In Vitro Identification of Vasoactive Intestinal Peptide Receptors in Human Tumors: Implications for Tumor Imaging

Jean Claude Reubi

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

In vitro receptor measurements in tumors were performed to evaluate the potential of the vasoactive intestinal peptide receptor (VIP-R) as an imaging tool in human cancer. **Methods:** Three hundred thirty-nine human tumors were investigated for their VIP-R content by in vitro receptor autoradiography on tissue sections with ^{125}I-VIP. For comparison, somatostatin receptors (SS-R) were measured in adjacent sections of these tumors with ^{125}I-[Tyr3]-octreotide. **Results:** VIP-R were characterized and localized in the neoplastic cells of most breast carcinomas, breast cancer metastases, ovarian adenocarcinomas, endometrial carcinomas, prostate carcinomas, prostate cancer metastases, bladder carcinomas, colonic adenocarcinomas, pancreatic adenocarcinomas, gastrointestinal squamous cell carcinomas, non-small-cell lung cancers, lymphomas, astrocytomas, glioblastomas and meningiomas. Among neuroendocrine tumors, all differentiated and one-half of undifferentiated gastroenteropancreatic tumors, pheochromocytomas, small-cell lung cancers, neuroblastomas and inactive pituitary adenomas were found to express VIP-R. In general, VIP-R were found much more frequently than SS-R, but only 5 of 19 growth hormone-producing adenomas and no medullary thyroid carcinomas or Ewing sarcomas had VIP-R. In all tumors tested, the VIP-R were of high affinity and specific for VIP and pituitary adenylate cyclase-activating peptide. No cross-competition between VIP and SS could be identified. **Conclusion:** Most human carcinomas express VIP-R, as measured by in vitro receptor autoradiography. These data represent the molecular basis for evaluation of VIP-R imaging of these tumors in vivo and predict its great potential value.

Key Words: vasoactive intestinal peptide receptors; somatostatin receptors; neuropeptides; tumors; in vitro receptor autoradiography

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Radiolabeled peptides represent a category of substances of increasing interest for nuclear medicine (*1*). Indeed, neuropeptide receptors are frequently overexpressed in pathologic states, particularly in tumors (*2*). This has led to the development of in vivo imaging methods to localize certain types of tumors by targeting their neuropeptide receptors with the corresponding radioactively labeled neuropeptide (*3*). Somatostatin receptor (SS-R) imaging with ^{111}In-diethylaminetriaminepentaacetic acid (DTPA)-octreotide as the ligand (Octreoscan, Mallinckrodt Inc., St. Louis, MO) has recently become an established in vivo method to visualize SS-R-containing tumors (*4,5*) and other diseases (*6,7*). Although SS-R imaging has demonstrated its value, it remains clear that, unfortunately, several frequently occurring and very devastating tumors, i.e., pancreatic adenocarcinomas, colonic adenocarcinomas or nonsmall-cell lung cancer (NSCLC) do not express sufficient amounts of SS-R (*8*) and therefore cannot be visualized in vivo with this method (*5*).

Recently, another neuropeptide, the 28-amino acid long vasoactive intestinal peptide (VIP) (*9*), was shown to be a promising imaging agent. VIP-R have been identified with binding assays in various tumoral cell lines (*10–18*), and selected human tumors, i.e., neuroendocrine tumors, breast cancers and intestinal adenocarcinomas have been shown to express VIP receptors (VIP-R) in vitro in homogenates, as reported recently (*19*). Moreover, using ^{123}I-VIP as a ligand, Virgolini et al. (*20*) were able to localize in vivo the tumors of patients with gastrointestinal neuroendocrine tumors and pancreatic and colonic adenocarcinomas. The tumor types visualized in this study appear to be characterized by a high incidence of VIP-R.

The aim of the present study was to identify and characterize VIP-R pharmacologically in a large number of individual human tumors of various origins with VIP receptor autoradiography. In contrast to homogenate binding, this method has the advantage of being able to identify the site of receptor localization in the tissue (*8*). Moreover, it allows the evaluation of the incidence of VIP-R in the various tumor types investigated because each tumor is tested individually. In addition, the VIP receptor content in individual tumors was compared with their SS-R content. Such a study should

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For correspondence or reprints contact: J.C. Reubi, MD, Professor of Pathology, Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Murtenstrasse 31, CH-3010 Berne, Switzerland.

give the basis for an evaluation of the potential value of in vivo VIP-R imaging in a wide range of human tumors.

MATERIALS AND METHODS

Aliquots of surgically resected tumors or of biopsy specimens submitted for diagnostic histopathologic analysis were frozen immediately after surgical resection and stored at −70°C. The specimens originated from different clinical institutions, and some had previously been used for other purposes. The following tumors were investigated: colonic adenocarcinomas (n = 21); pancreatic adenocarcinomas (n = 12); squamous cell carcinomas of the esophagus (n = 4); breast carcinomas (n = 39), including metastases (n = 15); ovarian adenocarcinomas (n = 24); endometrial carcinomas (n = 12); prostate carcinomas (n = 32), including metastases (n = 7); bladder carcinomas (n = 4); lung tumors, including small-cell lung cancers (SCLC) (n = 4) and NSCLC (n = 12); brain tumors, including astrocytomas (n = 13), glioblastomas (n = 16), meningiomas (n = 16); neuroendocrine tumors, including pituitary adenomas (n = 38), endocrine gastroenteropancreatic (GEP) tumors (n = 17), medullary thyroid carcinomas (MTCs) (n = 14), pheochromocytomas (n = 18), neuroblastomas (n = 14); lymphomas (n = 19); and Ewing sarcomas (n = 10).

VIP Receptor Autoradiography

Receptor autoradiography was performed on 10- and 20-μm thick cryostat sections of the tissue samples, as described previously (*21,22*). The drug [^{125}I]-VIP (2000 Ci/mmole; Anawa, Windisch, Switzerland) was used as the radioligand. Only the mono ^{125}I-[Tyr10]-VIP, eluated as single peak by high-performance liquid chromatography and analyzed by mass spectrometry, was used. The tissues were cut on a cryostat, mounted on microscope slides and then 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 m*M* Tris HCl, pH 7.4, which contained bovine serum albumin (BSA) 2%, EGTA 2 m*M*, bacitracin 0.1 m*M* and $MgCl_2$ 5 m*M* to inhibit endogeneous proteases, in the presence of 30 p*M* [^{125}I]-VIP, at room temperature, as described previously (*23*). To estimate nonspecific binding, paired serial sections were incubated as described above, except that 1 μ*M* VIP (Bachem, Bubendorf, Switzerland) was added to the incubation medium. After this incubation, the slides were rinsed with four washes in ice-cold 50 m*M* Tris HCl, pH 7.4, with 0.25% BSA dipped in ice-cold water and then quickly dried in a refrigerator under a stream of cold air. The sections were subsequently exposed to a ^{3}H-Ultrofilm (Amersham, Buckinghamshire, UK) for 1 wk. Validation of the method was shown on rat brain, a tissue with a well-defined distribution of VIP-R. Moreover, in each experiment, rat brain was added as an internal, positive control.

In selected cases, displacement experiments with successive sections of a tumor were performed with increasing concentrations of various biologically active or inactive peptides (*21,22*). In addition to VIP, pituitary adenylate cyclase-activating polypeptide (PACAP), growth hormone releasing factor (GRF) and SS-14, SS-28 and octreotide were used.

The autoradiograms were quantified with a computer-assisted image-processing system, as previously described (*21,22*). Radiolabeled tissue sections were exposed to ^{3}H-Ultrofilms together with standards (Autoradiographic [^{125}I]microscales, Amersham) that contained known amounts of isotope, cross-calibrated to tissue-equivalent ligand concentration. The image analyzer was calibrated to the standards; it performed interpolation to read values that lay between those of the film standards. A tumor was considered as VIP-R positive when the optical density measured over a tissue area in the total binding section was at least twice that of the nonspecific binding section. This threshold for receptor positivity was previously validated for SS-R in studies in which SS-R status was shown to correlate with functional parameters (*24*).

SS Receptor Autoradiography

SS receptor autoradiography was performed on adjacent sections by the same method as described previously (*21,22*). Sections were incubated for 2 hr at ambient temperature in 170 m*M* Tris HCl buffer, pH 8.2, that contained BSA 1%, bacitracin 40 μg/ml and $MgCl_2$ 5 m*M* to inhibit endogenous proteases in the presence of 50 p*M* of ^{125}I-[Tyr3]-octreotide ligand. Nonspecific binding was determined by addition of the unlabeled peptide [Tyr3]-octreotide or octreotide at a concentration of 1 μ*M*. Incubated sections were washed twice for 5 min in cold buffer that contained 0.25% BSA. The sections were then washed in buffer alone and dried quickly, apposed to ^{3}H-Ultrofilms and exposed for 1 wk in radiographic cassettes.

In selected cases, displacement experiments with successive tumor sections incubated with a defined tracer concentration were performed with increasing concentrations (range 0.1–1000 n*M*) of octreotide, SS-14, SS-28 and VIP. Two peptides related to the VIP family, PACAP and GRF, were also tested. The data were expressed as percent of specific binding, in which nonspecific binding, defined as the binding in presence of 10^{-6} *M* unlabeled octreotide, was substracted from all values.

The in vitro SS-R measurements were always performed with ^{125}I-[Tyr3]-octreotide (*25*), not with labeled SS-14 or SS-28 analogs, to allow a direct comparison with in vivo SS-R imaging data, which was performed also with an octreotide analog (*5*).

RESULTS

VIP-R Localization and Incidence in Tumors

Most human tumors investigated in this study expressed VIP receptors (Table 1). A high incidence of VIP-R was found in breast, endometrial and ovarian cancers. As seen in Figures 1 and 2, the VIP-R were expressed in high density and homogeneously in these tumor tissues. Even in breast cancers known to be frequently polyclonal, the VIP-R were homogeneously distributed in all parts of the tumor (Fig. 2). Lymph node metastases of breast cancers were also VIP-R positive in all cases (Table 1). Breast tumors were VIP-R positive regardless of the histologic type because both ductal (n = 12) and lobular carcinomas (n = 27) express VIP-R. All bladder and prostate cancers, including all bone metastases from prostate cancer, were also receptor positive (Fig. 2). It should be noted that all samples of normal prostate epithelium expressed VIP-R in large amounts, as shown in Figure 2. Furthermore, all colonic adenocarcinomas and a significant number of the exocrine pancreatic carcinomas were receptor positive (Table 1). An example of each is shown in Figure 3 and demonstrates the homogeneous distribution of VIP-R. All squamous cell carcinomas of the esophagus were receptor positive (Table 1). Most nSCLC also expressed VIP-R. In most cases, the receptor density in the tumors was com-

TABLE 1
Percentage of Vasoactive Intestinal Peptide Receptor-Expressing Tumors Measured with In Vitro Autoradiography: Comparison with Somatostatin Receptor Status

Tumor	Type	VIP-R-positive tumors (% Incidence)	SS-R-positive tumors* (% Incidence)
Breast ca	P	24/24 (100)	14/24 (58)
	M	15/15 (100)	6/15 (40)
Ovarian ca		20/24 (83)	0/24 (0)
Endometrial ca		12/12 (100)	NT
Prostate ca	P	25/25 (100)	0/25 (0)
	M	7/7 (100)	NT
Bladder ca		4/4 (100)	0/4 (0)
Colon ca		21/21 (100)	7/21 (33)†
Pancreatic ca		9/12 (75)	0/12 (0)
Esophageal ca		4/4 (100)	0/4 (0)
Lung cancers			
nSCLC		9/12 (75)	1/12 (8)†
SCLC		3/4 (75)	3/4 (75)
Brain tumors			
Astrocytomas		13/13 (100)	10/13 (77)
Glioblastomas		12/16 (75)	1/16 (6)†
Meningiomas		15/16 (94)	16/16 (100)†
Neuroendocrine tumors			
GEP tumors			
Differentiated		11/11 (100)	11/11 (100)
Undifferentiated		3/6 (50)	0/6 (0)
Pheochromocytomas		10/18 (55)	13/18 (72)
MTC		0/14 (0)	6/14 (43)
Neuroblastomas		8/14 (57)	12/14 (86)
Pituitary adenomas			
GH producing		5/19 (26)	19/19 (100)†
Inactive		16/19 (84)	8/19 (42)
Lymphomas		11/19 (58)	17/19 (89)
Ewing's sarcoma		0/10 (0)	0/10 (0)†

*SS-R measured with ^{125}I-[Tyr3]-octreotide.
†These results were taken from previous studies.
ca = carcinoma; P = primary; M = metastasis; GH = growth hormone; NT = not tested; nSCLC = non-small-cell lung cancer; SCLC = small-cell lung cancer; GEP = gastroenteropancreatic; MTC = medullary thyroid carcinomas.

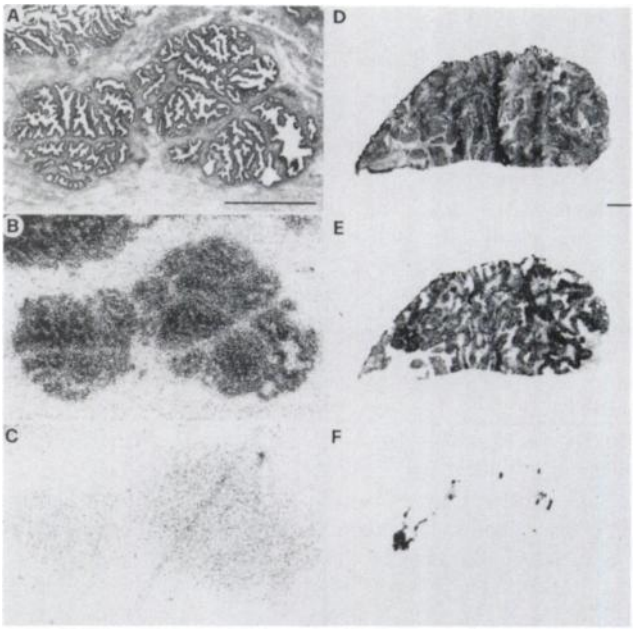

FIGURE 1. VIP-R in ovarian (A–C) and endometrial (D–F) carcinomas. (A, D) Hematoxylin-eosin-stained sections (bar = 1 mm). (B, E) Autoradiograms showing total binding of ^{125}I-VIP. Notice strong labeling of tumor tissue. (C, F) Autoradiograms showing nonspecific binding of ^{125}I-VIP (in the presence of 10^{-6} *M* VIP).

parable to that of the surrounding normal lung. Most astrocytomas and meningiomas and three quarters of the glioblastomas were also positive, usually with a high density of VIP-R. Normal human brain adjacent to the tumor was, as expected, also receptor positive. Most of the neuroendocrine tumors were all positive; all differentiated hormone-producing GEP tumors and one half of the undifferentiated GEP tumors were positive, in addition to many pheochromocytomas, SCLC and neuroblastomas (Table 1). All MTCs were negative in this study. Growth-hormone (GH)-producing pituitary adenomas rarely expressed the receptors and inactive pituitary adenomas, frequently. Two thirds of the lymphomas were VIP-R positive. Six of seven solid non-Hodgkin lymphomas, three of six follicular non-Hodgkin lymphomas and three of six Hodgkin lymphomas were receptor positive. These receptors were usually expressed in low density. The fact that the surrounding lymphocytes and venules always expressed VIP-R, usually more strongly than the tumor itself (Fig. 4), made it difficult in

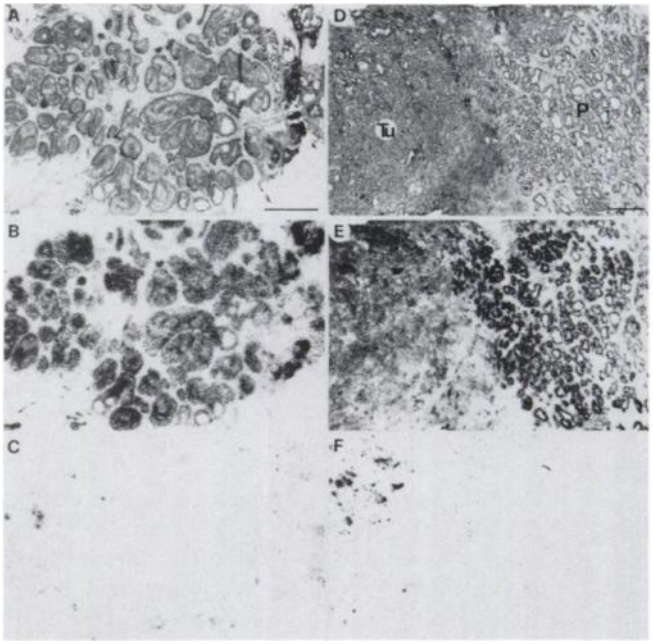

FIGURE 2. VIP-R in a breast (A–C) and prostate carcinoma (D–F). (A, D) Hematoxylin-eosin-stained sections. In D, one part is tumor tissue (Tu) and the other is normal prostate (P) (bar = 1 mm). (B, E) Autoradiograms demonstrate total binding of ^{125}I-VIP. Breast carcinoma, prostate carcinoma and the prostate are heavily and homogeneously labeled. (C, F) Autoradiograms demonstrate nonspecific binding of ^{125}I-VIP.

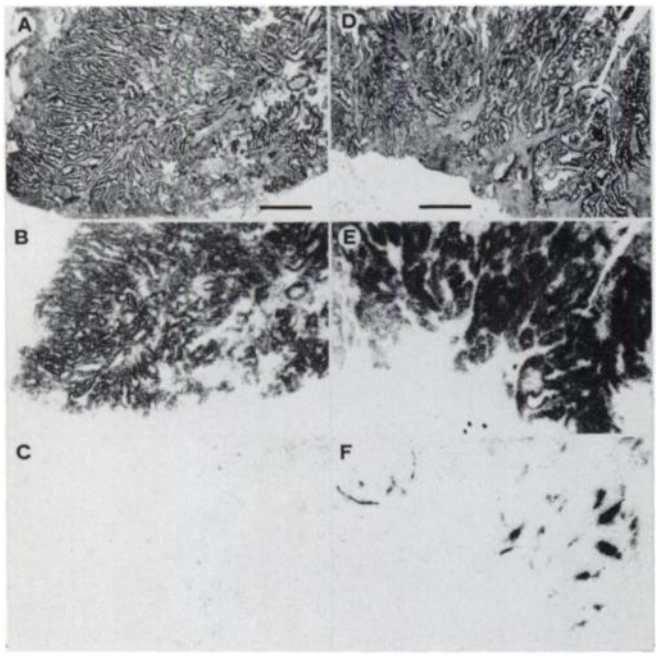

FIGURE 3. VIP-R in pancreatic (A–C) and colonic (D–F) adenocarcinomas. (A, D) Hematoxylin-eosin-stained sections (bar = 1 mm). (B, E) Autoradiograms showing total binding of ^{125}I-VIP. (C, F) Autoradiograms showing nonspecific binding of ^{125}I-VIP.

some cases to evaluate the receptor status in the tumor tissue. This was particularly true for the follicular lymphomas (Fig. 4), whereas the solid type of lymphomas, with a more homogeneous distribution of tumor tissue, were easier to evaluate. Ewing's sarcomas were all negative (Table 1).

Pharmacologic Characteristics of VIP-R

The VIP-R were of high affinity and specific for VIP and PACAP in the various types of cancers investigated. As seen in the four examples of Figure 5, displacement experiments in pancreatic, colonic, endometrial and prostate adenocarcinomas showed complete displacement of ^{125}I-VIP by unlabeled VIP and PACAP in the nanomolar range; 100 and 1000 n*M* of octreotide, SS-14 and SS-28 were inactive in the displacement of ^{125}I-VIP. The identified VIP-R probably correspond to the PACAP type II receptor subtype (*26*).

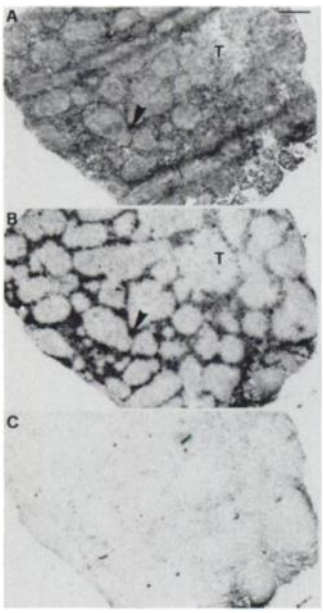

FIGURE 4. VIP-R in low-grade, follicular NHL. (A) Hematoxylin-eosin-stained section. Arrowheads identify stroma with lymphocytes and high endothelial venules surrounding tumoral follicles (T) (bar = 1 mm). (B) Autoradiogram demonstrates total binding of ^{125}I-VIP. Stroma, but not tumor (T), is labeled. (C) Autoradiogram shows nonspecific binding of ^{125}I-VIP.

Comparison of VIP-R and SS-R

The VIP-R status was compared in most of the tested cancers with the SS-R status performed on adjacent tissue sections. As seen in Table 1, significantly more tumors express VIP-R than (octreotide-preferring) SS-R. This was particularly true for ovarian, prostate, bladder and pancreatic cancers but also for colonic and breast cancers. Also glioblastomas, nSCLC and squamous cell carcinomas of the esophagus were much more frequently VIP-R positive than SS-R positive. Other tumors, such as meningiomas or astrocytomas had a similar expression of both receptors. Most GEP tumors expressed both receptors; however, it should be noted that several SS-R negative, undifferentiated "atypical" carcinoids were VIP-R positive (Table 1). SCLC and pheochromocytomas expressed both VIP and SS receptors, whereas MTC only expressed SS-R. Neuroblastomas and lymphomas had a lower incidence of VIP-R than SS-R. A trend toward an inverse relationship was seen within the pituitary adenomas (Table 1). In general, the tumoral VIP-R distribution, compared with the SS-R distribution in the same tumor, was often found to be more homogeneous over the whole tumor tissue. This was particularly true in breast cancers, which are characterized by a frequent nonhomogeneous SS-R distribution (*27*).

VIP-R in Nontumoral Tissues

As mentioned previously, VIP-R were also identified in several nontumoral tissues present in the tested tumor samples. Not only normal brain, lung and prostate tissues express VIP-R but also liver, gastrointestinal mucosa and smooth muscles, lymphocytes and vessels in several locations. Figure 6 shows gastrointestinal mucosa and submucosal vessels, which express VIP-R in a colonic carcinoma sample. It is also worth mentioning that the lymph nodes invaded by VIP-R-positive breast tumor metastases expressed VIP-R in the remaining normal, lymphocyte-rich areas.

Lack of Cross-Competition Between SS and VIP

Despite the fact that many tumors simultaneously expressed VIP-R and SS-R, it was not possible to detect any cross-competition between VIP and SS. As seen in Figure 7, a case of VIP-R- and SS-R-positive carcinoid, SS analogs did not displace ^{125}I-VIP binding nor did VIP or PACAP displace ^{125}I-[Tyr3]-octreotide binding.

Another example in which a VIP-R- and SS-R-positive breast tumor revealed identical results, were shown directly on autoradiograms (Fig. 8). Furthermore, Table 2 shows the list of further tumors in which a cross-competition between VIP and SS was ruled out. Such a cross-

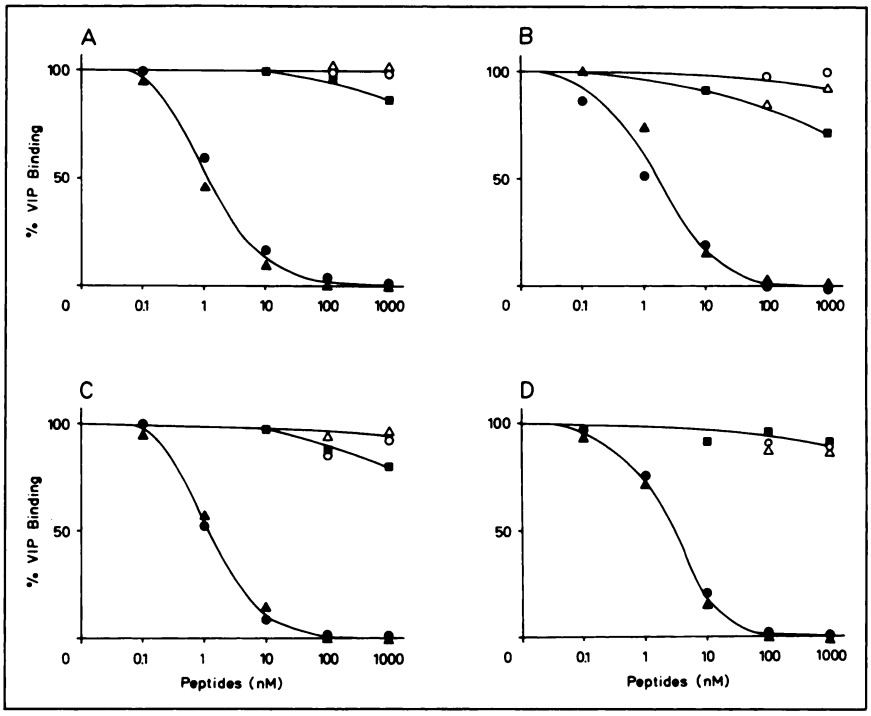

FIGURE 5. Displacement curves of specific ^{125}I-VIP binding to tissue sections from four different tumors. (A) Pancreatic adenocarcinoma. (B) Colonic adenocarcinoma. (C) Endometrial carcinoma. (D) Prostatic carcinoma. Tissue sections were incubated with 14,000 cpm/100 μl ^{125}I-VIP and increasing concentrations of unlabeled VIP (●), PACAP (▲), GRF (■), SS-14 (△) or octreotide (○). Each point represents the optical density of binding measured in the tumor area. Nonspecific binding was subtracted from all values. In all cases, high-affinity displacement of ligand is achieved by VIP and PACAP, but GRF, SS-14 or octreotide were inactive in the nanomolar range.

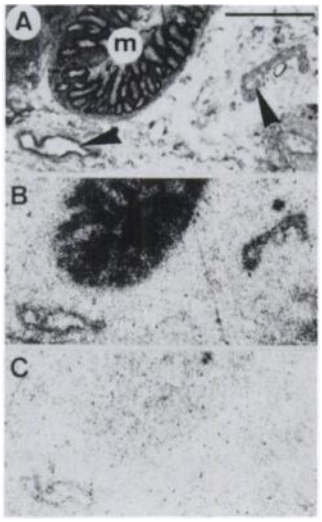

FIGURE 6. VIP-R in submucosal vessels near colonic adenocarcinoma. (A) Hematoxylin-eosin-stained section. m = mucosa, arrowheads = submucosal vessels (bar = 1 mm). (B) Autoradiogram showing total binding of ^{125}I-VIP. Mucosa and vessels are labeled. (C) Nonspecific binding.

competition was found neither in tumors that expressed both receptors nor in tumors that expressed only VIP-R or only SS-R.

DISCUSSION

The present in vitro study characterized and localized VIP-R in human tumors. It demonstrated that the incidence of VIP-R is extremely high in a large variety of tumors. These receptors were expressed in adenocarcinomas of the breast, endometrium, ovary, colon, pancreas, prostate, bladder and lung. They were also expressed in squamous cell carcinomas of the esophagus and of the lung, in neuroendocrine tumors and in brain tumors. The variety of human primary tumors that express VIP-R and the incidence of VIP-R-positive tumors were higher than previously known for other neuropeptide receptors (*2*). Therefore, the present results are not only relevant for tumor biology in general but should affect further applica-

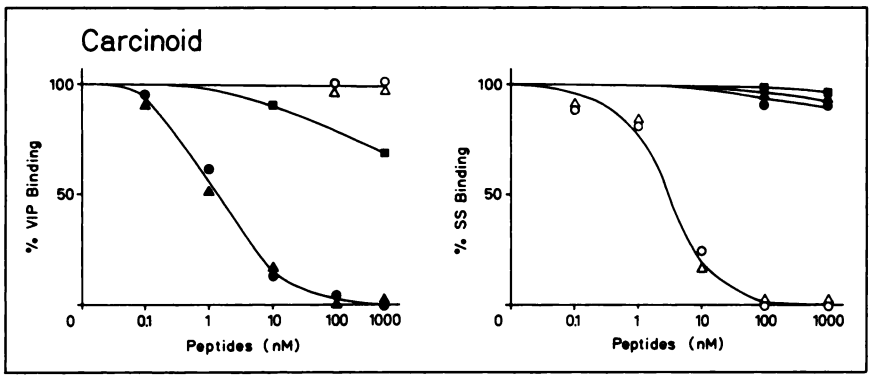

FIGURE 7. Lack of cross-competition in VIP-R- and SS-R-containing carcinoid. Displacement experiment on tissue sections with ^{125}I-VIP shows that VIP (●) and PACAP (▲) displace the ligand with high affinity, whereas GRF (■) is less active and SS-14 (▲) or octreotide (○) are inactive. Similarly, the displacement experiment with ^{125}I-[Tyr^3]-octreotide demonstrates that SS-14 (△) or octreotide (○) displaces the ligand with high affinity, whereas GRF (■), VIP (●) and PACAP (▲) are inactive. Nonspecific binding was subtracted from all values.

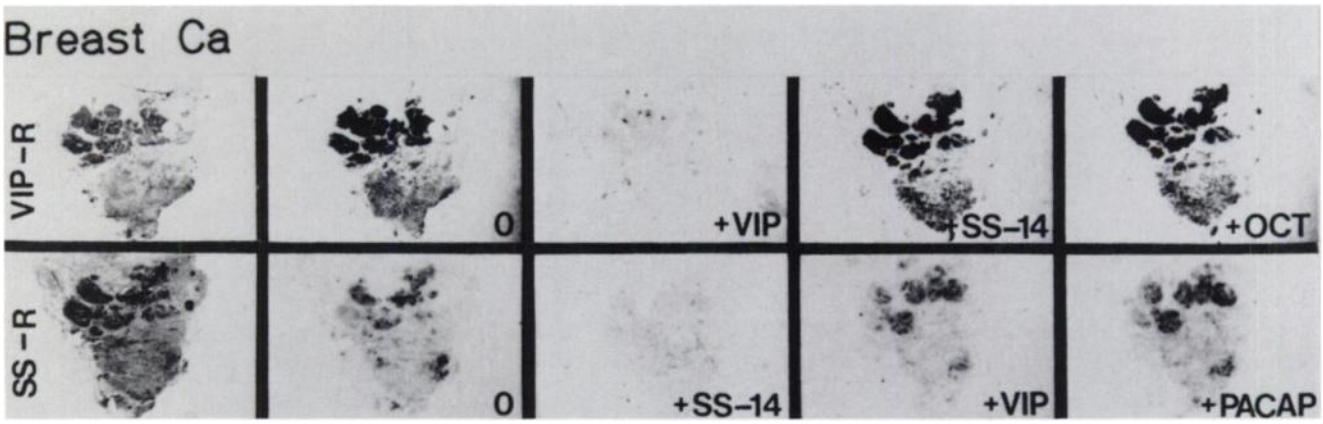

FIGURE 8. Lack of cross-competition in VIP-R- and SS-R-containing breast carcinomas. (Top) VIP-R. Total binding (○) of ^{125}I-VIP on tumor tissue is displaced by 1000 n*M* VIP but not by 1000 n*M* SS-14 or octreotide (OCT). (Bottom) SS-R. Total binding (○) of ^{125}I-[Tyr3]-octreotide on tumor tissue is displaced by 1000 n*M* SS-14 but not by 1000 n*M* VIP or PACAP (far left) stained sections.

tions of ^{123}I-VIP radioligands or other radiolabeled VIP analogs in nuclear medicine. For example, the 100% incidence of VIP-R in breast and prostate cancer metastases should warrant a high success rate of VIP-R imaging in the detection of such metastases. The present observations, therefore, represent the molecular basis for the evaluation of the potential usefulness of VIP-R in vivo imaging in a wide range of human cancer types.

The use of in vitro receptor autoradiography allows the clear identification of the type of tissue that expresses the receptors, i.e., the tumor itself on one side, the surrounding healthy tissue or infiltrating lymphocytes on the other side. Such histologic confirmation is essential when heterogeneous tissue samples such as tumor biopsy specimens are analyzed. Homogenate binding assays could indeed give false-positive results, i.e., a receptor-negative tumor tissue contaminated with healthy tissue or infiltrating blood cells that expressed the investigated receptor might falsely be considered as positive. This aspect is particularly important when VIP-R are evaluated because so many normal tissues, i.e., lung, gut mucosa, liver, brain and prostate (*28–32*), but also various inflammatory cells (*33*), express VIP-R.

VIP-R are of high affinity and specific for VIP. In the various tumors tested, VIP and PACAP could displace the iodinated natural ligand, ^{125}I-VIP, in the low nanomolar range. Peptides not related to the VIP family, such as the various SS analogs tested, i.e., octreotide, SS-14 or SS-28, were all inactive in the displacement of VIP binding at low concentrations. The reverse was also true, i.e., compounds of the VIP family such as VIP, PACAP or GRF, were unable to displace ^{125}I-[Tyr3]-octreotide binding. The investigated receptor subtypes in this study were most likely the PACAP II subtype (with high affinity for VIP and PACAP) on one hand and the SSTR2 and/or SSTR5 (with high affinity for octreotide) subtypes on the other hand. A cross-competition between other subtypes cannot completely be excluded, although the lack of cross-competition

TABLE 2
Lack of Cross-Competition between Vasoactive Intestinal Peptide and Somatostatin

Tissue*	^{125}I-[Tyr3]-octreotide binding		^{125}I-VIP binding	
	IC_{50} for VIP (n*M*)	IC_{50} for PACAP (n*M*)	IC_{50} for SS-14 (n*M*)	IC_{50} for octreotide (n*M*)
Tumoral				
Carcinoids, colon ca, islet cell ca, breast ca, neuroblastomas, pheochromocytomas, prostate ca, ovarian ca, endometrial ca, astrocytomas, pituitary adenomas	>1000	>1000	>1000	>1000
Nontumoral				
Gut mucosa, gut vessels, prostate, peritumoral vessels	>1000	>1000	>1000	>1000

*Three to eight different samples of each tissue type listed were investigated for SS-R or VIP-R binding. IC_{50} values for the four peptides were calculated. In these experiments, the displacement capacity of each peptide was compared with the five- to seven-point displacement curve of the radioligand by its corresponding unlabeled analog. Nonspecific binding: 10^{-6} *M* of unlabeled octreotide and VIP, respectively.

ca = carcinoma; IC_{50} = peptide dose required for 50% binding inhibition, PACAP = pituitary adenylate cyclase-activating polypeptide; SS-14 = somatostatin analog.

seen with SS-28, which binds with high affinity to all SS-R subtypes, speaks against it. A lack of cross-competition is of considerable importance for the in vivo specificity of the imaging signal given either by ^{111}In-DTPA-octreotide or by ^{123}I-VIP. This high specificity explains why it is possible to identify (with labeled ^{111}In-DTPA-octreotide) tumors in vivo that express SS receptors but not tumors that express only VIP-R such as pancreatic adenocarcinomas (*19*). Conversely, it explains why ^{123}I-VIP can identify tumors that express VIP receptors but not tumors that express only a high density of SS-R, such as GH-producing pituitary adenomas (*34*). The present results are, however, in contradiction with those of a recent study that measured VIP-R in tumoral homogenates, where VIP and SS were shown to cross-compete at comparable nanomolar concentrations (*19*). I cannot explain this discrepancy. If such a VIP-SS cross-competition does exist, all tumors that express SS-R would be expected to be similarly labeled in vivo with radioactive octreotide or VIP and all tumors that express VIP-R would also be labeled indifferently by VIP or by SS ligands. Obviously, this is not the case (*5,20*). One could, however, speculate that, in certain tumors, a receptor-receptor interaction takes place between VIP-R and SS-R, perhaps through a common G protein, which implies changes in the affinity state of one receptor when the other is activated. Possibly, the use of receptor transfectants may help investigators to understand this intriguing problem further.

In vivo SS-R imaging with ^{111}In-DTPA-octreotide can presently be considered the standard reference for neuropeptide receptor imaging because it is the first and the best documented method that uses such a neuropeptide radioligand in vivo (*5*). The comparison of the VIP-R status with the SS-R status (octreotide binding) in the present series of tumors is, therefore, of particular interest because it reveals the great potential of VIP-R for tumor imaging. Indeed, a large number of frequently occurring tumors do express VIP-R but not SS-R, at least not the octreotide-preferring SS-R. Most impressive examples are ovarian, endometrial, colonic and pancreatic adenocarcinomas and lung, bladder and prostate cancers. These tumors not only have a high incidence but usually also a moderate to high density of VIP-R, which makes in vivo imaging of such tumors very likely. Breast tumors, which are known to express SS-R nonhomogeneously in many patients (*27*), appear to express VIP-R diffusely over the whole tumor tissue; this is advantageous for tumor imaging. Ductal and lobular breast tumors, which were all VIP-R positive in this series, and their metastases, belong, therefore, to the tumors with a great potential for detection with in vivo VIP-R imaging.

Only a small number of the tested tumor types did not, or only rarely, expressed VIP-R. Ewing's sarcomas, which do not express SS-R either, belong to this group as do MTC; it should, however, be noted that MTC show a very high nonspecific ^{125}I-VIP binding to the secretory products of the gland, which makes the identification of a small amount of VIP-R difficult. Nevertheless, the lack of VIP-R in MTC, but the well-established SS-R expression in these tumors, suggests that MTC probably represent a group of neuroendocrine tumors that should preferentially be scanned with SS-R imaging. Another tumor type, the SS-R-expressing, GH-producing pituitary adenoma, does not frequently express VIP-R, whereas a tumor that originates in the same organ, the endocrine inactive pituitary adenoma, expresses VIP-R in virtually all cases. Although GH-producing adenomas are ideally visualized with SS-R imaging, the latter tumor type represents an excellent potential candidate for VIP-R imaging.

Because several normal human tissues, i.e., lung, brain, gut and lymphoid tissues, express VIP-R, it is an important issue to elucidate whether or not these receptors will interfere with the in vivo imaging of VIP-R-positive tumors. Interestingly, SS-R are also known to be expressed in high amounts in several normal tissues, such as brain, gut or lymphoid tissues; nevertheless, the available SS-R imaging studies have shown that the SS-R in normal tissues do not, or only minimally, interfere with the tumor imaging (*5,35*). By analogy, the recent VIP-R imaging study permitted clear in vivo identification of most gastrointestinal tumors despite VIP-R expression in the normal gut (*20*). This is an encouraging sign suggesting that, for a yet unclear reason, the normal VIP-R-positive (gut) tissues are not sufficiently labeled in vivo to interfere with the imaging of VIP-R-positive tumors significantly.

The functional role played by these tumoral VIP-R needs further investigation, although there is information emerging about the VIP regulation of cell proliferation and of tumor growth in various cell line systems. For instance, VIP was shown to stimulate the growth of glial cells, keratinocytes, lung carcinomas and mammary tumor cells in culture (*36–40*). Recently, a VIP antagonist was able to inhibit the growth of various nSCLC cell lines (*41*). These data suggest that VIP may have proliferative actions and that VIP antagonists may be promising therapeutic tools to inhibit tumor growth in the VIP-R-expressing tumors described in the present study.

CONCLUSION

This in vitro study provides evidence that a high incidence of VIP-R occurs in breast, ovarian, endometrial, prostate, bladder, lung, esophageal, colonic and pancreatic cancers and in neuroendocrine and brain tumors. This suggests that labeled VIP analogs may be promising candidates for VIP-R imaging of these tumors in vivo.

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