figure 1. Pharmacologic evidence of Y2 expression is based on the complete displacement of the universal ligand by the Y2-, but not by the Y1-, selective analog.

NPY receptor expression in tumor blood vessels

Y1 receptors were observed in the muscular wall of intratumoral, mid-sized and large vessels in 83% of RCCs and 20% of nephroblastomas (Tables I, II). These vessels corresponded mainly to arteries. Receptor density was moderate to high (Fig. 1*l–n*). Of note, Y1-expressing vessels were also present in otherwise receptor-negative RCCs, including the chromophobe and papillary types.

NPY receptor expression in nonneoplastic renal parenchyma

NPY receptors were also present in the nonneoplastic renal parenchyma adjacent to tumors in comparable density and distribution in all studied cases (Fig. 2). Strongest receptor expression was observed in the medulla, with higher density in the outer than the inner medullary zone (Fig. 2*a–d*). Here, NPY receptors were present in a radiated, striped pattern and corresponded mainly to tubules. In the cortex, NPY receptors were expressed in moderate density in arterioles and small arteries as well as weakly in the tubuli (Fig. 2*e–g*).

Pharmacologic characterization of NPY receptors

To further distinguish the different NPY receptor subtypes expressed in the tumors, competition experiments were performed with increasing concentrations of receptor subtype-selective analogs to assess their rank orders of potency at the receptors. Figure 3 shows a Y1-expressing nephroblastoma. The universal ligand ^{125}I-hPYY was displaced with high affinity by the Y1-selective analogs [Leu^{31}, Pro^{34}]-hPYY and BIBP 3226, whereas the Y2-selective analogs hPYY(3–36) and BIIE 0246 displaced it with low affinity. The low affinity of the Y4-preferring ligand hPP ruled out high amounts of Y4. The Y5-selective analog [Ala^{31}, Aib^{32}]-hNPY was inactive at the receptor (data not shown), excluding the presence of Y5 in this tumor. A similar rank order of potency was found in the control tissue, namely, Y1-expressing rat cortex. Conversely, in Y2-expressing tumors, as well as in the rat hippocampus as positive control, there was high-affinity displacement of ^{125}I-hPYY by the Y2-selective ligand hPYY(3–36) and low-affinity displacement by the Y1-selective ligand [Leu^{31}, Pro^{34}]-hPYY.

Immunohistochemical expression of NPY, PYY and PP in RCC and nephroblastoma

In the formalin-fixed, paraffin-embedded material, in 6 of 16 RCCs (3 clear cell, 2 chromophobe, 1 papillary RCC) and 1 of 8 nephroblastomas (triphasic differentiation), NPY peptide was present in nerve fibers around large, medium-sized and small intratumoral arteries (Fig. 4*a*) as well as nerve fibers in close proximity to the tumor cells (Fig. 4*b*). These nerve fibers were often numerous. They were found in and adjacent to the tumor capsule and in the tumor center. In one case, a large NPY-positive nerve was present in a thick intratumoral fibrovascular septum which was in direct contact with the adjacent renal parenchyma (Fig. 4*c*), suggesting that this nerve originated in preexisting intrarenal nerves. Figure 4*d* shows numerous small nerve fibers spreading from this main branch into the tumor tissue. Conversely, no immunoreactivity for NPY peptide was observed in the tumor cells of RCCs or nephroblastomas. No PYY or PP was detected in tumor cells or nerve fibers.

To evaluate simultaneous NPY receptor and NPY neurotransmitter expression in the same tumors, serial tissue sections were assessed by NPY receptor autoradiography and NPY immunohistochemistry. Unfortunately, the results of NPY immunohistochemistry performed on frozen tissue sections were unsatisfactory and could not be adequately evaluated.

Discussion

The present study shows that NPY receptors are highly expressed in tumor cells and blood vessels of adult and embryonal

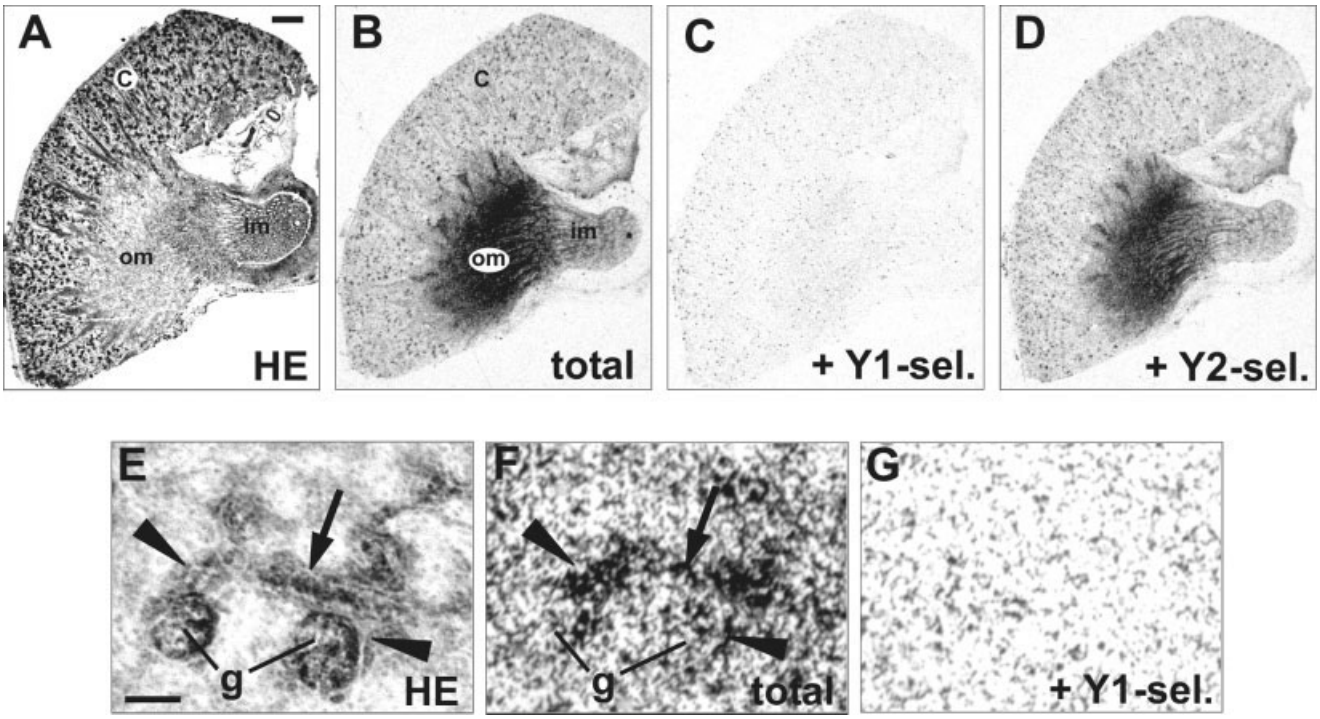

FIGURE 2 – NPY receptor expression in the nonneoplastic kidney in an overview (*a–d*) and in various cortical structures (*e–g*). (*a*) At low magnification (scale bar = 1 mm), hematoxylin and eosin (HE)–stained section shows the cortex (c) on the left, the outer medulla (om) in the middle and the inner medulla (im) on the right. (*b*) Total binding of ^{125}I-hPYY is strong in the outer medulla and weak in the cortex and inner medulla. (*c*) ^{125}I-hPYY is completely displaced by the Y1-selective analog [Leu^{31}, Pro^{34}]-hPYY. (*d*) In contrast, there is only marginal displacement in the presence of the Y2-selective analog hPYY(3–36). Therefore, the receptors in the nonneoplastic human kidney correspond mainly to Y1 receptors. (*e*) The higher magnification (scale bar = 0.1 mm) of the cortex shows 2 glomeruli (g) with polar arterioles (arrowheads) and a small interlobular artery (arrow). Adjacent to these structures, convoluted tubules are present. (*f*) The interlobular and polar arterioles are strongly labeled with the universal ligand ^{125}I-hPYY, in contrast to the glomeruli. The weak and diffuse signal in the rest of the tissue corresponds to convoluted tubules. (*g*) Complete displacement of ^{125}I-hPYY in the presence of the Y1-selective analog [Leu^{31}, Pro^{34}]-hPYY provides evidence for the presence of the Y1 receptor subtype.

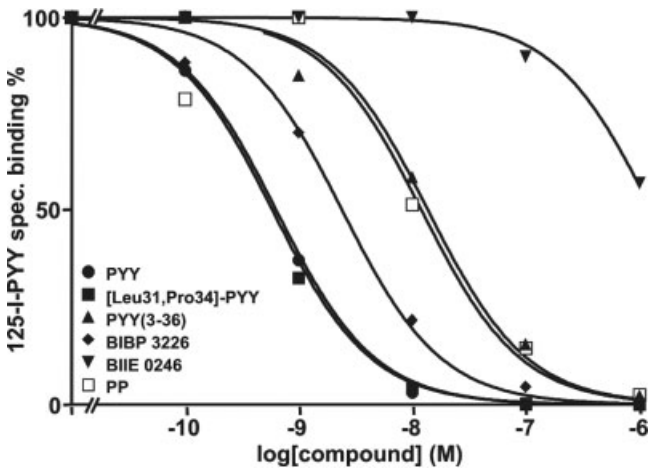

FIGURE 3 – Competition experiments in a Y1-expressing nephroblastoma. There is high-affinity displacement of ^{125}I-hPYY by hPYY (circles) as well as the Y1-selective analogs [Leu^{31}, Pro^{34}]-hPYY (solid squares) and BIBP 3226 (diamonds), whereas the Y2-selective analogs hPYY(3–36) (upward triangles) and BIIE 0246 (downward triangles) displace ^{125}I-hPYY with low and very low affinity, respectively. hPP (open squares) also shows only low affinity. This rank order of potencies is consistent with the presence of the Y1 receptor subtype.

cancers of the kidney. Clear cell RCCs express Y1 in moderate incidence and density, and nephroblastomas express Y1 and Y2 in high incidence and density. Moreover, in intratumoral blood vessels, Y1 is also frequently present in high density. In the nonneoplastic kidney, NPY receptors are identified in small arteries as well as in tubuli corresponding to proximal and distal nephron segments. These data confirm previous studies, which report Y1 mRNA in human kidney tubules in similar distribution.[27] From this NPY receptor distribution, a regulatory role of NPY in renal perfusion and tubular function can be suggested, in accordance with scarce experimental data on the functional renal effects of NPY.[28] Moreover, as we and others provide *in vitro* evidence for Y1 receptor expression in the precursor cells of RCCs and nephroblastomas, *i.e.,* in tubules and nephrogenic blastema,[27] respectively, it can be assumed that the Y1 receptors in renal tumors are probably not expressed *de novo* as opposed to the NPY receptors in pheochromocytomas and paragangliomas.[10] Since nephroblastomas express Y1 as well as Y2, one may speculate that a switch in NPY receptor subtype expression occurs in embryonal tumor cells. The possibility of a receptor subtype switch from Y2 to Y1 has been reported also for breast cancer.[8]

Displacement experiments in the present study provide strong evidence that the subtypes expressed in RCC and nephroblastoma correspond mainly to Y1 and Y2. Pharmacologic evidence for the presence of Y1 and Y2 receptors consists of high-affinity and complete displacement of the universal ligand ^{125}I-hPYY by the Y1-selective analog [Leu^{31}, Pro^{34}]-hPYY and the Y2-selective analog hPYY(3–36), respectively. These results are confirmed by comparable findings with the highly specific nonpeptide analogs BIBP 3226 for Y1[29] and BIIE 0246 for Y2.[30] The presence of Y4

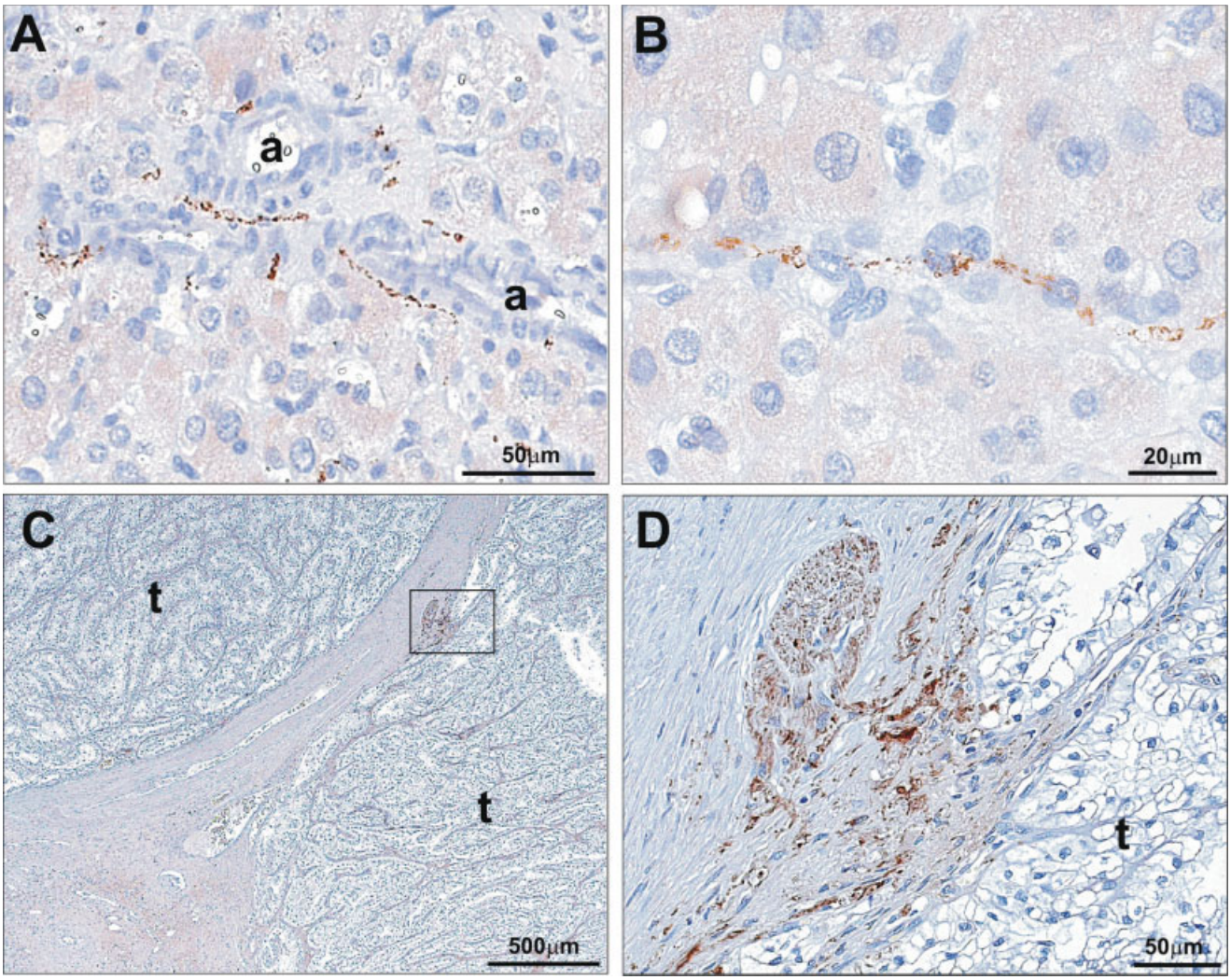

FIGURE 4 – Immunohistochemistry for NPY peptide in a chromophobe (*a,b*) and in a clear cell carcinoma (*c,d*). Within the tumor tissue, NPY-reactive nerves are present along small arteries (a) directly adjacent to the smooth muscle cells (*a*) as well as in very close association with tumor cells (*b*). There is no NPY immunoreactivity in tumor cells however. (*c*) Two tumor nodules (t) separated by a fibrovascular septum originating from the adjacent renal parenchyma in the lower left corner. (*d*) Higher magnification of the indicated area (rectangle) in (*c*) shows within the septum a large nerve strongly reactive for NPY, from which numerous small NPY-positive nerve fibers spread into the tumor mass.

or Y5 can be virtually ruled out based on the low affinity of the Y4-preferring hPP[6] and the inactivity of the Y5-selective analog [Ala[31], Aib[32]]-hNPY.[31] These results correspond well to the previously assessed rank orders of potency in other Y1- and Y2-expressing human tumors.[8,9] Moreover, studies performing *in situ* hybridization for Y1 and Y2 mRNA could confirm the results obtained from the above-mentioned pharmacologic approach to detect Y1 and Y2.[8]

For the Y1 and Y2 receptors to be functional in tumors, sufficient endogenous NPY peptide should be available on site. Circulating NPY is unlikely to play a major role for tumoral NPY receptors since there are very few NPY production sites in the periphery and NPY is rapidly inactivated enzymatically in the blood. More likely sources of intratumoral NPY could be either nerves or the tumor cells themselves. Therefore, the presence of NPY and related peptides in renal cancers was assessed immunohistochemically. None of the peptides of the NPY/PYY family (NPY, PYY or PP) was immunohistochemically present in the tumor cells of RCC or nephroblastoma. However, in a subset of RCCs and nephroblastomas, a dense network of nerve fibers reactive for NPY was found, presumably originating from preexisting NPY-containing renal nerves.[7] This is an unexpected finding since, to our knowledge, nerve fibers within RCC have not been described previously using other immunohistochemical neural markers.[32] In general, nerve fibers have been rarely observed in human tumors.[33–35] Interestingly, in adrenal cortical tumors, also known to express NPY receptors,[10] nerve fibers have been identified that were also shown to contain NPY.[35] These reports, together with the present results, demonstrate that NPY-positive nerve fibers can grow into certain human tumors. Furthermore, the present observations may also suggest that NPY released from perivascular and interstitial intratumoral nerve fibers could bind to the NPY receptors of tumor vessels and tumor cells, respectively.

At present, the *in vivo* effects of NPY on tumor vessels and tumor cells are largely unknown. While it is possible that binding of NPY to vascular receptors could result in vasoconstriction,[28] tumor ischemia and necrosis, potential direct effects *via* tumor cell receptors on cell metabolism and proliferation are unclear. It was shown that NPY can inhibit the growth of tumor cell lines under certain conditions[8] and stimulate it in others.[36] In this context, it is interesting that RCCs have long been con-

sidered to undergo host defense based on the possibility of spontaneous tumor regression[37,38] and response to immune therapy.[14] It may be that regulative systems other than those mediating host immunity are additionally involved, *e.g.,* peptide hormones or neurotransmitters and their receptors.

The observed NPY receptors in RCC and nephroblastoma can be the molecular basis for potential future therapeutic applications of NPY analogs in these tumors. Both RCC and nephroblastoma represent current oncologic problems. The response of RCC to conventional chemotherapy, radiation and immune therapy is unsatisfactory,[14,15,39] whereas the treatment of nephroblastoma, though highly effective,[40] is afflicted with considerable late side effects such as cardiomyopathy, secondary leukemia, infertility and scoliosis.[17–19] Thus, novel treatment strategies with a more favorable benefit–toxicity profile are needed. This requirement could perhaps be fulfilled by peptide hormone receptor targeting.[41] *Via* binding to the corresponding receptors expressed on tumor cells, radioisotopes or cytotoxic molecules coupled to peptide hormone analogs are delivered directly into the tumor cells. This results in high intratumoral drug concentrations, while systemic side effects may be significantly reduced.[1,42] At the same time, the high intratumoral drug concentration may diminish the drug-resistance mechanisms. It was shown in animal models that anthracycline-resistant, somatostatin receptor–expressing RCC responds well to anthracyclines when coupled to somatostatin analogs.[43] A corresponding anthracycline-coupled NPY analog suitable for this purpose has been synthesized[12] and would be ready for testing in RCC. Another promising approach is radiotherapeutic targeting of NPY receptors on tumor blood vessels, in particular knowing that other antiangiogenic therapies are effective in highly vascularized RCC.[39] Even those tumors which do not express the receptors on tumor cells could respond to therapy. Also, one may take advantage of the multiple peptide receptor expression in RCC. Indeed, in half of the RCCs investigated in the present series, NPY receptors were coexpressed with somatostatin receptors.[23] Therefore, multireceptor targeting,[1] *i.e.,* simultaneous NPY and somatostatin receptor targeting, may show a higher therapeutic effect. Finally, in view of the potential NPY receptor targeting of RCC, it is important to know that NPY receptors are expressed also in RCC characterized by features associated with an unfavorable outcome, such as clear cell type, sarcomatoid differentiation, higher tumor grade and presence of metastases, *i.e.,* in patients who are less likely to be cured by surgery alone.[16,44]

Although expression of NPY receptors in the normal, nonneoplastic kidney should not be ignored when targeting intrarenal tumors with radioactive peptide analogs, major consideration should be given to the NPY receptor-independent renal accumulation of the excreted radiolabeled compounds in the course of imaging renal tumors located within the kidney. This problem was identified previously for *in vivo* somatostatin receptor targeting of renal cell cancers. While it was difficult to image RCCs within the kidney, their extrarenal metastases were well identified.[45,46] Renal side effects due to binding of exogenous NPY analogs to physiologically expressed renal NPY receptors are expected to be negligible because very low peptide doses need to be applied.[1]

In conclusion, RCCs and nephroblastomas express Y1 and/or Y2 receptors in moderate and high incidence and density, respectively. Y1 is also present in high amounts in tumor blood vessels. These receptors may be the target of endogenous NPY released by intratumoral nerve fibers. In addition, they represent the molecular basis for NPY receptor-targeted therapy of tumors, which still represent an unsolved oncologic problem.

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