[CANCER RESEARCH 60, 3105–3112, June 1, 2000]

Vasoactive Intestinal Peptide/Pituitary Adenylate Cyclase-activating Peptide Receptor Subtypes in Human Tumors and Their Tissues of Origin[1]

Jean Claude Reubi,[2] Ursula Läderach, Beatrice Waser, Jan-O. Gebbers, Patrick Robberecht, and Jean A. Laissue

Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, CH-3010 Berne, Switzerland [J. C. R., U. L., B. W., J. A. L.]; Institute of Pathology, Kantonsspital Luzern, CH-6000 Luzern, Switzerland [J-O. G.]; and Departement de Chimie Biologique et de la Nutrition, Université Libre de Bruxelles, B-1070 Bruxelles, Belgium [P. R.]

ABSTRACT

The evaluation of peptide receptors in man is needed not only to discover the physiological target tissues of a given peptide but also to identify diseases with a sufficient receptor overexpression for diagnostic or therapeutic interventions. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) receptors have been evaluated in human tumors and in their tissues of origin using *in vitro* receptor autoradiography with ^{125}I-VIP or ^{125}I-acetyl-PACAP-27 in tissue sections. The VIP/PACAP receptor subtypes $VPAC_1$, $VPAC_2$, and PAC_1 were evaluated in these tissues by determining the rank order of potencies of VIP and PACAP as well as $VPAC_1$- and $VPAC_2$-selective analogues. The VIP/PACAP receptors expressed in the great majority of the most frequently occurring human tumors, including breast (100% receptor incidence), prostate (100%), pancreas (65%), lung (58%), colon (96%), stomach (54%), liver (49%), and urinary bladder (100%) carcinomas as well as lymphomas (58%) and meningiomas (100%), are predominantly of the $VPAC_1$ type. Their cells or tissues of origin, *i.e.*, hepatocytes, breast lobules and ducts, urothelium, prostate glands, pancreatic ducts, lung acini, gastrointestinal mucosa, and lymphocytes, also predominantly express $VPAC_1$. Leiomyomas predominantly express $VPAC_2$ receptors, whereas paragangliomas, pheochromocytomas, and endometrial carcinomas preferentially express PAC_1 receptors. Conversely, $VPAC_2$ receptors are found mainly in smooth muscle (*i.e.*, stomach), in vessels, and in stroma (*e.g.*, of the prostate), whereas PAC_1 receptors are present in the adrenal medulla and in some uterine glands. Whereas the very wide distribution of VIP/PACAP receptors in the normal human body is indicative of a key role of these peptides in human physiology, the high VIP/PACAP receptor expression in tumors may represent the molecular basis for clinical applications of VIP/PACAP such as *in vivo* scintigraphy and radiotherapy of tumors as well as VIP/PACAP analogue treatment for tumor growth inhibition.

INTRODUCTION

A majority of human tumors, in particular the frequently occurring carcinomas, express VIP[3] receptors (1–4). Based on this high occurrence of tumoral VIP receptors, a number of potential clinical applications have been evaluated. First, it could be demonstrated that selected tumors, in particular, the VIP receptor-positive colorectal cancers, can be visualized in the patient by means of *in vivo* VIP receptor scintigraphy (5). Moreover, several studies have reported an effect of VIP and PACAP analogues on tumor growth in animal tumor models, mediated by specific receptors (6, 7). Therefore, VIP and the related peptide PACAP may be of great potential importance for oncology.

In the last few years, molecular biology has provided evidence for the existence of several receptor subtypes within the VIP/PACAP family (8). There are two VIP receptors, $VPAC_1$ and $VPAC_2$, both with high affinity for VIP and PACAP that can be distinguished pharmacologically by the $VPAC_1$-selective analogue [K^{15},R^{16},L^{27}]VIP(1–7)/GRF(8–27) (KRL-VIP/GRF) and the $VPAC_2$-selective RO 25-1553 (9, 10). There is at least one PACAP receptor, PAC_1, that is characterized by high affinity for PACAP but low affinity for VIP (8, 11).

Recently, a high incidence of PAC_1 was found in human gliomas, neuroblastomas, and pituitary adenomas (11–14), whereas $VPAC_1$ was identified in pancreatic cancers (15). However, it is presently unknown which subtype of receptor is expressed by the great majority of the other human tumors having VIP/PACAP receptors. Generally, this information is also lacking for the normal tissues of origin of the tumors. Such data would not only be important as additional biological information on these tumors and their tissues of origin but may be decisive for the formulation of a number of clinical applications for VIP/PACAP, such as *in vivo* VIP/PACAP receptor scintigraphy or long-term treatment with VIP/PACAP analogues, two approaches based on receptor-selective targeting of tumors by labeled or unlabeled VIP/PACAP molecules.

To be able clinically to take advantage of a high peptide receptor expression in human tumors, a particularly high "tumor to background" ratio (where background represents nontumor tissue) is preferable for diagnostic as well as radiotherapeutic applications. It is therefore a prerequisite to also have data on the VIP/PACAP receptor expression in the normal human tissues to identify which types of receptor-positive human tumors are most adequate for clinical investigations. Although peptide receptors, including VIP/PACAP receptors, have usually been investigated extensively in normal tissues of laboratory animals, systematic investigations in human tissues are much less frequent, being limited by the difficulty in obtaining and analyzing such tissues as well as by the often great individual variability in receptor expression observed in human tissue samples.

The aim of the present investigation was to evaluate VIP/PACAP receptors and identify their subtypes in a large number of different types of human tumors, including the most frequently occurring cancers, in comparison with the VIP/PACAP receptor subtypes expressed in the normal tissues of origin of these tumors. More than 400 human primary tumors and metastases as well as numerous samples of normal tissue were therefore evaluated *in vitro* by means of VIP/PACAP receptor autoradiography with the use of subtype-selective analogues to differentiate $VPAC_1$, $VPAC_2$, and PAC_1.

MATERIALS AND METHODS

Aliquots of surgically resected tumors or of biopsy specimens submitted for diagnostic histopathological analysis were frozen immediately after surgical resection and stored at −70°C. The following tumors were investigated: (*a*) colonic adenocarcinomas ($n = 26$); (*b*) gastric carcinomas ($n = 26$); (*c*) ductal pancreatic adenocarcinomas ($n = 40$); (*d*) non-small cell carcinomas of the lung ($n = 40$); (*e*) breast carcinomas ($n = 68$); (*f*) endometrial carcinomas ($n = 12$); (*g*) prostate carcinomas ($n = 37$); (*h*) liver carcinomas (HCCs;

Received 12/6/99; accepted 4/4/00.

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[1] Supported in part by an Interuniversity Poles of Attraction, Prime Minister Office, Federal Government, Belgium.

[2] To whom requests for reprints should be addressed, at Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Murtenstrasse 31, P. O. Box 62, CH-3010 Berne, Switzerland. Phone: 41-31-632-3242; Fax: 41-31-632-8999; E-mail: reubi@patho.unibe.ch.

[3] The abbreviations used are: VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating peptide; HCC, hepatocellular carcinoma; ^{125}I-VIP, ^{125}I-Tyr^{10}-VIP; ^{125}I-PACAP, ^{125}I-Ac-His^1-PACAP-27; GI, gastrointestinal.

Table 1 *Incidence of VIP/PACAP receptor-positive tumors*[a]

Tumor type	Incidence
Lung carcinoma (NSCLC)[b]	23/40 (58%)
Colorectal carcinoma	25/26 (96%)
Breast carcinoma	68/68 (100%)
Gastric carcinoma	14/26 (54%)
Prostate carcinoma	37/37 (100%)
Liver carcinoma (HCC)	29/59 (49%)
Ductal pancreatic carcinoma	26/40 (65%)
Urinary bladder carcinoma	4/4 (100%)
Non-Hodgkin's lymphoma	11/19 (58%)
Meningioma	27/27 (100%)
Leiomyoma	10/15 (66%)
Endometrial carcinoma	12/12 (100%)
Pheochromocytoma	18/25 (72%)
Paraganglioma	18/18 (100%)

[a] Positive tumors include those labeled with ^{125}I-VIP and ^{125}I-PACAP and those labeled with ^{125}I-PACAP only.
[b] NSCLC, non-small cell lung carcinoma.

$n = 59$); (*i*) non-Hodgkin's lymphomas ($n = 19$); (*j*) bladder carcinomas ($n = 4$); (*k*) meningiomas ($n = 27$); (*l*) pheochromocytomas ($n = 25$); (*m*) paragangliomas ($n = 18$); and (*n*) leiomyomas ($n = 15$). We also tested 11 lymph node metastases of breast cancer. Although many of the tumor samples contained adjacent normal tissue, we have also evaluated normal tissue from noncancerous patients, including tissue from the lymph nodes, gastric and colonic wall, breast, lung, prostate, liver, pancreas, bladder, uterus, and adrenal gland.

VIP and PACAP Receptor Autoradiography. Receptor autoradiography was performed on 10- and 20-μm-thick cryostat sections of the tissue samples, as described previously (16, 17). All tissue samples were analyzed with ^{125}I-VIP, eluted as a single peak by high-performance liquid chromatography, and analyzed by mass spectrometry (2000 Ci/mmol; Anawa, Wangen, Switzerland) and ^{125}I-PACAP (2000 Ci/mmol; Anawa). The tissues were cut on a cryostat, mounted on microscope slides, and stored at −20°C for at least 3 days to improve adhesion of the tissue to the slide. The slide-mounted tissue sections were allowed to reach room temperature and then incubated for 90 min in a solution of 50 mM Tris-HCl (pH 7.4) containing 2% BSA, 2 mM EGTA, 0.1 mM bacitracin, and 5 mM $MgCl_2$ to inhibit endogenous proteases in the presence of 30 pM ^{125}I-VIP or ^{125}I-PACAP at room temperature, as described previously (18). To estimate nonspecific binding, paired serial sections were incubated as described above, except that 1 μM VIP or PACAP, respectively (Bachem, Bubendorf, Switzerland), was added to the incubation medium. After the incubation, the slides were rinsed with four washes (1 min each) in ice-cold 50 mM Tris-HCl (pH 7.4) with 0.25% BSA, dipped in ice-cold water, and then dried quickly in a refrigerator under a stream of cold air. The sections were subsequently exposed to ^{3}H-Hyperfilms (Amersham, Aylesbury, United Kingdom) for 1 week.

The autoradiograms were quantified with a computer-assisted image-processing system, as described previously (16, 17). Radiolabeled tissue sections were exposed to ^{3}H-Hyperfilms, together with standards (Autoradiographic [^{125}I]microscales; Amersham) that contained known amounts of isotope cross-calibrated to tissue-equivalent ligand concentration. A tissue was considered VIP receptor-positive when the absorbance measured over a tissue area in the total binding section was at least twice that of the nonspecific binding section.

To distinguish PAC_1 receptors from $VPAC_1$ and $VPAC_2$ subtypes, all cases demonstrating binding with the ^{125}I-PACAP ligand were evaluated for high ($VPAC_1$ or $VPAC_2$) or low (PAC_1) affinity for VIP in complete displacement curves or, in selected cases, using a single VIP concentration of 20 nM.

In addition, a large and representative selection of each type of tumor found to be positive using ^{125}I-VIP as ligand was characterized in terms of $VPAC_1$ and $VPAC_2$ subtypes. Complete displacement curves (or, in selected cases, displacement with a single concentration of 20 nM) were performed using $VPAC_1$-selective KRL-VIP/GRF (9) and $VPAC_2$-selective RO 25-1553 (10). This pharmacological evaluation of VIP/PACAP receptor subtypes permitted us to identify with confidence the predominantly expressed subtype in a tissue. For further confirmation of the data obtained using the above-mentioned method, a number of $VPAC_1$- and $VPAC_2$-expressing human tissues were tested with ^{125}I-labeled $VPAC_1$-selective KRL-VIP/GRF or $VPAC_2$-selective RO 25-1553 used as radioligands. KRL-VIP/GRF and RO 25-1553 were both iodinated using the lactoperoxidase method (2000 Ci/mmol; Anawa); receptor autoradiography conditions, including radioligand concentration, were the same as those described above for the VIP receptor autoradiography. Whereas ^{125}I-KRL-VIP/GRF gave a very high nonspecific binding inadequate to identify $VPAC_1$ receptors in tissues, ^{125}I-RO 25-1553 was found to be a very valuable radioligand to detect $VPAC_2$-expressing normal and neoplastic human tissues.

RESULTS

Table 1 summarizes the incidence of a series of VIP/PACAP receptor-expressing tumors, including all of the most frequently occurring carcinomas. It is striking to see that the overall incidence for tumors displaying ^{125}I-VIP and/or ^{125}I-PACAP binding was very high for colorectal cancers; breast, prostate, and bladder carcinomas; meningiomas (both meningothelial and fibroblastic meningiomas); paragangliomas; and endometrial carcinomas. More than half of the lung, gastric, and pancreatic carcinomas; lymphomas; leiomyomas; and pheochromocytomas and almost half of the HCCs expressed VIP/PACAP receptors. As seen in the subtype characterization shown in Table 2, almost all listed tumors had predominantly $VPAC_1$ (defined by a high affinity for KRL-VIP/GRF), except for the leiomyomas with $VPAC_2$ (defined by a high affinity for RO 25-1553). In the tumors listed in Table 2, the detection of a predominant PAC_1 expression was extremely rare (PAC_1 was detected in two HCCs listed in Table 2).

Table 3 lists an additional group of tumors expressing predominantly PAC_1, including most of the paragangliomas, pheochromocytomas, and endometrial cancers. In all cases, as seen in displacement curves using ^{125}I-PACAP, VIP bound with low affinity, compared to

Table 2 *Characterization of the main VIP/PACAP receptor subtype expressed by tumors*

Tumor type	Tumor labeled by ^{125}I-VIP and ^{125}I-PACAP			Tumor labeled by ^{125}I-PACAP only	Receptor subtypes in normal tissue of origin
	$VPAC_1$[a]	$VPAC_2$[b]	PAC_1[c]	PAC_1[c]	
Lung carcinoma (NSCLC)[d]	11/11	0/11	0/11	0/15	$VPAC_1$ (Acini)
Colorectal carcinoma	23/23	0/23	0/23		$VPAC_1$ (Mucosa)
Breast carcinoma	21/21	0/21	0/21		$VPAC_1$ (Lobules + ducts)
Gastric carcinoma	13/13	0/13	0/13	0/12	$VPAC_1$ (Mucosa)
Prostate carcinoma	17/17	0/17	0/17		$VPAC_1$ (Glands)
Liver carcinoma (HCC)	26/26	0/26	1/26	1/28	$VPAC_1$ (Hepatocytes)
Ductal pancreatic carcinoma	13/13	0/13	0/13	0/12	$VPAC_1$ (Ducts)
Urinary bladder carcinoma	3/3	0/3	0/3		$VPAC_1$ (Urothelium)
Non-Hodgkin's lymphoma	6/6	0/6	0/6	NA	$VPAC_1$ (Lymphocytes)
Meningioma	6/6	0/6	0/6		NA
Leiomyoma	0/7	7/7	0/7	0/4	$VPAC_2$ (Smooth muscle)

[a] $VPAC_1$ is defined by its high affinity for KRL VIP/GRF and low affinity for RO-25-1553 using ^{125}I-VIP tracer.
[b] $VPAC_2$ is defined by its high affinity for RO-25-1553 and low affinity for KRL VIP/GRF using ^{125}I-VIP tracer.
[c] PAC_1 is defined by its high affinity for PACAP 27 and low affinity for VIP using ^{125}I-PACAP tracer.
[d] NSCLC, non-small cell lung carcinoma; NA, not assessed.

Table 3 *Incidence of PAC_1-expressing tumors and comparison with the main subtype expressed in their tissue of origin*

Tumor type	Incidence of PAC_1-positive tumors[a]	Main VIP/PACAP-R subtype present in tissue of origin
Catechol-secreting tumors		
Pheochromocytoma	18/25 (72%)	PAC_1 (Adrenal medulla)
Paraganglioma	18/18 (100%)	PAC_1 (Adrenal medulla)
Endometrial carcinoma	12/12 (100%)	$VPAC_1$ PAC_1 (Glands)

[a] PAC_1-positive tumors were defined by high affinity for PACAP 27 and low affinity for VIP using ^{125}I-PACAP tracer.

the high affinity seen with PACAP. Interestingly, in the majority of the cases, there was nevertheless a specific ^{125}I-VIP binding displaced by nanomolar concentrations of VIP, as described previously for PAC_1 transfected cells by Hashimoto *et al.* (19). However, this ^{125}I-VIP binding was poorly displaced by KRL-VIP/GRF or RO 25-1553. It may correspond to the PAC_1 splice variant reported recently (20).

The VIP/PACAP receptor status of the tissues of origin for the above-mentioned tumors is also summarized in Tables 2 and 3. It is striking to observe that VIP/PACAP receptors are found in most human epithelial tissues. In the majority of these tissues, the VIP/PACAP receptor subtype preferentially expressed is the $VPAC_1$ receptor, as detected by the differential affinity of the $VPAC_1$- and $VPAC_2$-selective analogues. This is the case for hepatocytes, GI mucosa, lobules and ducts of the breast, prostatic glands, urothelium of bladder and ureter, acini of the lung, and pancreatic ducts. None of the investigated epithelial tissues have been found to have a predominant $VPAC_2$ receptor expression (Tables 2 and 3). However, several human tissues have a predominant PAC_1 receptor expression. This is the case for the adrenal medulla, the tissue of origin of the catecholamine-secreting tumors. Another case is the uterus, which focally expresses PAC_1 and $VPAC_1$ in glands, whereas the stroma expresses mainly $VPAC_2$ receptors (Table 3).

Conversely, smooth muscle in various locations preferentially expresses $VPAC_2$ receptors as documented by ^{125}I-VIP binding displaced by nanomolar concentrations of the $VPAC_2$-selective RO 25-1553 but not of the $VPAC_1$-selective analogue KRL-VIP/GRF. ^{125}I-PACAP binding is displaced by nanomolar concentrations of PACAP and of VIP in these tissues. Such $VPAC_2$ receptors are observed in smooth muscle in locations as different as the GI tract (the stomach in particular, but not the colon) and the seminal vesicle. Moreover, several blood vessels (arteries more than veins) express VIP receptors, located primarily in the smooth muscle. However, not all identified blood vessels show VIP receptor expression, and a considerable subtype variability is noticed: whereas the majority of the vessels express $VPAC_2$ receptors, few preferentially express

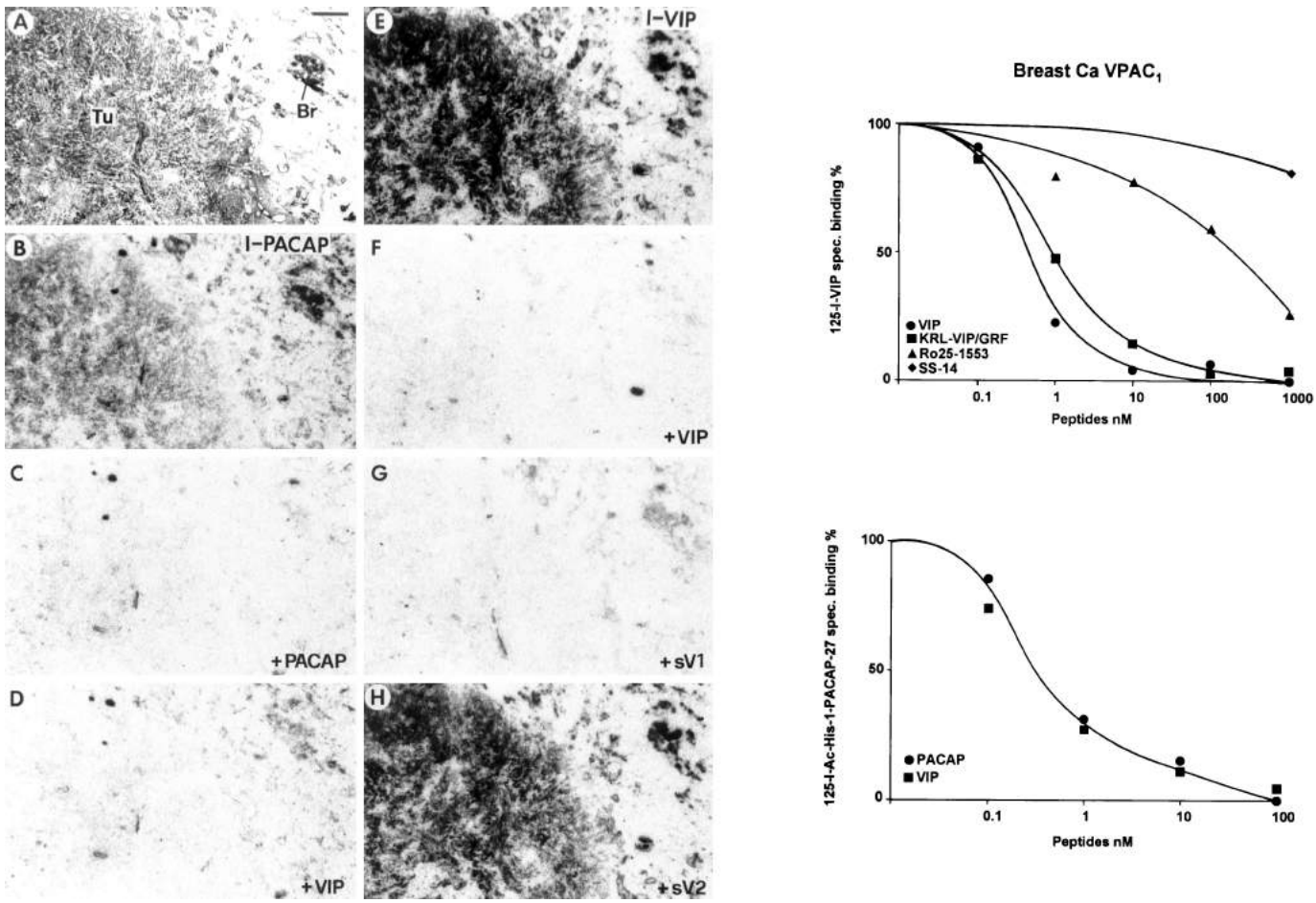

Fig. 1. Detection of the $VPAC_1$ receptor subtype in invasive ductal breast carcinoma and the surrounding breast tissue. *A*, H&E-stained section. *Tu*, carcinoma; *Br*, normal breast tissue. *Bar*, 1 mm. *B–D*, autoradiograms showing ^{125}I-PACAP binding. *B*, ^{125}I-PACAP total binding. *C*, binding in the presence of 20 nM PACAP. *D*, binding in the presence of 20 nM VIP. *E–H*, autoradiograms showing ^{125}I-VIP binding. *E*, ^{125}I-VIP total binding. *F*, binding in the presence of 20 nM VIP. *G*, binding in the presence of 20 nM of the $VPAC_1$-selective analogue (*sV1*) KRL-VIP/GRF. *H*, binding in the presence of 20 nM of the $VPAC_2$-selective analogue (*sV2*) RO 25-1553. The tumor and normal breast tissues are labeled with ^{125}I-PACAP and ^{125}I-VIP. Complete displacement by 20 nM PACAP and VIP excludes the presence of PAC_1. Complete displacement by 20 nM KRL-VIP/GRF but not RO 25-1553 strongly suggests expression of $VPAC_1$ in both tissues. On the *right*, the two competition curves reveal the $VPAC_1$ expression of breast carcinoma: the *top graph* shows high affinity displacement of ^{125}I-VIP by KRL-VIP/GRF but not by RO 25-1553; the *bottom graph* shows high affinity displacement of ^{125}I-PACAP by PACAP and VIP.

$VPAC_1$ receptors, and some others express a mixture of $VPAC_1$ and $VPAC_2$ receptors. Furthermore, stromal tissue can also express $VPAC_2$ receptors: they are found, in particular, in the stroma of the uterus and prostate, whereas the glands of the prostate preferentially express $VPAC_1$ (see above). These $VPAC_2$-expressing tissues, namely, gastric smooth muscles, vessels, and the uterine and prostatic stroma, are all specifically labeled by the $VPAC_2$-selective ^{125}I-RO 25-1553 radioligand (data not shown).

Most human solid lymphoid tissues express VIP/PACAP receptors at high density, as shown previously (21, 22). In the present study, all of the investigated lymph nodes, which were removed from the axillary region in most cases, have a strong predominance of $VPAC_1$ receptors (Table 2).

Fig. 1 illustrates a typical $VPAC_1$-expressing breast carcinoma with $VPAC_1$-expressing adjacent breast tissue. PACAP and VIP completely displace ^{125}I-PACAP binding in the high affinity range, indicating the absence of PAC_1. VIP and KRL-VIP/GRF displace ^{125}I-VIP in the nanomolar range, whereas RO 25-1553 displaces it with low affinity, indicating a predominance of $VPAC_1$. Fig. 2 shows a $VPAC_1$-expressing ductal pancreatic carcinoma next to a $VPAC_1$-positive normal pancreatic duct. Fig. 3 is an autoradiography of a $VPAC_1$-expressing gastric carcinoma with, for comparison, a normal stomach expressing $VPAC_1$ in the mucosa and $VPAC_2$ in the smooth muscle. Complete displacement curves are shown in Fig. 4 for gastric cancer and normal stomach, including mucosa and smooth muscles. The leiomyoma in Fig. 5 expresses the same $VPAC_2$ subtype as found in the smooth muscle shown in Fig. 4, characterized by the low affinity of KRL-VIP/GRF and high affinity of RO 25-1553. This type of tumor can also be specifically labeled with ^{125}I-RO 25-1553. In contrast to the examples cited above, Fig. 6 shows a PAC_1-expressing pheochromocytoma next to normal human adrenal medulla, both characterized by a low affinity of VIP. Fig. 7 shows a PAC_1-expressing paraganglioma in a displacement experiment.

Lymph nodes are important metastasis sites of tumors. As shown in Table 4, we have measured the VIP receptor density of metastatic breast cancer tissue in axillary lymph nodes, and compared with the nonmetastatic lymphoid tissue of lymph nodes: the density of $VPAC_1$ receptors in lymphoid tissue was twice as high as that in the cancer tissue.

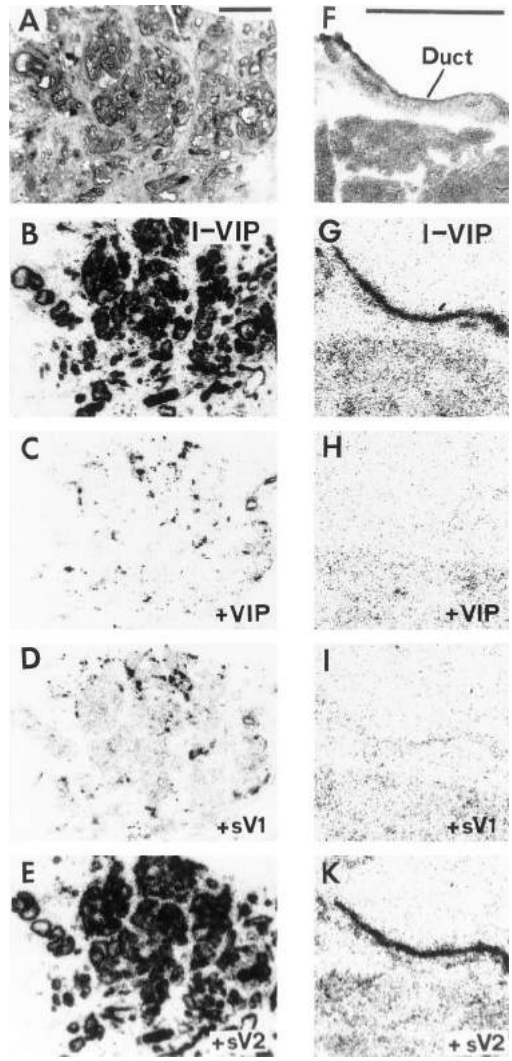

Fig. 2. $VPAC_1$ in human ductal pancreatic carcinoma (*A–E*) and its tissue of origin, the pancreatic duct (*F–K*). *A* and *F*, H&E-stained sections. *Bars*, 1 mm. *B–E* and *G–K*, autoradiograms showing ^{125}I-VIP binding. *B* and *G*, ^{125}I-VIP total binding. *C* and *H*, binding in presence of 20 nM VIP. *D* and *I*, binding in presence of 20 nM of the $VPAC_1$-selective analogue (*sV1*) KRL-VIP/GRF. *E* and *K*, binding in presence of 20 nM of the $VPAC_2$-selective analogue (*sV2*) RO 25-1553. Complete displacement by 20 nM KRL-VIP/GRF but not RO 25-1553 in the carcinoma and in the duct suggest the presence of $VPAC_1$.

DISCUSSION

The present study shows that the great majority of frequently occurring carcinomas predominantly express $VPAC_1$ receptors, as do their normal tissues of origin. This is the case for breast, prostate, colon, lung, and bladder carcinomas as well as gastric, ductal pancreatic, and HCCs. It is rare to find tumors predominantly expressing $VPAC_2$. In our series, we could identify only leiomyomas as a benign mesenchymal neoplasm expressing $VPAC_2$, probably in relation to the $VPAC_2$-expressing smooth muscles.

Conversely, other benign and malignant neoplasms located in the brain or endocrine and neuroendocrine system appear to predominantly express PAC_1. It had previously been reported that gliomas, neuroblastomas, and pituitary adenomas express PAC_1 (11–13). We can now add to this list catecholamine-secreting tumors, pheochromocytomas, paragangliomas, and endometrial cancers. There is evidence that their respective tissues of origin can express PAC_1, often at high density, as seen in the human adrenal medulla.

The presence of the various VIP/PACAP receptor subtypes not only in tumors but also in the great majority of the nonneoplastic, normal tissues of origin may have a number of important implications, both advantageous and disadvantageous, with regard to the potential clinical applications for VIP/PACAP.

The diagnostic localization of tumors and their metastases using receptor scintigraphy requires a sufficiently high density of tumoral receptors, as well as a high tumor to background ratio. Whereas most tumors yield the high VIP/PACAP receptor density necessary for their visualization, the optimal tumor to background ratio is more of a concern because VIP/PACAP receptors are expressed by so many normal tissues. It may therefore be necessary to limit VIP/PACAP receptor scintigraphy to those tumors located in sites where an optimal tumor:tissue ratio of receptor density can be expected. $VPAC_1$-expressing colorectal cancers are probably good candidates because the normal GI tract has a relatively moderate density of $VPAC_1$ receptors located in very distinct areas of the mucosa. This statement is supported by the study of Virgolini *et al.* (5) showing that human colorectal cancers can be localized by *in vivo* VIP receptor scintigraphy. Conversely, lung cancers are poor candidates because of the high density of VIP/PACAP receptors in lung acini. $VPAC_1$-expressing prostate cancers are also inadequate candidates due to the high $VPAC_1$ receptor expression in normal prostatic glands. Furthermore, $VPAC_1$-expressing neoplasms located in the liver may not be ade-

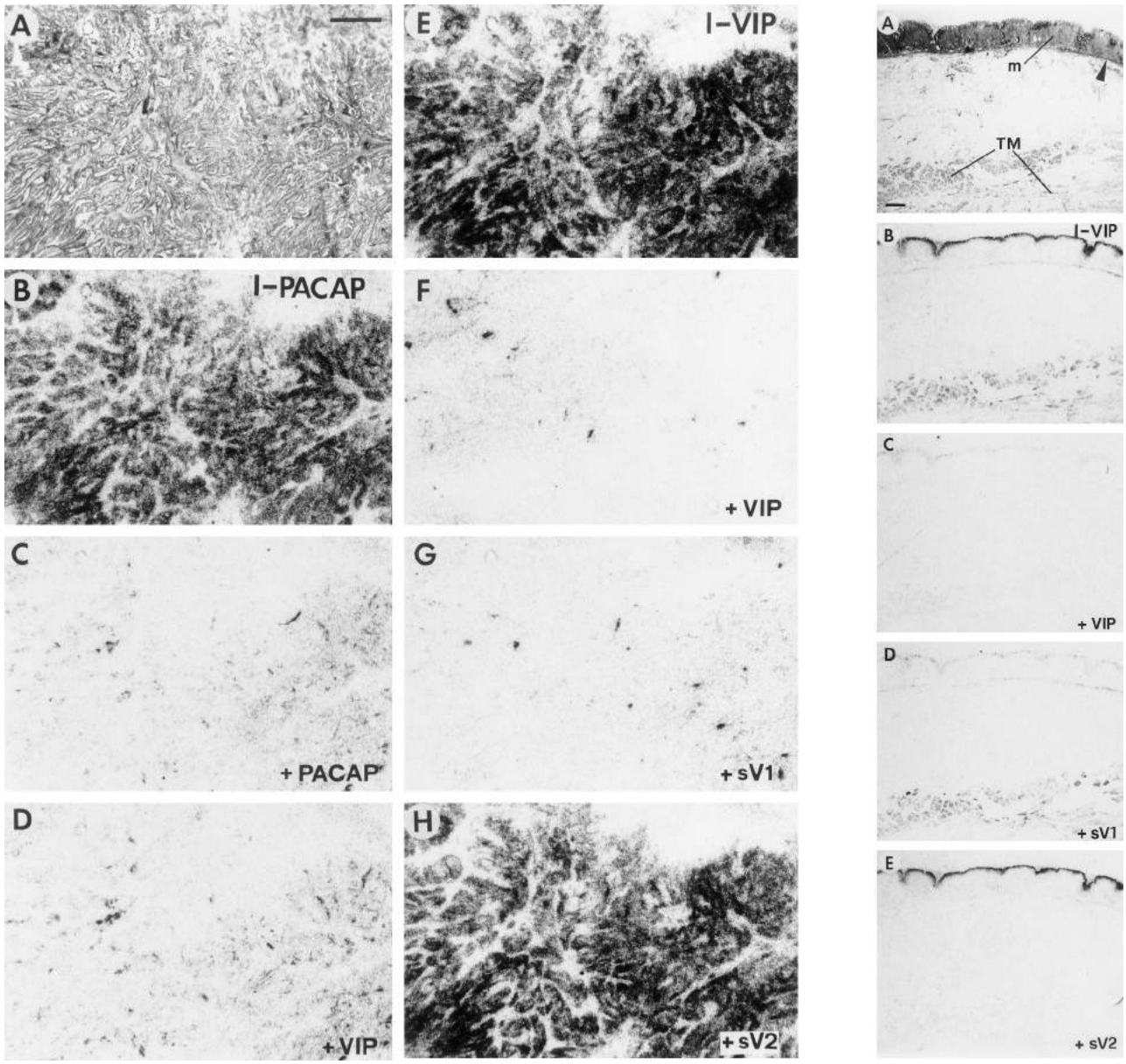

Fig. 3. *Left panels A–H*, $VPAC_1$ in human gastric carcinoma. *A*, H&E-stained section. *Bar*, 1 mm. *B–D*, autoradiograms showing ^{125}I-PACAP binding. *B*, ^{125}I-PACAP total binding. *C*, binding in the presence of 20 nM PACAP. *D*, binding in the presence of 20 nM VIP. *E–H*, autoradiograms showing ^{125}I-VIP binding in gastric carcinoma tissue. *E*, ^{125}I-VIP total binding. *F*, binding in the presence of 20 nM VIP. *G*, binding in the presence of 20 nM of the $VPAC_1$-selective analogue (*sV1*) KRL-VIP/GRF. *H*, binding in the presence of 20 nM of the $VPAC_2$-selective analogue (*sV2*) RO 25-1553. *Right panels A–E*, normal gastric wall with $VPAC_1$ in mucosa (*m*) and $VPAC_2$ in the tunica muscularis (*TM*). *A*, H&E-stained section. *Arrowhead*, muscularis mucosae. *Bar*, 1 mm. *B–E*, autoradiograms showing ^{125}I-VIP binding in analogy to *left panels E–H*. *B*, ^{125}I-VIP total binding. *C*, binding in the presence of 20 nM VIP. *D*, binding in the presence of 20 nM of the $VPAC_1$-selective analogue (*sV1*) KRL-VIP/GRF. *F*, binding in the presence of 20 nM of the $VPAC_2$-selective analogue (*sV2*) RO 25-1553. The mucosa has the same $VPAC_1$ characteristics as the gastric carcinoma, whereas the smooth muscle expresses $VPAC_2$.

quate for VIP receptor scintigraphy because of the high density of $VPAC_1$ receptors in the normal liver. We have shown that HCCs have approximately one-fourth the density of VIP receptors in the liver (3). The same low ratio is found between pancreatic or colorectal carcinomas and the normal liver,[4] suggesting that in many cases, liver metastases of these two types of cancers as well as HCCs will not be identified as positive hot spots with VIP receptor scintigraphy but rather as cold spots. We can conclude from the present study that lymph node metastases, for example, lymph node metastases of breast cancers, will also be difficult to assess with VIP receptor scintigraphy because of the high VIP receptor content of normal lymphoid tissue (21, 22).

These density ratios identified in the present study are, of course, based on *in vitro* data measuring a nondynamic receptor condition in sections of normal and tumoral tissues. One cannot exclude that, *in vivo*, VIP receptors expressed in tumoral tissues will have characteristics distinct from those expressed in normal tissues, *e.g.*, because of different internalization rates, different ligand dissociation rates, or different receptor turnover; this would lead to an accumulation of radioligand in both tissues at a rate different from that predicted by the *in vitro* measurement of receptor density. It would, of course, be particularly useful for imaging purposes if a differential receptor characteristic between tumor and normal tissue led to a higher *in vivo* accumulation in the tumor than in normal tissue. Experimental evidence for such mechanisms are presently lacking; it is much needed, but difficult to obtain.

[4] J. C. Reubi, unpublished data.

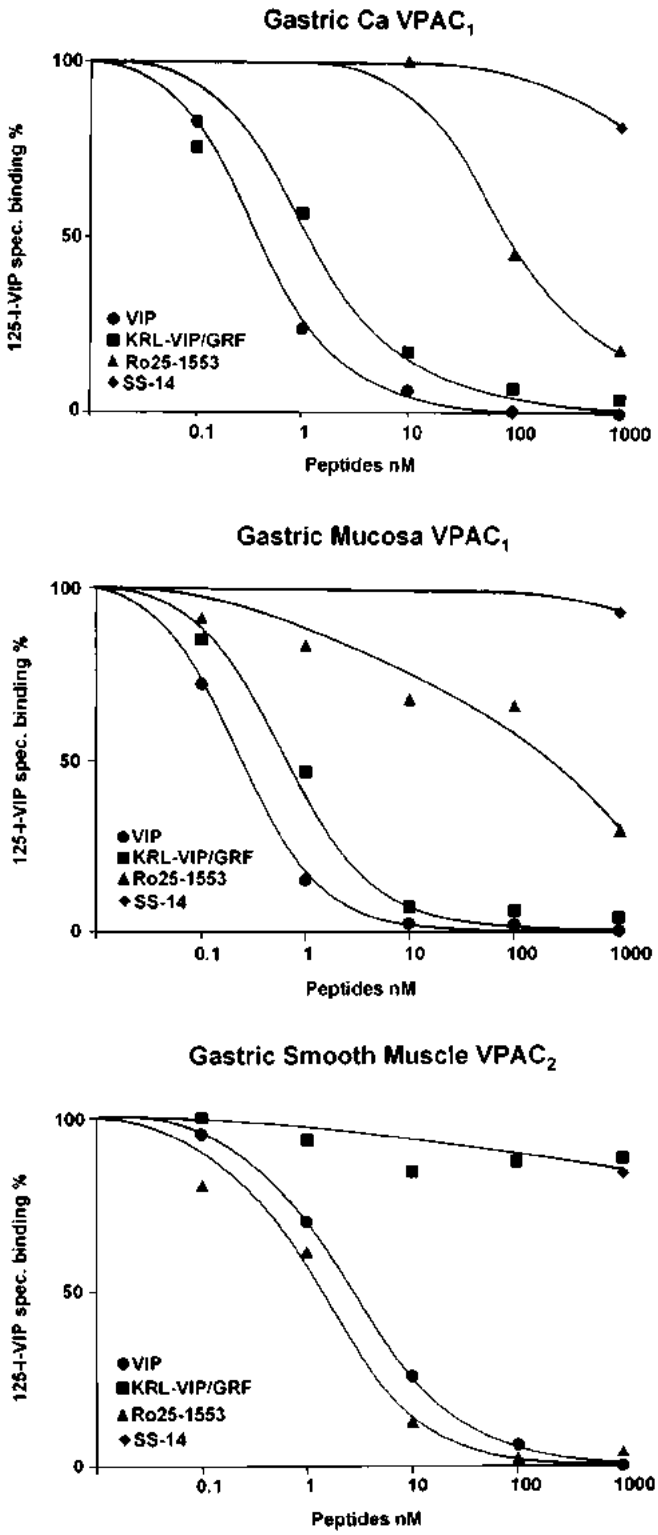

Fig. 4. Competition curves showing $VPAC_1$ in human gastric carcinoma (*top graph*) and gastric mucosa (*middle graph*) and $VPAC_2$ in gastric smooth muscle (*bottom graph*). *Top* and *middle graphs* show high affinity displacement of ^{125}I-VIP by KRL-VIP/GRF but not by RO 25-1553. Somatostatin (*SS-14*) is inactive. *Bottom graph* shows high affinity displacement of ^{125}I-VIP by RO 25-1553 but not by KRL-VIP/GRF. Somatostatin (*SS-14*) is inactive.

In the case of PAC_1 receptor-expressing tumors, one may overcome the problem of high background in liver or nodal metastases if the receptor scintigraphy is performed with a PAC_1 receptor-selective ligand such as maxadilan (23). One may assume that, under these circumstances, the background given by the liver and/or lymph node, which consists mainly of $VPAC_1$ receptors, may remain low: the selective PAC_1 radioligand would label the PAC_1 tumor, but not the adjacent $VPAC_1$ tissues.

Because it is possible to target VIP/PACAP receptor-positive tumors with radiolabeled VIP/PACAP analogues (5, 24), it should also be possible to treat these receptor-positive tumors selectively with high doses of adequately radiolabeled VIP analogues. Preliminary studies using radiolabeled somatostatin analogues suggest that both β emitters as well as Auger emitters (25, 26) can give promising results in terms of stabilization or reduction of the growth of somatostatin receptor-positive tumors. A prerequisite is that the tumor expresses a particularly high density of receptors. The limitations of such a radiotherapy include the destruction by irradiation of surrounding and distant receptor-positive normal target tissues, in particular, radiosensitive tissues such as the VIP/PACAP receptor-positive immune system. Other critical organs that may be destroyed by such a radiotherapy include the kidney and liver, not only because they express $VPAC_1$, but also because they excrete and eliminate large amounts of peptide

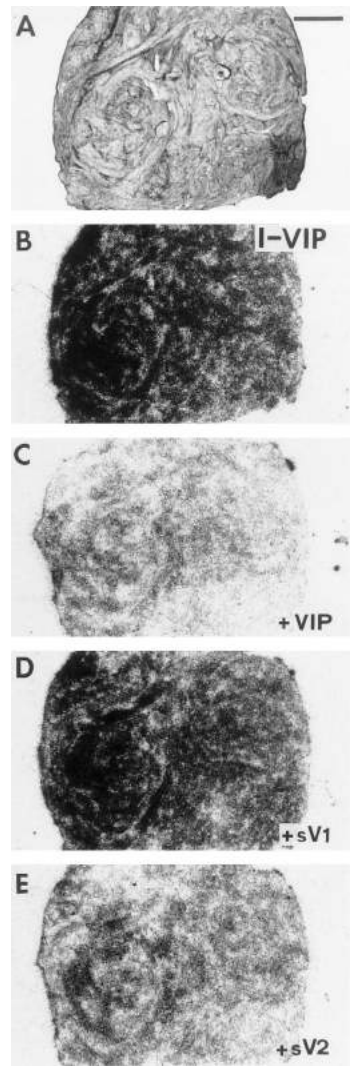

Fig. 5. $VPAC_2$ in a human leiomyoma. *A*, H&E-stained section. *Bar*, 1 mm. *B–E*, autoradiograms showing ^{125}I-VIP binding. *B*, ^{125}I-VIP total binding. *C*, binding in the presence of 20 nM VIP. *D*, binding in the presence of 20 nM of the $VPAC_1$-selective analogue (*sV1*) KRL-VIP/GRF. *E*, binding in the presence of 20 nM of the $VPAC_2$-selective analogue (*sV2*) RO 25-1553. Displacement by 20 nM RO 25-1553 but not KRL-VIP/GRF suggests the predominance of $VPAC_2$.

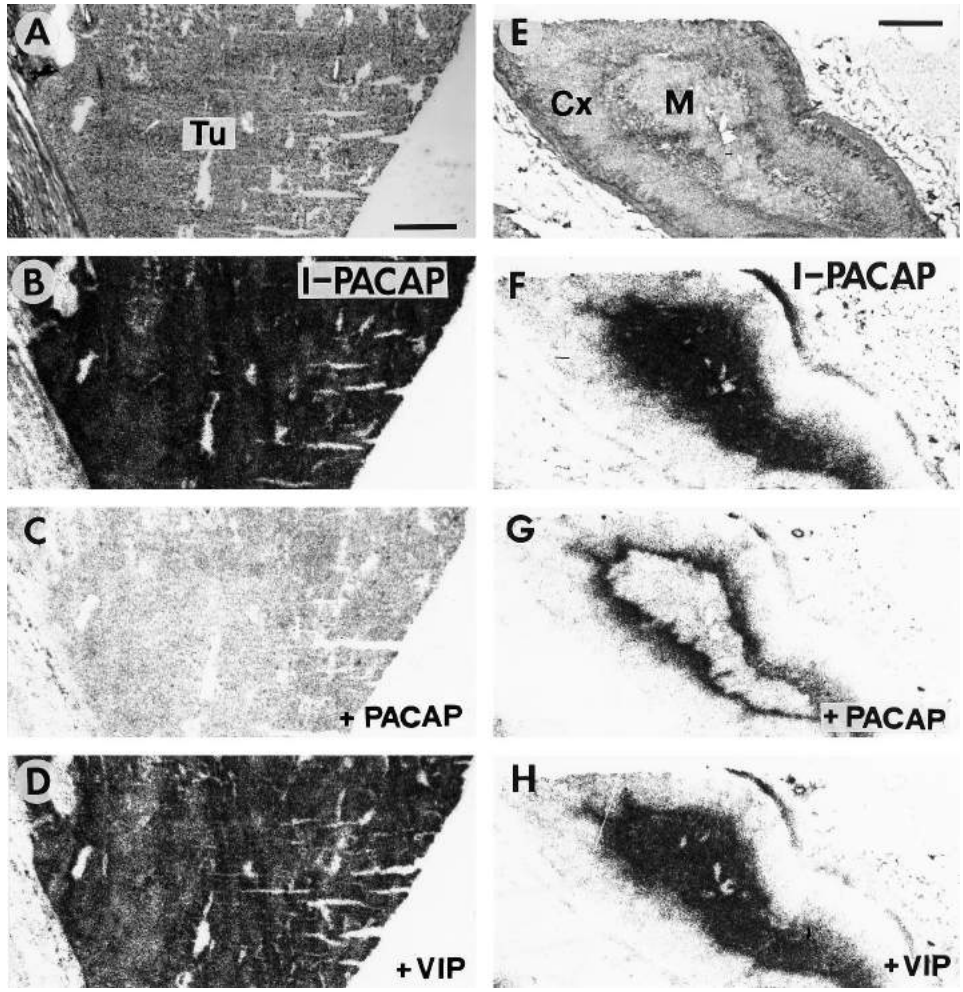

Fig. 6. PAC_1 receptors in a human pheochromocytoma (*A–D*) and its tissue of origin, the adrenal medulla (*E–H*). *A* and *E*, H&E-stained sections. *Tu*, pheochromocytoma; *Cx*, cortex; *M*, medulla. *Bars*, 1 mm. *B–D* and *G–H*, autoradiograms showing ^{125}I-PACAP binding. *B* and *F*, ^{125}I-PACAP total binding. *C* and *G*, binding in the presence of 20 nM PACAP. *D* and *H*, binding in the presence of 20 nM VIP. 20 nM PACAP but not VIP displaces the ligand in the pheochromocytoma and in the adrenal medulla, suggesting the presence of PAC_1.

radiotracers from the body. It is to be hoped, however, that a careful limitation of the radiation dose given to these vital organs may partly overcome the potential side effects.

VIP and PACAP can affect the growth of normal and neoplastic tissues. Whereas several groups have reported tumor growth-promoting activities of VIP and growth inhibition properties of VIP antagonists in various tumor models (6, 15, 27–29), recent evidence by Maruno *et al.* (7) has suggested that VIP itself may be an inhibitor of tumor growth under certain conditions. Based on the presence of VIP/PACAP receptors in the majority of the most common human tumors, the postulate to use high doses of a growth-inhibiting VIP/PACAP analogue (agonist or antagonist) is therefore highly attractive. One crucial question is whether there will be an equally good growth-inhibiting effect in human tumors as that seen in animal tumor models or cell cultures. The present study will help to choose the type of human cancer that will be most promising for clinical trials with VIP/PACAP.

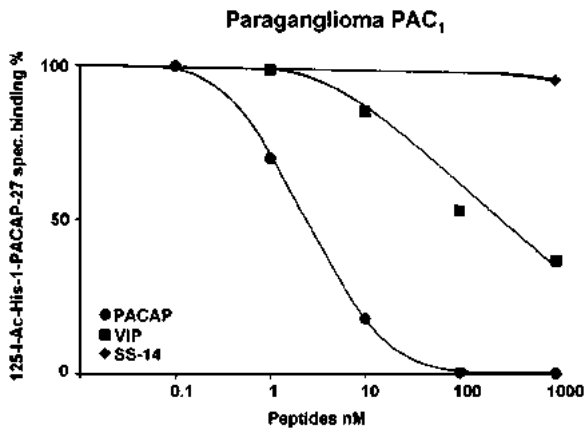

Fig. 7. High affinity displacement of ^{125}I-PACAP by PACAP but not VIP in a paraganglioma suggests the presence of PAC_1. Somatostatin (*SS-14*) is inactive.

Table 4 *VIP receptor density in breast cancer lymph node metastases compared with nonneoplastic lymph nodes*

Tissues	Density (dpm/mg tissue; mean ± SE)
Breast cancer metastases	2672 ± 311 (n = 11)
Nonneoplastic lymph nodes	5563 ± 562 (n = 13)

Clinical indications in which high doses of nonradioactive VIP/PACAP analogues are necessary for long-term peptide treatment should be considered carefully, due to the possible side effects related to the high number of VIP target tissues in the human body. To be able to predict such side effects, we need a better understanding of VIP/PACAP actions in various locations. Conversely, in diagnostic or radiotherapeutic indications where radiolabeled VIP/PACAP analogues can be used in very low peptide doses, a considerably lower potential risk of side effects due to undesired peptide actions may be expected. These latter indications may be an advantage when dealing with the VIP/PACAP system.

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Vasoactive Intestinal Peptide/Pituitary Adenylate Cyclase-activating Peptide Receptor Subtypes in Human Tumors and Their Tissues of Origin[1]

Jean Claude Reubi, Ursula Läderach, Beatrice Waser, et al.

Cancer Res 2000;60:3105-3112.