Concomitant expression of several peptide receptors in neuroendocrine tumours: molecular basis for in vivo multireceptor tumour targeting

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Abstract. Peptide receptors have been found to represent excellent targets for in vivo cancer diagnosis and therapy. Recent in vitro studies have shown that many cancers can overexpress not only one but several peptide receptors concomitantly. One of the challenges for nuclear medicine in this field in the coming decade will be to take advantage of the co-expression of peptide receptors for multireceptor tumour targeting. In vitro receptor studies can reveal which peptide receptor is overexpressed in which tumour and which receptors are coexpressed in an individual tumour; such knowledge is a prerequisite for successful in vivo development. One group of tumours of particular interest in this respect is the neuroendocrine tumours, which have previously been shown often to express peptide receptors. This review summarises our investigations of the concomitant expression of 13 different peptide receptors, in more than 100 neuroendocrine tumours of the human intestine, pancreas and lung, using in vitro receptor autoradiography with subtype-selective ligands. The incidence and density of the somatostatin receptors sst_1–sst_5, the VIP receptors $VPAC_1$ and $VPAC_2$, the CCK_1 and CCK_2 receptors, the three bombesin receptor subtypes BB_1 (NMB receptor), BB_2 (GRP receptor) and BB_3, and GLP-1 receptors were evaluated. While the presence of $VPAC_1$ and sst_2 was detected in the majority of these neuroendocrine tumours, the other receptors, more differentially expressed, revealed a characteristic receptor pattern in several tumour types. Ileal carcinoids expressed sst_2 and $VPAC_1$ receptors in virtually all cases and had CCK_1, CCK_2, sst_1 or sst_5 in approximately half of the cases; they were the only tumours of this series to express NMB receptors. Insulinomas were characterised by a very high incidence of GLP-1, CCK_2 and $VPAC_1$ receptors, with the GLP-1 receptors expressed in a particularly high density; they expressed sst_2 in two-thirds and sst_1 in approximately half of the cases and lacked CCK_1 and NMB receptors. All gastrinomas had sst_2 and GLP-1 receptors; they expressed GRP receptors in three-quarters of the cases and CCK_1 or $VPAC_1$ in approximately half of the cases. Most bronchial carcinoids had $VPAC_1$, while sst_1, sst_2 and CCK_2 were found in two-thirds of the cases and BB_3 in one-third of the cases. These data provide evidence for the vast biological diversity of these neuroendocrine tumours. Moreover, the results represent a basis for starting and/or optimising the in vivo targeting of these tumours by selecting the suitable radiopeptides for tumour diagnosis and/or therapy. Finally, the data strongly encourage concomitant application of several radiopeptides to permit more efficient targeting of these tumours.

Keywords: Peptide receptors – Neuroendocrine tumours – Tumour targeting – Somatostatin

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Introduction

The presence of somatostatin receptors in neuroendocrine tumours of the intestine, pancreas and lung has led to development of the field of somatostatin receptor targeting in oncology, at both the diagnostic [1] and the therapeutic level [2]. The success of this novel approach has also triggered interest in studying the in vitro expression of other peptide receptors, e.g. vasoactive intestinal peptide (VIP) receptors, cholecystokinin (CCK) receptors and bombesin receptors [3, 4, 5], and in evaluating their potential for peptide receptor targeting in vivo [6, 7, 8]. Specifically, neuroendocrine tumours can express

various peptide receptors [3, 9, 10], apart from somatostatin receptors [11].

Up to now, however, the peptide receptor most frequently targeted in vivo has been the somatostatin receptor. Various somatostatin radioligands have been used for this purpose, with different levels of success. Octreoscan has been considered the gold standard for detection of somatostatin receptors in many neuroendocrine tumours [12, 13]; other radiotracers, such as ^{111}In-DOTA-lanreotide or ^{99m}Tc-P829, are being used less frequently, due in part to a lower sensitivity and higher background [14]. However, even Octreoscan, which binds primarily to sst_2 receptors, does not allow the detection of every neuroendocrine tumour: while virtually all gastrinomas and their metastases can be precisely visualised [12], a much lower percentage of insulinomas can be identified with this method [13]. It has been argued that this may be due to the lower frequency of sst_2 receptor expression in insulinomas [12]. These data indicate that the success of in vivo somatostatin receptor targeting is very much dependent on the presence in the tumour of the appropriate receptor subtype in a sufficient amount and on the particular receptor affinity profile of the used radioligand.

Only a very small number of studies have tried to visualise neuroendocrine tumours through peptide receptors other than somatostatin receptors. It has been shown that VIP receptor scintigraphy is able to detect gut neuroendocrine tumours [6]. However, the high background over many VIP receptor-positive tissues, such as lung, and the very unstable radioligand are likely to prevent successful development of this technique. In addition, in vivo CCK and bombesin receptor scintigraphy, although not yet evaluated in gut neuroendocrine tumours, have successfully been used to target medullary thyroid cancers [7] and prostate and breast cancers [8], respectively.

For each of these peptide receptors, the proof of principle has been established that their respective radioligands can be used, separately, to successfully target tumours. As a further step, it is tempting to speculate that the tracers may also be used as a cocktail to target several co-expressed peptide receptors in a single tumour, in order to obtain a much more efficient and powerful means of diagnosis and therapy. A prerequisite for such successful in vivo development is knowledge of which receptor is expressed in which tumour and which receptors are co-expressed in an individual tumour. Recently, taking breast cancers as example, in vitro studies have reported the concomitant expression of several of these peptide receptors [15], in particular gastrin-releasing peptide (GRP) receptors and neuropeptide Y (NPY) Y_1 receptors, in individual tumours. As neuroendocrine tumours are known to express various peptide receptors, it may be of particular interest to know the extent of peptide receptor co-expression in these types of tumour.

The present review summarises the data obtained in a large number of neuroendocrine tumours of the intestine, pancreas and lung in which we evaluated the concomitant expression of various peptide receptors that are of established or potential interest in nuclear medicine and oncology, namely somatostatin, VIP, CCK, bombesin and glucagon-like peptide (GLP) receptors. Because most of these peptide receptors exist as multiple subtypes [16, 17, 18, 19], it is crucial to evaluate as many of the subtypes as possible; for this study, these are the five somatostatin receptor subtypes sst_1–sst_5 [11], the three bombesin receptors, namely BB_1 [or neuromedin B (NMB) receptors], BB_2 (or GRP receptors) and BB_3 receptors [10], the CCK_1 and CCK_2 receptors [3], the VIP receptor subtypes $VPAC_1$ and $VPAC_2$ [9] and, finally, the GLP-1 receptors [20]. The choice of a series of more than 100 gastroenteropancreatic and lung neuroendocrine tumours, including bronchial carcinoids, ileal carcinoids and functioning neuroendocrine pancreatic tumours consisting of insulinomas, gastrinomas, glucagonomas and vipomas, was made on the basis that these tumours have previously been shown often to express, individually, various somatostatin receptor subtypes [11], as well as VIP receptors [9] or CCK receptors [3]. Moreover, the bombesin receptor subtypes have recently been found to be expressed differently in these types of tumour, with GRP receptors preferentially found in gastrinomas, NMB receptors in gut carcinoids and BB3 in lung carcinoids [10]. Furthermore, GLP-1 receptors, although never investigated in human cancers, have previously been shown to be expressed in rat insulinomas. In vitro information on concomitant receptor expression in these tumours not only should allow the nuclear physician to choose the appropriate radiopeptides for optimal targeting of the respective tumours, but also may give a better insight into the pathobiological behaviour of these different neuroendocrine tumours.

Methodological aspects

Which in vitro methodology and which parameters are best able to yield the required receptor information? It is likely that a method detecting proteins is more relevant than one detecting mRNA. A method that can quantify the number of receptors is also of prime importance. Further, the method should be sensitive enough to detect small amounts of receptors. Finally, the method should preferably identify the receptor binding sites. Among the available techniques, the first choice is likely to be in vitro receptor autoradiography, a highly sensitive method that has the advantage of identifying and quantifying peptide receptor proteins rather than the mRNA [21]. Moreover, it recognises the binding sites of the receptor protein that correspond precisely to the molecular targets reached by the radioligands, as used in vivo by nuclear physicians both for diagnosis and for therapy of tumours. It is also possible and advantageous to use subtype-selective receptor autoradiography to identify the various peptide receptor subtypes [3, 9, 10, 11].

In this study, frozen neuroendocrine tumours of the intestine, pancreas and lung, including 27 ileal carcinoids (most of them metastatic to lymph nodes and/or liver), 29 bronchial carcinoids, 27 insulinomas, 10 gastrinomas, 4 glucagonomas and 4 vipomas, were cut into 20-µm-thick successive cryostat sections and prepared to be used for in vitro receptor autoradiography of the various peptide receptors, as described below. Subtype-selective somatostatin receptor autoradiography was performed as described recently [11] using ^{125}I-[Leu^{8}, D-Trp^{22}, Tyr^{25}]-somatostatin-28 (^{125}I-LTT-SS-28; 2,000 Ci/mmol; Anawa, Wangen, Switzerland) as radioligand and the following sst-selective analogues: the sst_1-selective CH288, the sst_2-selective L-779-976, the sst_3-selective sst_3-ODN-8, the sst_4-selective L-803,087 and the sst_5-selective L-817,818 [11]. Also subtype-selective VIP receptor autoradiography was performed as described previously [9] using ^{125}I-VIP (2,000 Ci/mmol; Anawa, Wangen, Switzerland) as radioligand with the $VPAC_1$-selective [K^{15}, R^{16}, L^{27}]VIP(1–7)/GRF(8–27) and the $VPAC_2$-selective Ro25-1553. Subtype-selective CCK receptor autoradiography was performed as described previously [3] using ^{125}I-[D-Tyr-Gly, Nle^{28,31}]-CCK26–33 (^{125}I-CCK; 2,000 Ci/mmol; Anawa, Wangen, Switzerland) as radioligand, displaced with CCK-8 and/or gastrin to discriminate between CCK_1 and CCK_2 receptors. Subtype-selective bombesin receptor autoradiography was performed using ^{125}I-[D-Tyr^{6}, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin(6–14) (2,000 Ci/mmol; Anawa, Wangen, Switzerland) as radioligand [10] and unlabelled GRP, NMB and [D-Tyr^{6}, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin(6–14) to discriminate between GRP, NMB and BB_3 receptors. GLP-1 receptor autoradiography is briefly summarised below, as it has not been published previously. Twenty-micrometre-thick sections were incubated for 2 h at ambient temperature in the presence of 32 p*M* ^{125}I-GLP-1 (2,000 Ci/mmol; Anawa, Wangen, Switzerland). The incubation solution was 170 m*M* Tris-HCI buffer (pH 8.2) containing 1% bovine serum albumin, bacitracin (40 µg/ml) and $MgCl_2$ (10 m*M*) to inhibit endogenous proteases. Non-specific binding was determined by adding 100 n*M* solution of unlabelled GLP-1. Incubated sections were washed twice for 5 min in cold incubation buffer containing 0.25% bovine serum albumin, then in buffer alone, and dried quickly. Finally, the sections were apposed to Biomax MR films (Kodak) and exposed for 1 week in X-ray cassettes. In selected cases, displacement experiments were performed in successive tissue sections using increasing concentrations of GLP-1, GLP-2, exendin 4 and glucagon 1–29 (Bachem, Bubendorf, Switzerland), in order to identify the GLP-1 receptor subtype.

In all experiments, the autoradiograms were quantified using a computer-assisted image processing system, as described previously [9, 22]. Tissue standards for iodinated compounds (Amersham, Aylesbury, UK) were used for this purpose. A tissue was defined as receptor-positive when the absorbance measured in the total binding section was at least twice that of the non-specific binding section. When multiple peptide receptor subtypes of a single family were detected in a tumour, only those present in a density equal to or higher than 10% of the density of the most abundantly expressed receptor subtype in that tumour were considered positive. Moreover, in tumours expressing sst_1 and sst_5 simultaneously, it was necessary to take into account the cross-reactivity of the sst_5-selective L-817,818 with sst_1 and to correct the sst_5 value measured at 10 n*M* L-817,818 by subtracting 15% of the sst_1 density value measured in that tumour [11]. Finally, it should be remembered that it cannot be completely excluded, by using subtype-selective receptor autoradiography with universal radioligands, that a receptor subtype expressed in very low amounts may be masked by another subtype expressed in very high density in the same tumour.

Incidence and density of peptide receptors in neuroendocrine tumours

Tables 1, 2 and 3 report the incidence and density of the 13 peptide receptors investigated in each individual tumour tested in this study, i.e. in ileal carcinoids (Table 1), functioning pancreatic neuroendocrine tumours (Table 2) and bronchial carcinoids (Table 3). We did not find a single neuroendocrine tumour that did not express at least one of these peptide receptors. In most cases, several peptide receptors were concomitantly expressed. While the great majority of the tested tumours expressed $VPAC_1$ and sst_2, the more selective expression pattern of the other peptide receptors may allow pathobiochemical distinction between various tumour types. For a better overview, Fig. 1 shows the incidence and mean receptor density for the four main groups of tumours tested, namely ileal carcinoids, insulinomas, gastrinomas and bronchial carcinoids.

Virtually all ileal carcinoids expressed $VPAC_1$, while $VPAC_2$ was absent (Table 1, Fig. 1). They all expressed sst_2, but in half of the cases sst_1 and/or sst_5 was also present. They rarely expressed sst_3 and sst_4. The highest receptor densities were found for sst_2, followed by sst_1. Characteristic for ileal carcinoids was the expression of NMB receptors, as seen in 11/27 of the cases. Such receptors were virtually not expressed by any of the other tested neuroendocrine tumour types (Table 1, Fig. 1). The ileal carcinoids also expressed GLP-1 receptors in one-third of the cases and CCK_1 and CCK_2 in half and two-thirds of the cases, respectively, with the density of CCK_1 receptors being several times higher than that of the CCK_2 receptors (Table 1, Fig. 1). Figure 2 shows a typical example of the heterogeneous CCK receptor expression in an ileal carcinoid. Very high expression of CCK_1 was seen in one area of the tumour, while another area had CCK_2 receptors in low density. Histopathologi-

Table 1. Peptide receptor expression in ileal carcinoids

Case	VIP-R		Somatostatin-R					Bombesin-R			CCK-R		GLP-1-R
	$VPAC_1$	$VPAC_2$	sst_1	sst_2	sst_3	sst_4	sst_5	NMB	GRP	BB_3	CCK_1	CCK_2	
1 CL	7,353	–	2,487	9,477	1,752	–	2,437	–	–	–	–	847	831
2 MK	6,322	–	3,619	5,014	–	–	1,909	751	–	–	–	1,099	–
3 MT	6,517	–	4,698	–	1,666	–	7,476	1,993	–	–	–	–	–
4 JM	602	–	652	822	–	–	–	–	–	–	–	–	–
5 JS	3,907	–	5,056	8,963	–	–	2,895	–	–	–	–	436	–
6 DV	1,145	–	4,511	8,121	1,954	–	2,231	–	–	–	–	–	–
7 ST	6,014	–	8,413	591	–	–	2,782	–	–	–	–	295	1,260
8 OS	4,528	–	4,641	3,200	–	–	–	1,034	–	–	–	–	1,058
9 PB	2,151	–	2,027	1,469	–	–	1,526	–	–	–	–	–	–
10 KF	1,682	–	2,145	7,586	–	–	–	–	–	–	1,159[b]	1,419[b]	–
11 BZ	4,089	–	–	1,559	–	–	–	632	–	–	–	–	–
12 PM	2,699	–	–	5,962	–	–	896	968	–	–	1,054[b]	194[b]	–
13 MS	650	–	–	6,506	–	–	–	–	–	–	5,917[b]	–	–
14 HM	1,102	–	–	10,759	–	–	–	–	–	–	2,430[b]	686[b]	–
15 WS	1,740	–	–	6,106	–	–	–	–	–	–	–	–	–
16 BS	11,081	–	–	5,129	–	1,459	1,803	1,456	–	–	–	918	–
17 EM	4,828	–	1,219	7,633	–	–	–	–	–	–	–	945	303
18 HZ	1,127	–	–	4,750	–	–	–	1,038	–	–	4,325[b]	–	384
19 GV	1,688	–	–	3,563	–	–	1,307	1,746	–	–	–	1,584	–
20 GN	174	–	–	10,710	–	–	–	–	–	–	4,096[b]	1,026[b]	2,955[a]
21 JZ	7,380	–	5,485	3,499	–	–	4,229	341	–	–	1,403[b]	1,385[b]	1,029
22 LW	547	–	–	16,915	–	–	–	2,860	–	–	–	458	–
23 SV	2,341	–	–	1,318	–	–	365	–	–	–	1,831[b]	1,555[b]	–
24 HM	–	–	–	2,570	–	–	–	–	–	–	5,576[b]	–	–
25 SK	7,491	–	940	1,459	–	–	1,805	4,583	–	–	–	–	–
26 AN	1,718	–	–	6,393	–	–	–	–	–	–	3,338[b]	895[b]	395
27 FB	2,484	–	1,015	1,173	1,051	–	–	–	–	–	3,025[b]	834[b]	–
Incidence	26/27	0/27	14/27	26/27	4/27	1/27	13/27	11/27	0/27	0/27	11/27	16/27	8/27
Mean density	3,516		3,351	5,433	1,606	1,459	2,435	1,582			3,105	911	1,027

Numbers represent receptor density measured as dpm/mg tissue; – signifies absence of receptors; NA, not assessed ; R, receptors

[a] GLP-1 receptors were heterogeneously distributed in the tumour samples

[b] CCK_1 and/or CCK_2 receptors were heterogeneously distributed in the tumour samples

Table 2. Peptide receptor expression in functioning pancreatic neuroendocrine tumours

Case	VIP-R		Somatostatin-R					Bombesin-R			CCK-R		GLP-1-R
	$VPAC_1$	$VPAC_2$	sst_1	sst_2	sst_3	sst_4	sst_5	NMB	GRP	BB_3	CCK_1	CCK_2	
Insulinomas													
1 A9	1,706	NA	11,673	–	2,763	–	8,125	–	–	2,746	–	396	10,146
2 BS	293	–	1,975	2,582	–	–	–	–	956	–	–	2,705	7,779
3 12	2,682	–	1,757	–	408	–	524	–	–	–	–	462	9,665
4 12	635	–	–	5,775	–	–	–	–	–	–	–	1,467	6,005
5 AS	650	–	–	6,541	1,095	–	–	–	–	–	–	–	9,555
6 WM	1,192	–	–	4,579	–	–	–	–	–	–	–	586	3,604
7 A3	253	–	2,767	755	1,547	–	–	–	727	–	–	1,051	7,710
8 34	387	–	–	6,521	–	–	–	–	–	–	–	6,696	5,807
9 34	1,576	–	–	4,398	–	–	–	–	–	–	–	5,428	8,490
10 AF	385	–	727	4,534	–	–	–	–	–	–	–	1,154	5,384
11 32	–	–	–	1,261	–	–	–	–	–	–	–	1,490	7,440
12 HF	1,859	–	NA	NA	NA	NA	NA	–	–	–	–	4,108	10,236
13 GY	3,761	–	–	1,057	405	–	–	–	–	–	–	6,432	7,129

Table 2. (continued)

Case	VIP-R		Somatostatin-R					Bombesin-R			CCK-R		GLP-1-R
	$VPAC_1$	$VPAC_2$	sst_1	sst_2	sst_3	sst_4	sst_5	NMB	GRP	BB_3	CCK_1	CCK_2	
14 SC	662	–	419	–	191	–	–	–	–	–	–	3,446	9,580
15 BD	1,783	–	–	6,268	–	–	NA	–	–	–	–	2,389	6,142
16 P4	7,764	–	1,379	2,359	–	–	–	–	–	–	–	1,145	9,854
17 47	156	–	–	374	303	–	391	–	–	–	–	325	8,424
18 48	1,619	–	–	–	–	–	–	–	–	–	–	1,227	9,567
19 48	242	–	1,300	8,167	–	1,038	–	–	–	–	–	1,742	6,575
20 49	261	–	994	–	–	–	–	–	–	–	–	6,800	6,804
21 P5	1,145	–	5,395	–	–	–	–	–	–	–	–	836	10,205
22 P5	4,395	–	3,292	1,661	3,131	–	1,483	–	–	–	–	543	9,944
23 PL	366	–	2,262	435	–	–	–	–	320	–	–	575	8,658
24 GI	3,748	–	1,821	–	–	–	–	–	–	–	–	377	10,184
25 AN	4,365	–	638	–	–	–	–	–	–	–	–	3,508	8,430
26 EM	1,615	–	1,284	6,477	988	–	2,080	–	–	–	NA	NA	–
27 33	2,772	–	1,858	4,778	–	–	–	–	–	4,680	–	287	–
Incidence	26/27	0/26	16/26	18/26	9/26	1/26	5/25	0/27	3/27	2/27	0/26	25/26	25/27
Mean density	1,780		2,471	3,807	1,203	1,038	2,521		668	3,713		2,207	8,133
Gastrinomas													
1 39	–	–	–	11,305	–	–	–	–	4,116	–	1,032	–	1,718[a]
2 40	2,125	–	–	1,881	1,453	–	NA	–	2,909	–	–	–	4,426[a]
3 43	–	–	2,345	12,679	–	–	–	–	5,401	3,106	2,037	–	1,532
4 MT	3,111	–	–	6,204	–	–	–	–	–	–	204	–	412
5 P4	4,619	–	–	7,431	–	–	–	–	–	–	–	–	428
6 WT	–	–	–	9,118	–	–	2,512	–	2,644	–	2,266	–	641
7 DM	2,050	–	–	11,543	–	–	–	–	6,627	–	–	–	4,712
8 P3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	4,504
9 HI	3,551	–	–	5,106	–	–	–	–	5,301	–	–	–	487
10 JI	–	–	–	7,853	–	–	5,017	–	2,093	–	152	–	5,745[a]
11 MT	2,883	–	–	8,811	1,429	–	2,071	–	–	3,053	–	–	NA
Incidence	6/10	0/10	1/10	10/10	2/10	0/10	3/9	0/10	7/10	2/10	5/11	0/11	10/10
Mean density	3,057		2,345	8,193	1,441		3,200		4,156	3,080	1,138		2,461
Glucagonomas													
1 GT	2,550	–	4,669	8,043	–	–	–	–	–	1,313	–	–	–
2 LH	1,290	–	6,152	3,664	1,712	–	–	–	–	3,312	–	162	899[a]
3 JS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	941
4 KP	2,333	–	–	–	–	–	–	–	–	1,166	–	171	–
Incidence	3/3	0/3	2/3	2/3	1/3	0/3	0/3	0/3	0/3	3/3	0/3	2/3	2/4
Mean density	2,058		5,411	5,854	1,712					1,930		167	920
Vipomas													
1 KT	825	–	9,324	6,380	3,436	–	2,499	–	618	–	–	403	–
2 PM	393	–	–	20,166	–	–	–	–	–	3,045	1,050	2,527	–
3 MT	636	–	–	18,013	–	–	–	–	–	3,924	–	6,603	–
4 WM	4,299	–	–	20,207	–	–	–	–	311	–	–	603	3,028
Incidence	4/4	0/4	1/4	4/4	1/4	0/4	1/4	0/4	2/4	2/4	1/4	4/4	1/4
Mean density	1,538		9,324	16,192	3,436		2,499		465	3,485	1,050	2,534	3,028

Numbers represent receptor density measured as dpm/mg tissue; – signifies absence of receptors; NA, not assessed; R, receptors
[a] GLP-1 receptors were heterogeneously distributed in the tumour samples

Table 3. Peptide receptor expression in bronchial carcinoids

Case	VIP-R		Somatostatin-R					Bombesin-R			CCK-R		GLP-1-R
	$VPAC_1$	$VPAC_2$	sst_1	sst_2	sst_3	sst_4	sst_5	NMB	GRP	BB_3	CCK_1	CCK_2	
1 WT	7,979	–	–	8,395	–	–	–	–	–	–	–	1,787	2,333[a]
2 DF	3,311	–	–	–	–	–	177	–	–	–	–	933	–
3 TW	841	–	11,036	2,497	–	–	–	–	2,281	2,064	–	817	7,179[a]
4 BX	2,361	–	–	8,849	–	–	NA	–	–	–	–	–	4,339[a]
5 HL	–	–	1,699	2,590	–	–	–	–	–	1,191	–	380	–
6 SH	58	–	1,771	2,475	–	–	–	508	–	–	–	8,779	–
7 DT	2,764	–	511	–	–	–	–	–	–	288	–	3,418	–
8 HL	836	–	897	7,582	–	–	–	–	–	2,202	–	720[b]	–
9 KN	4,997	–	4,780	3,468	–	–	1077	–	–	3,368	–	1,238	–
10 BR	–	–	2,998	4,738	–	–	–	–	–	–	–	2,904	–
11 VM	6,021	NA	–	6,914	–	–	–	–	–	–	–	2,775	–
12 SH	4,555	–	327	–	–	–	–	–	–	–	–	–	2,725[a]
13 WP	3,226	–	221	–	–	–	282	–	–	–	–	–	–
14 KH	5,945	–	NA	NA	NA	NA	NA	–	–	–	1,408	–	–
15 TK	1,880	–	465	–	–	–	–	–	–	–	–	–	–
16 BT	1,600	–	–	7,630	–	–	–	–	–	2,394	424	–	–
17 RS	3,618	–	1,472	4,359	–	–	–	–	–	–	–	2,040	253
18 WS	6,524	–	3,756	–	–	–	–	–	–	–	1,382	–	–
19 CM	3,545	–	4,002	891	–	–	–	–	–	6,337	–	561	3,699[a]
20 JT	4,382	–	–	3,837	–	–	–	–	–	–	–	181[b]	–
21 KM	NA	NA	–	–	–	–	–	–	–	–	–	2,127	456
22 SM	5,745	–	4,002	5,904	1,743	–	2,939	–	–	4,732	–	–	–
23 MN	2,393	–	5,184	–	–	–	–	–	–	4,650	–	616	3,089
24 SD	4,752	–	1,264	1,366	–	–	–	–	–	–	–	43	589[a]
25 01	6,773	–	6,701	–	–	–	1,740	–	–	1,476	–	324	–
26 KG	2,549	–	8,185	3,128	–	–	–	–	–	–	–	–	1,901[a]
27 LR	4,943	–	2,934	709	–	–	–	–	–	–	929	–	–
28 KC	6,161	–	5,630	2,844	–	–	1,396	–	–	–	–	–	118
29 SD	986	–	–	7,412	–	–	NA	–	–	–	–	1,451	–
Incidence	26/28	0/27	20/28	19/28	1/28	0/28	6/26	1/29	1/29	10/29	4/29	18/29	11/29
Mean density	3,796		3,392	4,505	1,743		1,269	509	2,281	2,870	1,036	1,727	2,456

Numbers represent receptor density measured as dpm/mg tissue; – signifies absence of receptors; NA, not assessed; R, receptors

[a] GLP-1 receptors were heterogeneously distributed in the tumour samples

[b] CCK_1 and/or CCK_2 receptors were heterogeneously distributed in the tumour samples

cal evaluation revealed that the CCK_1 receptor-expressing tumour area consisted of a more differentiated, cribriform, tubulo-acinar carcinoma, compared with the more solid and less differentiated CCK_2-expressing part. Similar histopathological observations were made in several other ileal carcinoids with a heterogeneous CCK receptor distribution. Furthermore, the whole tumour sample in Fig. 2 also expressed a high density of sst_2 and a moderate density of $VPAC_1$ receptors.

Insulinomas were characterised by the expression of $VPAC_1$, CCK_2 and GLP-1 receptors in almost all cases, whereas they were devoid of CCK_1 and $VPAC_2$ (Table 2, Fig. 1). Of 26 insulinomas, 18 expressed sst_2, an incidence which is considerably lower than that found in ileal carcinoids (26/27). However, another somatostatin receptor subtype, sst_1, was found in more than half of the insulinoma cases, often in high density. Interestingly, sst_1 was expressed in all but one of the sst_2-lacking insulinomas, often in high amounts. An extremely high receptor density was found for GLP-1 receptors, followed, in a subgroup of patients only, by sst_2 and CCK_2 receptors. Conversely, bombesin receptors were extremely rarely expressed in insulinomas (Table 2, Fig. 1). Figure 3 is a typical example of an insulinoma expressing multiple peptide receptors, in particular CCK_2, GLP-1, sst_2 and $VPAC_1$ receptors. Figure 4 shows a typical displacement curve characterising GLP-1 receptors in an insulinoma with high-affinity displacement of the radioligand by GLP-1 or exendin 4 but not by GLP-2 or glucagon 1–29.

Gastrinomas were characterised by a high expression of sst_2 receptors in all cases, whereas the other somatostatin receptors were rarely detected (Table 2, Fig. 1).

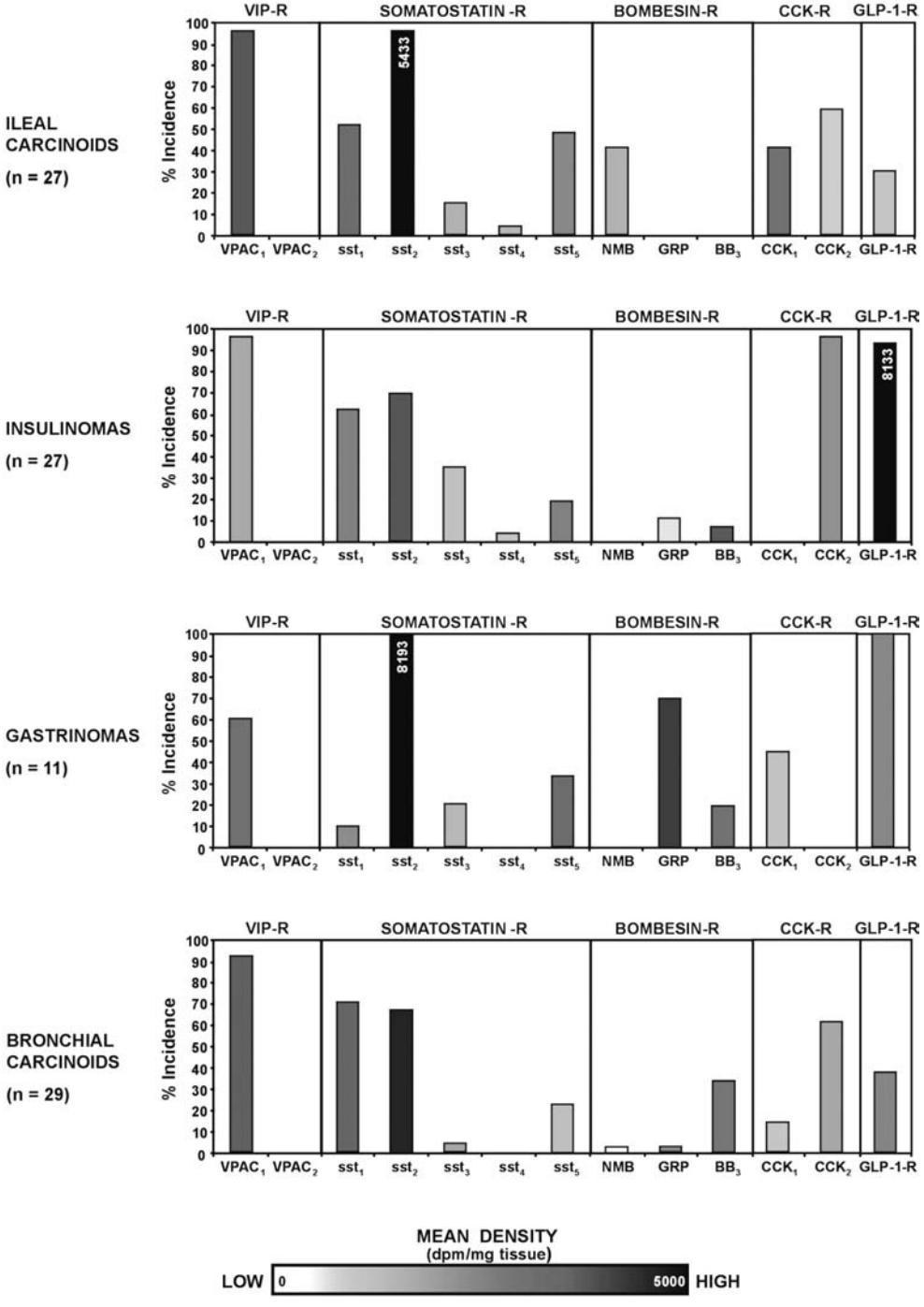

Fig. 1. Histograms summarising the incidence and the mean density of each of the 13 peptide receptors tested in ileal carcinoids, insulinomas, gastrinomas and bronchial carcinoids. The mean density (dpm/mg tissue) is visualised as relative darkness ranging from 0 to 5,000 dpm/mg tissue. Those cases with density values above 5,000 dpm/mg are represented by *dark bars* in which the numbers of the mean density values have been inserted (see sst_2 and GLP-1 receptors). *n*, Number of tumours tested

GLP-1 receptors were also expressed in all cases. $VPAC_1$ receptors were detected in two-thirds of the cases while $VPAC_2$ receptors were absent. Perhaps most characteristic for gastrinomas was the frequent expression of GRP receptors, as compared with the rare expression of the other bombesin receptor subtypes, and that of CCK_1 receptors, while CCK_2 are undetectable (Table 2, Fig. 1). Figure 5 shows a gastrinoma expressing sst_2, CCK_1 and GRP receptors.

Since the number of tested vipomas and glucagonomas was limited, only a trend towards a pattern can be proposed, with vipomas expressing $VPAC_1$, sst_2, CCK_2 and at least one of the bombesin receptor subtypes in all cases, whereas glucagonomas contained $VPAC_1$ and BB_3 in all cases but also a very high density of sst_1 and sst_2 in two of three cases (Table 2).

Bronchial carcinomas also expressed several peptide receptors in high amounts. Most of them had $VPAC_1$ and

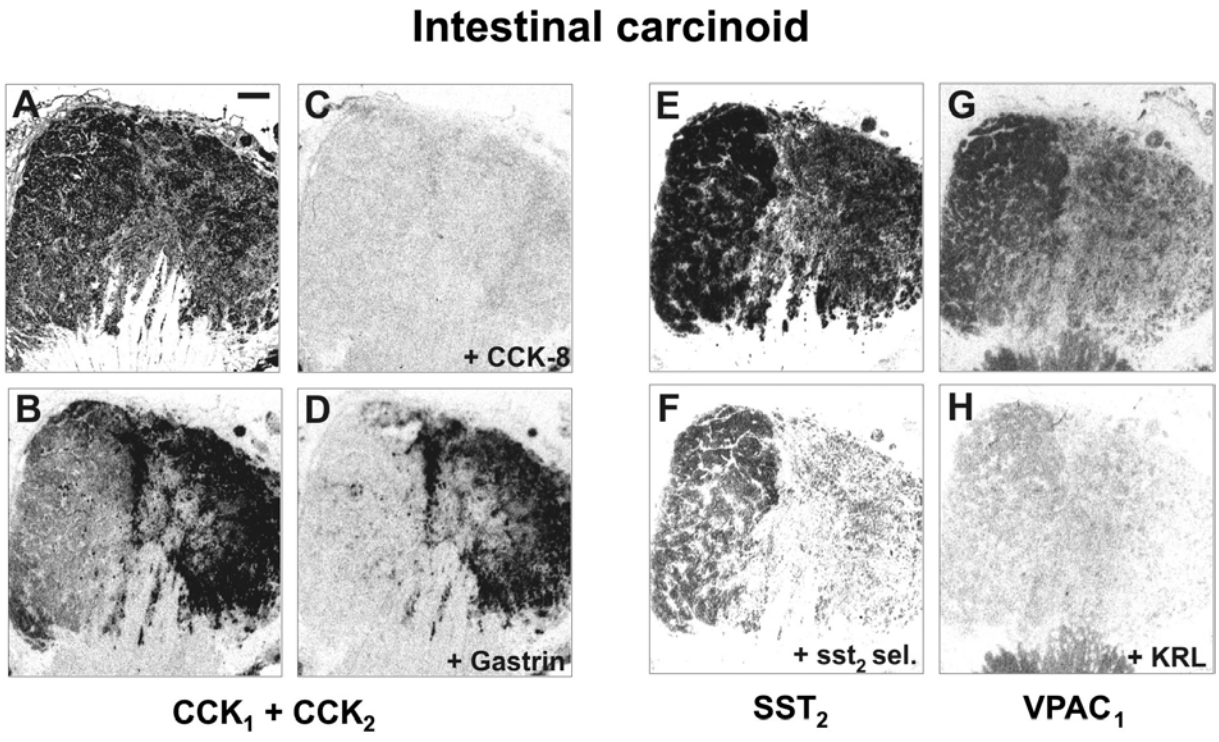

Fig. 2A–H. Receptor autoradiography of an ileal carcinoid expressing CCK_1 and CCK_2 receptors (**A–D**) simultaneously with sst_2 (**E, F**) and $VPAC_1$ (**G, H**). **A** Haematoxylin-eosin stained section showing the tumour. *Bar* =1 mm. **B** Autoradiogram showing total binding of ^{125}I-CCK in the tumour tissue. The right part is more intensively labelled than the left one. **C** Autoradiogram showing ^{125}I-CCK binding in the presence of 50 n*M* cold CCK-8. All the labelling is displaced. **D** Autoradiogram showing ^{125}I-CCK binding in the presence of 50 n*M* of gastrin. Gastrin displaces the radioligand in the left part of the tumour, but not in the right part, indicating that the left part expresses CCK_2 while the right part has CCK_1. **E, F** Autoradiograms showing total binding of ^{125}I-LTT-SS-28 (**E**) displaced by 100 n*M* of the sst_2-selective L-779,976 (**F**), indicating a very strong expression of sst_2. In **F**, the left side of the tumour shows a significant residual non-specific binding. **G, H** Autoradiograms showing total binding of ^{125}I-VIP (**G**) displaced by 20 n*M* of the $VPAC_1$-selective [K^{15}, R^{16}, L^{27}]VIP(1–7)/GRF(8–27) (KRL; **H**), indicating moderate expression of $VPAC_1$

somatostatin receptors of either the sst_1 or the sst_2 type while $VPAC_2$, sst_3 and sst_4 were virtually not detected. More than one-third of the cases had GLP-1 receptors, which were, however, often heterogeneously distributed. Most characteristic for bronchial carcinoids was the preferential expression of the bombesin receptor subtype BB_3 and of CCK_2 receptors (Table 3, Fig. 1). Figure 6 shows the multiple receptor expression seen in one bronchial carcinoid with a high density of sst_1, BB_3 and CCK_2 receptors, and in another with BB_3, GLP-1 and $VPAC_1$ receptor expression.

In vitro receptor profile as a predictor for in vivo tumour targeting

The above-mentioned in vitro data clearly demonstrate that neuroendocrine tumours of the small intestine, pancreas and lung can concomitantly express multiple peptide receptors, often in high density, and that the various types of tumour appear to have rather characteristic receptor profiles often distinct from each other. This knowledge may be used by nuclear physicians to select a radioligand, or a mixture of radioligands, suitable for each individual case, in order to achieve efficient and optimal in vivo tumour targeting.

Somatostatin receptors

The high incidence and high density of the sst_2 protein reported for the various tumours in Tables 1, 2 and 3 and Fig. 1 can be seen as one of the main keys to the success of Octreoscan in diagnosing the majority of neuroendocrine tumours of the small intestine, pancreas and lung, since Octreoscan has a preferential affinity for sst_2. The particularly high incidence and density of sst_2 in gastrinomas may be the explanation for the extremely good results found with in vivo Octreoscan imaging of these tumours [12]. The same may be true for ileal carcinoids. Conversely, the lower incidence and density of sst_2 in insulinomas may explain the lower rate of detection with Octreoscan in vivo. One can also foresee that precisely those tumours in Fig. 1 with the highest sst_2 density will be particularly amenable to successful radiotherapy with ^{111}Y-labelled DOTATOC or ^{177}Lu-labelled DOTATATE [2, 23]. In

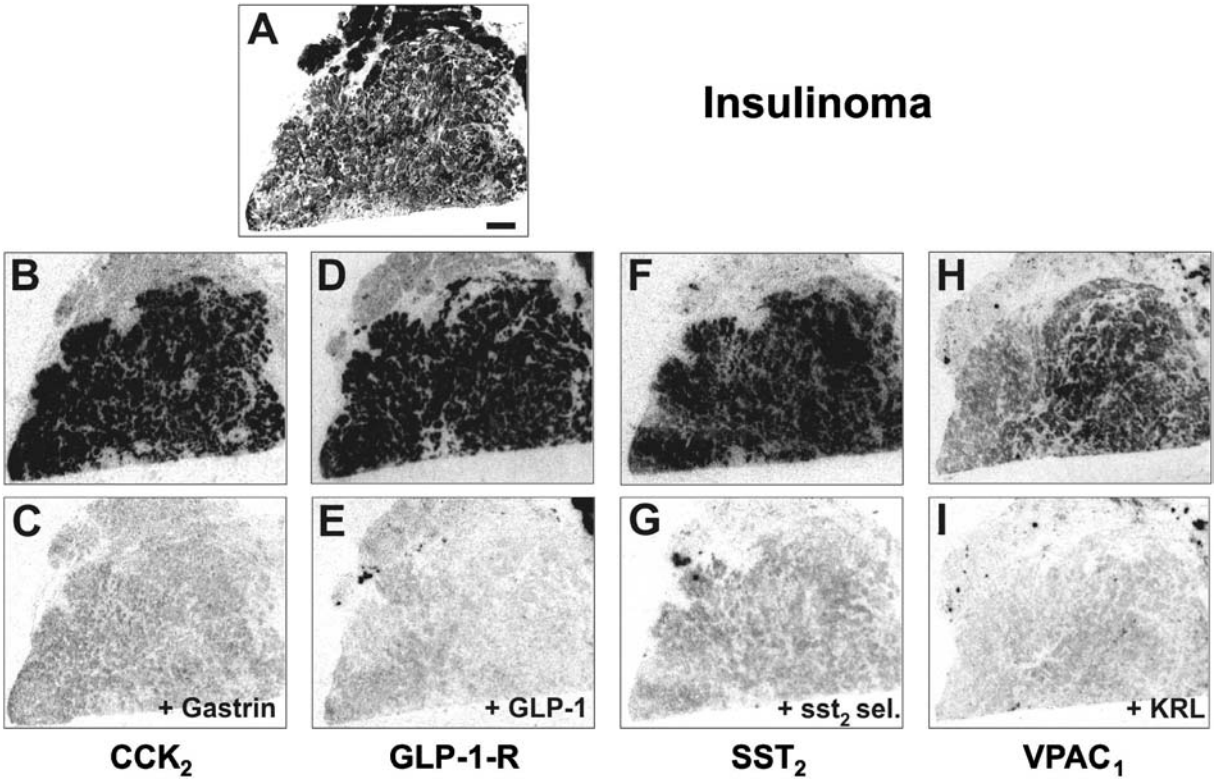

Fig. 3A–I. Insulinoma expressing concomitantly CCK_2 receptors (**B, C**), GLP-1 receptors (**D, E**), sst_2 receptors (**F, G**) and $VPAC_1$ receptors (**H, I**). **A** Haematoxylin-eosin stained section showing the tumour tissue. *Bar* =1 mm. **B, C** Autoradiograms showing total binding of ^{125}I-CCK (**B**) completely displaced by 50 n*M* of gastrin (**C**), thus indicating the presence of CCK_2 receptors. **D, E** Autoradiograms showing total binding of ^{125}I-GLP-1 (**D**) completely displaced by 100 n*M* of GLP-1 (**E**). **F, G** Autoradiograms showing total binding of ^{125}I-LTT-SS-28 (**F**) displaced by 100 n*M* of the sst_2-selective L-779,976 (**G**); this indicates the presence of sst_2 receptors. **H, I** Autoradiograms showing total binding of ^{125}I-VIP (**H**) displaced by 20 n*M* of the $VPAC_1$-selective [K^{15}, R^{16}, L^{27}]VIP(1–7)/GRF(8–27) (**I**), indicating the presence of $VPAC_1$ receptors. Note the very high density of CCK_2, GLP-1 and sst_2 receptors, compared with $VPAC_1$ receptors

many of these tumours, sst_2 may even be targeted concomitantly with other peptide receptors (see below).

Whereas the present study confirmed the predominance of sst_2 protein expression in neuroendocrine tumours [11, 24, 25, 26], it also revealed that sst_1 is the second most abundant somatostatin receptor subtype after sst_2 in many gut and lung neuroendocrine tumours, and in particular in bronchial carcinoids. In insulinomas, it was even more abundant than sst_2 and was most often expressed in tumours lacking sst_2. Commercially available somatostatin analogues for scintigraphy, including Octreoscan, are unable to bind to sst_1 receptors [19]. However, either sst_1-selective compounds, such as CH-288 [27], or pan-somatostatins, such as KE108 [28], that would be coupled to chelators, may be developed for this indication. Compared with other sst_1-expressing tumours such as prostate cancers or sarcomas [29, 30], the neuroendocrine tumours of the present study often had a much higher density of sst_1 receptors; it is probable, therefore, that the sst_1 targeting in vivo of these particular tumours may be successful.

VIP receptors

The great majority of the tested neuroendocrine tumours expressed $VPAC_1$. In theory, it can be predicted that most neuroendocrine tumours should be targeted with radiolabelled VIP analogues. This has at least been shown previously for a group of intestinal neuroendocrine tumours [6]. However, high expression of VIP receptors is found in a large number of normal tissues and organs [9], and it is unlikely that VIP receptor scintigraphy will be of great help in detecting distant metastases of neuroendocrine tumours, i.e. lymph node or liver metastases, owing to high background activity. Moreover, neuroendocrine lung tumours would be difficult to visualise, as their receptors would be masked by the high VIP binding to the lungs [9]. Also, combination of VIP radioligands with other peptide ligands, with the aim of achieving increased sensitivity for tumour detection, may not be an advantage owing to the high VIP background in healthy tissues.

Bombesin receptors

This study confirms and extends the results of an earlier investigation showing that bombesin receptor subtypes

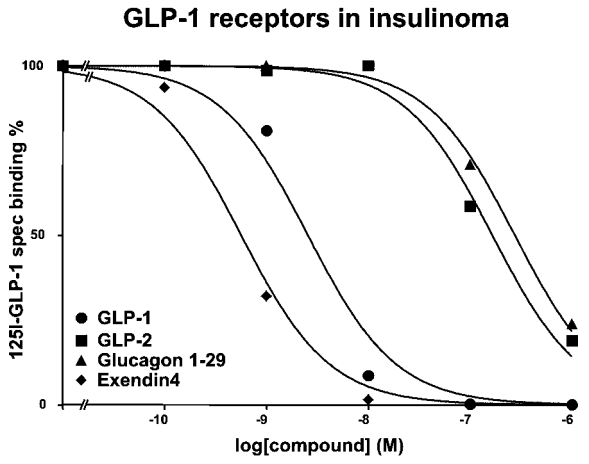

Fig. 4. GLP-1 receptors in an insulinoma. Competition experiment using successive tissue sections incubated with ^{125}I-GLP-1 and increasing concentrations of unlabelled GLP-1 (*circles*), GLP-2 (*squares*), glucagon 1–29 (*triangles*) or exendin 4 (*diamonds*). The high affinity for exendin 4 or GLP-1 and the low affinity for GLP-2 or glucagon 1–29 clearly indicates the presence of GLP-1 in this insulinoma

are differentially overexpressed in neuroendocrine tumours. BB_3 is frequently found in bronchial carcinomas, glucagonomas and vipomas, but is absent in ileal carcinoids and insulinomas. Conversely, NMB receptors are expressed in ileal carcinoids but are absent in other neuroendocrine tumours, whereas the high incidence and density of GRP receptors found in gastrinomas and some vipomas should be particularly stressed. These results point towards different biological characteristics of these tumours. They also indicate that it will be of great utility to know the bombesin receptor subtype affinity profile of newly developed bombesin radioligands foreseen for in vivo tumour targeting [31]. Up to now, only radioligands with strong GRP receptor affinity have been developed for in vivo targeting [8, 31, 32].

CCK receptors

The results of this study with respect to CCK receptors suggest that selected tumour types may become potential targets for CCK_2 receptor labelling in vivo. Insulinomas and vipomas appear to be highly promising CCK_2 targets

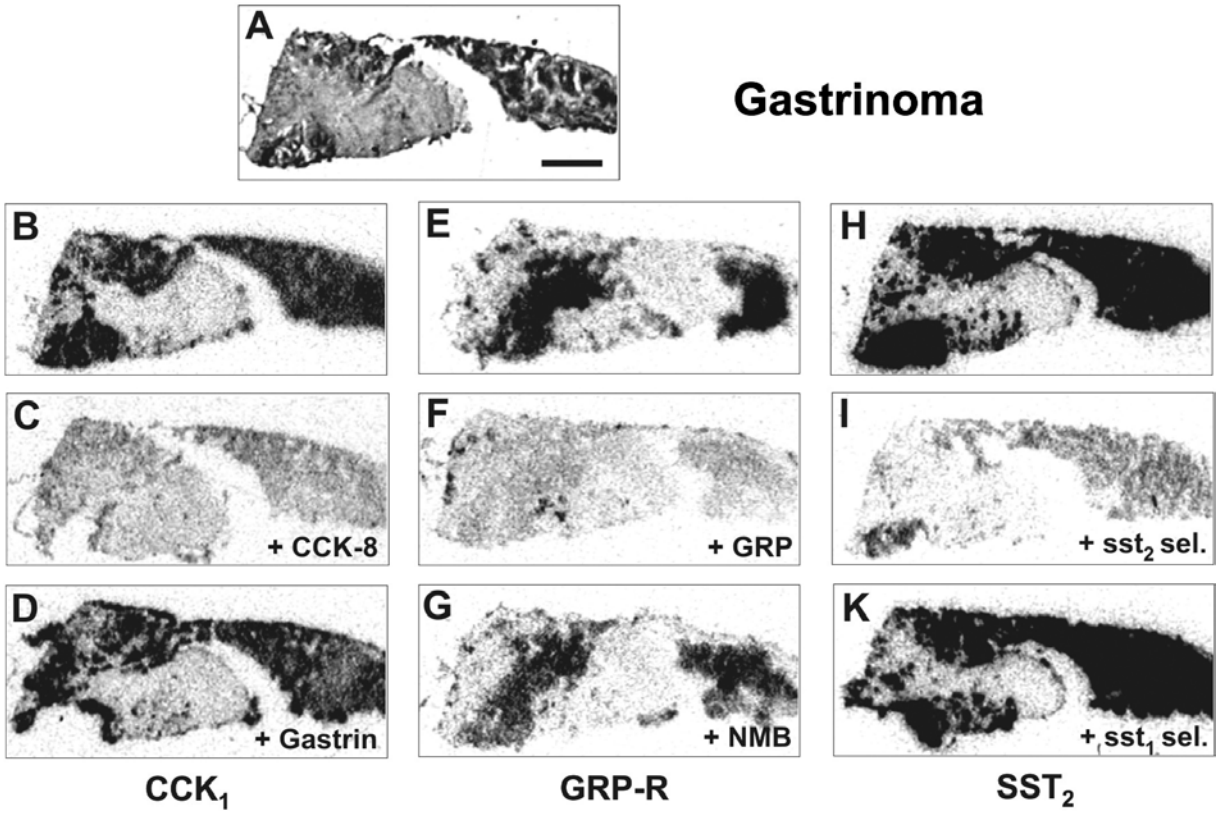

Fig. 5A–K. Gastrinoma (**A**) expressing concomitantly CCK_1 receptors (**B–D**), GRP receptors (**E–G**) and sst_2 receptors (**H–K**). **A** Haematoxylin-eosin stained section. *Bar* =1 mm. **B–D** Autoradiograms showing total binding of ^{125}I-CCK (**B**) displaced by 50 n*M* of CCK-8 (**C**) but not by 50 n*M* of gastrin (**D**), indicating the presence of CCK_1 receptors. **E–G** Autoradiograms showing total binding of ^{125}I-[D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin 6–14 (**E**) displaced by 50 n*M* of GRP (**F**) but not by 50 n*M* NMB (**G**), indicating the presence of GRP receptors. **H–K** Autoradiograms showing total binding of ^{125}I-LTT-SS-28 (**H**) displaced by 100 n*M* of the sst_2-selective L-779,976 (**I**) but not by the sst_1-selective CH-288 (**K**), indicating the predominance of sst_2 receptors

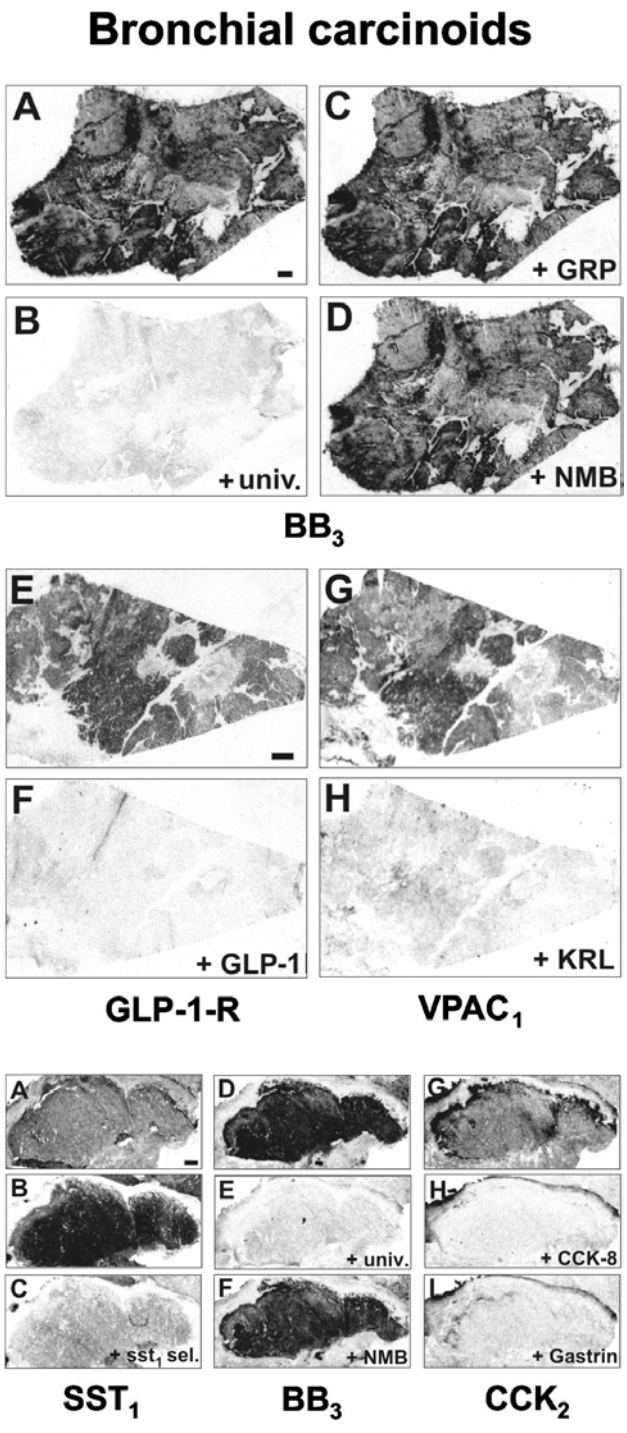

Fig. 6. Peptide receptor pattern in two bronchial carcinoids. *Upper figure:* Bronchial carcinoid expressing BB_3 (**A–D**), GLP-1 (**E, F**) and $VPAC_1$ receptors (**G, H**). **A–D** Autoradiograms showing total binding of ^{125}I-[D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin 6–14 (**A**) displaced completely by 50 n*M* of [D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin 6–14 (univ.; **B**) but not displaced by 50 n*M* of GRP (**C**) or NMB (**D**), indicating the presence of BB_3 receptors. **E, F** Autoradiograms showing total binding of ^{125}I-GLP-1 (**E**) completely displaced by 100 n*M* of GLP-1 (**F**). **G, H** Autoradiograms showing total binding of ^{125}I-VIP (**G**) displaced by 20 n*M* of the $VPAC_1$-selective [K^{15}, R^{16}, L^{27}]VIP(1–7)/GRF(8–27) (**H**), indicating the presence of $VPAC_1$ receptors. *Lower figure:* Bronchial carcinoid (**A**) expressing sst_1 receptors (**B, C**), BB_3 receptors (**D–F**) and CCK_2 receptors (**G–I**). **A** Haematoxylin-eosin stained section. *Bar* =1 mm. **B, C** Autoradiograms showing total binding of ^{125}I-LTT-SS-28 (**B**) displaced by 100 n*M* of the sst_1-selective analogue CH288, indicating the presence of sst_1. **D–F** Autoradiograms showing total binding of ^{125}I-[D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin 6–14 (**D**) displaced by 50 n*M* of [D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin 6–14 (univ.; **E**) but not by 50 n*M* of NMB (**F**) or GRP (not shown), indicating the presence of BB_3 receptors. **G–I** Autoradiograms showing total binding of ^{125}I-CCK (**G**) displaced by 50 n*M* of CCK-8 (**H**) and gastrin (**I**), indicating the presence of CCK_2 receptors

in most cases, as do some bronchial and ileal carcinoids. CCK_2 receptor scintigraphy may even be preferable to Octreoscan in those neuroendocrine tumours with few or no sst_2 receptors.

GLP-1 receptors

The GLP-1 receptor, which is massively overexpressed in virtually all insulinomas and gastrinomas and in a large number of intestinal and bronchial carcinoids, is a novel peptide receptor with a high potential for tumour targeting. The present in vitro study describes for the first time the overexpression of this receptor in human cancer. It is reasonable to expect successful in vivo targeting of these tumours with radiolabelled GLP-1 receptor-selective analogues; indeed, a GLP-1 receptor-containing rat insulinoma could be visualised recently with the radiolabelled GLP-1-selective ^{123}I-exendin 4 [33]. The present in vitro results predict that the use of GLP-1 receptor targeting in vivo should permit not only the efficient visualisation of all insulinomas, but also, because of the extraordinarily high receptor density, their successful radiotherapy; it may represent a considerable improvement over Octreoscan in these tumours.

The present data therefore strongly indicate that there may be several options for the targeting of neuroendocrine tumours, aside from somatostatin receptor scintigraphy. For insulinomas, the first choice should be not Octreoscan but GLP-1 receptor scintigraphy, since the incidence and density of these receptors are very close to those of sst_2 in gastrinomas, the gold standard indication for Octreoscan. Another alternative to Octreoscan in insulinomas may be CCK_2 receptor scintigraphy. However, in those insulinomas expressing sst_2, GLP-1 (and CCK_2) receptor targeting may be used advantageously together with Octreoscan (see below).

Another interesting aspect of the very high expression of the GLP-1 receptor in insulinomas and other tumours is related to its biological role. Knowing the potent effect of GLP-1 in stimulating insulin release from normal pancreatic beta cells [20], it is probable that GLP-1 will also massively affect insulin release from insulinoma tissue

through the numerous GLP-1 receptors. On the one hand, such release may play a significant pathophysiological role in this disease. On the other hand, it may be used as a potent diagnostic strategy: a GLP-1 stimulation test using a single injection of GLP-1 would trigger a release of large amounts of insulin from the insulinoma that could be detected in the circulation. This might offer a useful and easy test for the detection of insulinomas in the early stage of the disease, in analogy with the pentagastrin test, which stimulates calcitonin release from medullary thyroid cancers through CCK_2 receptors [3, 7].

Receptor co-expression as a basis for in vivo multireceptor targeting

The co-expression of multiple receptors in human tumours may be a ubiquitous feature of peptide receptors, as it is not confined to various neuroendocrine tumours but has been shown previously in other cancers, such as breast cancers [15]. Its in vivo application may be extremely attractive as a means to improve the efficacy of peptide targeting in tumours; the concomitant application of multiple radioligands will selectively increase the radioactivity accumulation in tumours, an advantage not only for diagnostic but especially for radiotherapeutic purposes. Specifically, the present data predict the combination of GLP-1 and CCK_2 receptors to be highly efficient targets in all insulinomas, and indicate that the use of a mixture of sst_2, GLP-1 and GRP radioligands would offer optimal targeting of gastrinomas. As some of the receptors are non-homogeneously expressed by tumours, such as CCK_1 and CCK_2 in ileal carcinoids, a combination of the corresponding receptor-selective radiopeptides may further improve the targeting efficacy during radiotherapy by destroying more than one receptor-expressing tumour area. Furthermore, a cocktail of different peptides may reduce the risk of a loss of efficacy during peptide radiotherapy, which may be due to tumour dedifferentiation with a resulting loss of some but not all peptide receptors. Finally, an advantage of using a cocktail of radioligands is the possibility of labelling each of them with different isotopes, namely with β-emitters of different ranges, in order to achieve optimal radiotherapy for large and small tumoural lesions [34]. One could conceive that the use of ^{177}Lu-labelled DOTATATE [23] together with ^{188}Re- or ^{90}Y-labelled GRP analogues [8] may be of benefit in gastrinoma patients with multiple, large and small metastases. Whenever possible, prior to the concomitant use of several radiopeptide ligands in vivo, it may be worth determining the individual peptide receptor affinity profile of the tumour under consideration by in vitro receptor determination using the described methodology in a surgically resected biopsy sample.

A prerequisite for development of multireceptor tumour targeting in vivo is, however, the availability of adequate radioligands. During the past few years, novel and more potent somatostatin radioligands such as ^{177}Lu-labelled DOTATATE [23] or ^{90}Y-DOTANOC [35] have been reported. In addition, analogues with affinity for the GRP receptor, such as Demobesin [31] or RP527 [8], NPY(Y_1)-selective analogues such as the one reported by Soll et al. [36] and more potent CCK_2-selective analogues [37] have recently been developed, which may be used for more efficient and powerful in vivo multireceptor targeting of tumours.

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