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Immunohistochemical distribution of chromogranins A and B and secretogranin II in neuroendocrine tumours of the gastrointestinal tract

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Abstract The aim of the present study was to investigate immunohistochemically the distribution of chromogranin A, chromogranin B, and secretogranin II in a series of 152 neuroendocrine tumours of the gastrointestinal tract. Tumour tissues from 25 argyrophil gastric carcinoids, 18 gastrin and 5 somatostatin-producing tumours, 4 'gangliocytic paragangliomas', 49 classical argentaffin and 2 L cell appendiceal carcinoids, 27 classical ileal carcinoids, 17 rectal carcinoids, and 5 poorly differentiated neuroendocrine tumours of the stomach and rectum were immunostained with antibodies against chromogranin A, chromogranin B, and secretogranin II. Chromogranin A was the major granin expressed in gastric carcinoids and in serotonin-producing carcinoids of the appendix and the ileum. In contrast, strong chromogranin B and secretogranin II immunoreactivity was found in rectal carcinoids, in which chromogranin A was rarely expressed. Since chromogranin A is a widely used marker for neuroendocrine differentiation, it is of diagnostic importance that some gastrin-producing tumours, 'gangliocytic paragangliomas', poorly differentiated neuroendocrine carcinomas, and appendiceal L cell carcinoids completely lacked chromogranin A positivity. It is concluded that the various neuroendocrine tumours of the gastrointestinal tract show distinctly different patterns of granin expression, probably reflecting their histogenetical origin.

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Introduction

Solcia et al. [34] recently proposed a classification of gut endocrine tumours based on histological, histochemical, ultrastructural, and clinicopathological parameters. Gut endocrine tumours consist mainly of cells that produce and secrete the same hormones as are expressed by the tissues from which they derive. Thus, knowledge of the exact site of origin is often essential for establishment of the nature of the respective tumour.

In the human gastrointestinal mucosa 14 endocrine cell types have been characterised morphologically and, to some extent, functionally [33]. Not all of these cell types give rise to gastrointestinal endocrine tumours, perhaps because of the tendency of endocrine tumours to retain or lose their proliferative capacity when undergoing differentiation [34]. Some cell types may show spontaneous proliferation (e.g. somatostatin- and gastrin-producing cells, intestinal EC or ECL cells), whilst others undergo differentiation only in the post-mitotic stage (e.g. pyloric EC cells, secretin cells).

The aim of the present study was to investigate the distribution patterns of chromogranin A, chromogranin B, and secretogranin II in the various types of neuroendocrine tumours occurring in the gastrointestinal tract [5, 34]. Chromogranins A and B and secretogranin II belong to a class of acidic proteins collectively named granins, which are widely distributed in a variety of normal tissues and in tumours of neuroendocrine and neuronal origin in vertebrates. They are stored within the cells in large dense core vesicles (LDV). Knowledge of the function(s) of these proteins is still incomplete, but there is evidence that they are involved in hormone secretion and hormone packaging within secretory granules, and that they may serve as the prohormones of more active proteolytic cleavage products [12, 18, 19, 40].

Materials and methods

Tissues

Routinely processed tissues from 152 neuroendocrine tumours of the gastrointestinal tract were retrieved from the files of the Department of Pathology, University of Münster, Germany. The tumours, which were classified according to a recently published revised classification of neuroendocrine tumours of the gut [5], are listed in Table 1. Serial sections 4 μm thick were cut and mounted on protein-coated glass slides. Prior to immunostaining sections were dewaxed, rehydrated in a series of alcohols, and rinsed in distilled water and TRIS buffer.

Chromogranin/secretogranin antibodies

Monoclonal chromogranin A antibody was purchased from Bio-Genex (San Ramon, Calif., USA), and the preparation of the polyclonal antibody (raised in rabbits) against a synthetic peptide present in the amino acid sequence of human chromogranin B (amino acids 306–326; designated as DK-21) has been described previously [30].

A peptide corresponding to rat secretogranin II amino acids 154–186 [14] was synthesised by standard solid phase t-BOC chemistry and purified by reversed phase HPLC. The synthetic peptide (designated as secretoneurin [21]) was coupled via an additional N-terminal cysteine to maleimide-activated keyhole limpet haemocyanin. Subsequently, rabbits were immunized following a standard protocol [11]. The antiserum has been characterized in detail elsewhere [21]. Anti-rat secretoneurin cross-reacts very well with human secretoneurin as there is only one amino acid substitution between these species.

The tissues were also immunostained with antibodies against gastrin, 5HT (serotonin), substance P, somatostatin, pancreatic polypeptide, insulin, glucagon, vasoactive intestinal peptide, and prostatic acid phosphatase (all purchased from Dako, Copenhagen, Denmark).

Immunohistochemical staining procedure

Primary chromogranin A, chromogranin B, and secretoneurin antibodies were applied overnight at 4°C in a humidified chamber (dilutions in PBS containing 0.6% bovine serum albumin: monoclonal chromogranin A antibody 1:800, polyclonal chromogranin B and secretoneurin antibodies 1:2000), followed by a goat-anti-mouse or goat-anti-rabbit bridging antibody (1:30 in PBS; 30 min at room temperature; Dako, Copenhagen, Denmark) and a polyclonal mouse- or rabbit-APAAP complex (1:100 in PBS; 60 min at room temperature; Dianova, Hamburg, Germany). The bridging antibody and the APAAP complex were applied on a semi-automatic immunostaining device ("Omnibus"; Quartett, Berlin, Germany). Subsequently the enzyme reaction was developed for 25 min at room temperature in a freshly prepared new fuchsin solution containing naphthol-bi-as-phosphate. Finally, the sections were counterstained with haematoxylin and mounted in Kayser's glycerin gelatin.

Omission of primary antibodies was used as the negative control and normal adrenal medulla as the positive control for the various granin antibodies.

Results

Stomach carcinoids

Out of the 25 cases, 17 arose against a background of atrophic fundus-body gastritis, 2 were associated with ZES-MEN1, and 6 were sporadic cases [29]. 5HT was immunolocalized in 3 sporadic cases. Gastrin- and somatostatin-immunoreactive cells were absent in all 25 cases. A strong chromogranin A immunoreactivity was demonstrated in virtually all cells of these 25 cases, whereas a weak to moderate chromogranin B and secretogranin II reactivity (also present in all 25 cases) was found in a focal distribution. Occasionally chromogranin B and/or secretogranin II immunoreactivity was restricted to a few tumour cells. In 13 cases there was associated pronounced NE cell hyperplasia of the oxyntic mucosa, demonstrated by a strong chromogranin A positivity; chromogranin B and/or secretogranin II was only found in a few cells.

Gastrin-producing tumours

Of 18 gastrin-producing tumours in the present series, 17 were located in the duodenum and the upper jejunum, and 1 was found in the gastric antrum. In only 11 tumours did the majority of cells contain immunoreactive gastrin. Thirteen were associated with Zollinger-Ellison syndrome (ZES). Whenever gastrin was immunohistochemically detectable chromogranin A, chromogranin B and secretogranin II were present at least focally. In

Table 1 Neuroendocrine tumours of the gastrointestinal tract (*EC* enterochromaffin, *ECL* enterochromaffin-like, *G* gastrin, *D* somatostatin; *PP* pancreatic polypeptide, *5HT* 5-hydroxytryptamine, *PYY* PP-like peptide, *subst.P* substance P, *L* PP- or PYY-producing cells)

Tumour	*n*	Prevalent cell type	Main hormone
Well differentiated			
Gastric carcinoid	25	ECL	
Gastrin producing tumour	18	G	Gastrin
Somatostatin producing tumour	5	D	Somatostatin
'Gangliocytic paraganglioma'	4	PP,D,?	Variable
Appendiceal EC carcinoid	49	EC	5HT, subst. P
Appendiceal L cell carcinoids	2	L	glicentin, PP,PYY
Carcinoid of the ileum	23	EC	5HT, subst. P
Carcinoid of the ceacum	4	EC	5HT, subst. P
Rectal carcinoids	17	L	glicentin, PP, PYY
	147		
Poorly differentiated			
Neuroendocrine carcinoma, stomach	2	?	Variable
rectum	3	?	Variable

cases with low gastrin immunoreactivity associated with ZES, chromogranins/secretogranin were either completely absent (2 cases) or found only in a few scattered tumour cells (Fig. 1b).

Somatostatin-producing tumours

These tumours were located in the duodenal periampullary region of the duodenum. None of the 5 cases was associated with the so-called somatostatinoma syndrome. All tumours showed glandular structures and psammoma body-like structures. In 4 tumours chromogranin A was only found in a few tumour cells (Fig. 1a), and in 1 tumour focal staining was noted. Chromogranin B immunoreactivity was observed in 3 tumours, where it was found in more than 50% of tumour cells. Secretogranin II was found in a few scattered tumour cells in 3 cases.

'Gangliocytic paraganglioma'

Four tumours (all located in the second portion of the duodenum) with an admixture of epithelial neuroendocrine cells, mature ganglion cells and a varying proportion of Schwann-like spindle cells were classified as 'gangliocytic paraganglioma'. In all these tumours somatostatin and PP immunoreactive cells were detected. Two also contained insulin-positive cells. Clusters of chromogranin A-positive cells were found in 3 of the 4 tumours and chromogranin B, in 2. Secretogranin II was found in a few tumour cells in 2 cases.

EC cell carcinoids of the appendix

All 49 cases in the present series showed a typical solid pattern and expressed 5HT, chromogranin A, and chromogranin B. The strongest immunoreactivity was found with chromogranin A (Fig. 1c); chromogranin B and 5HT immunoreactivity was strongest in solid tumour nests with peripheral tumour cell palisading. Secretogranin II was found in single cells or small clusters in 32 of the 49 cases; the staining intensity of secretogranin II in this tumour type was generally weak. Substance P was focally present in 47 tumours.

Enteroglucagon-producing (L cell) carcinoids of the appendix

Two carcinoids displaying a predominantly glandular pattern were strongly chromogranin B and secretogranin II positive, whereas chromogranin A immunoreactivity was detectable only in a few scattered tumour cells. Both cases contained cells with PP immunoreactivity. Prostatic acid phosphatase was not detectable.

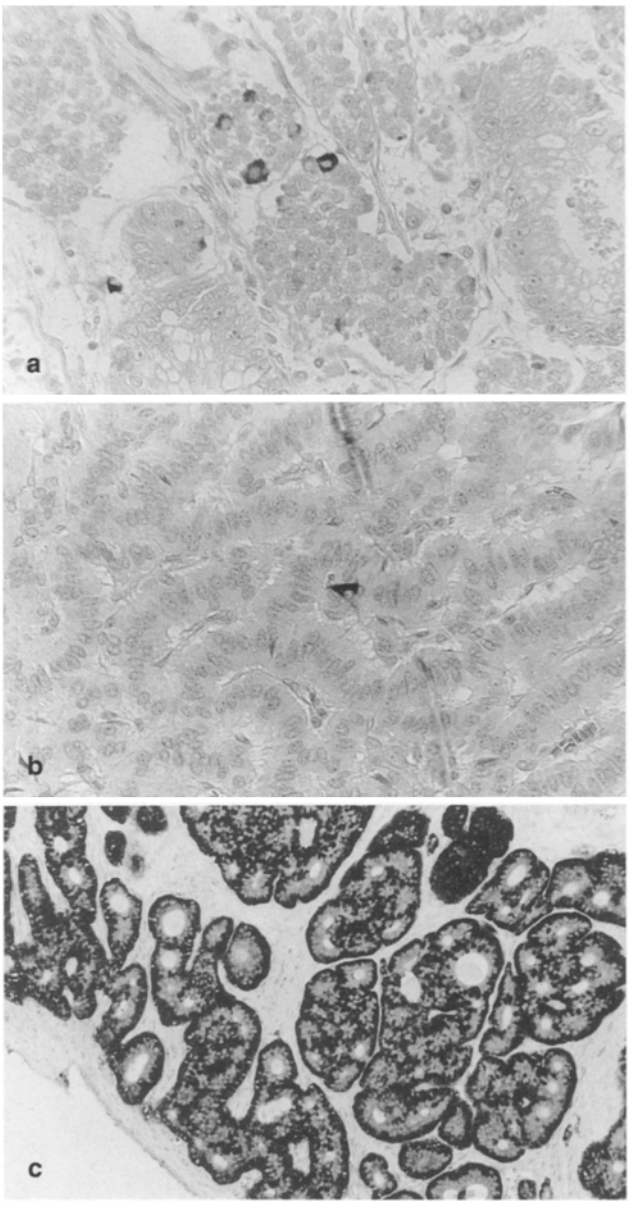

Fig. 1a–c Patterns of chromogranin A immunoreactivity in different intestinal carcinoids. **a** Somatostatin-rich duodenal carcinoid with a few scattered chromogranin A-positive tumour cells. On serial sections these cells were shown to contain gastrin, insulin, or PP. (APAAP technique, ×250). **b** Gastrin-producing duodenal carcinoid associated with Zollison Ellison syndrome. In tumours with low immunohistochemical gastrin content, chromogranins and secretogranin II were usually completely absent or only present a in few tumour cells. (chromogranin A; APAAP technique, ×250). **c** Classical appendiceal carcinoid. Strong chromogranin A positivity. (APAAP technique, ×100)

Jejunal carcinoids

A strong chromogranin A immunoreactivity was found in all 23 tumours investigated (Fig. 2a). Chromogranin B and 5HT was also present in these cases; however, moderate to strong staining of the latter two markers was mainly restricted to the periphery of tumour islets with typical palisading (Fig. 2b, c). A weak to moderate se-

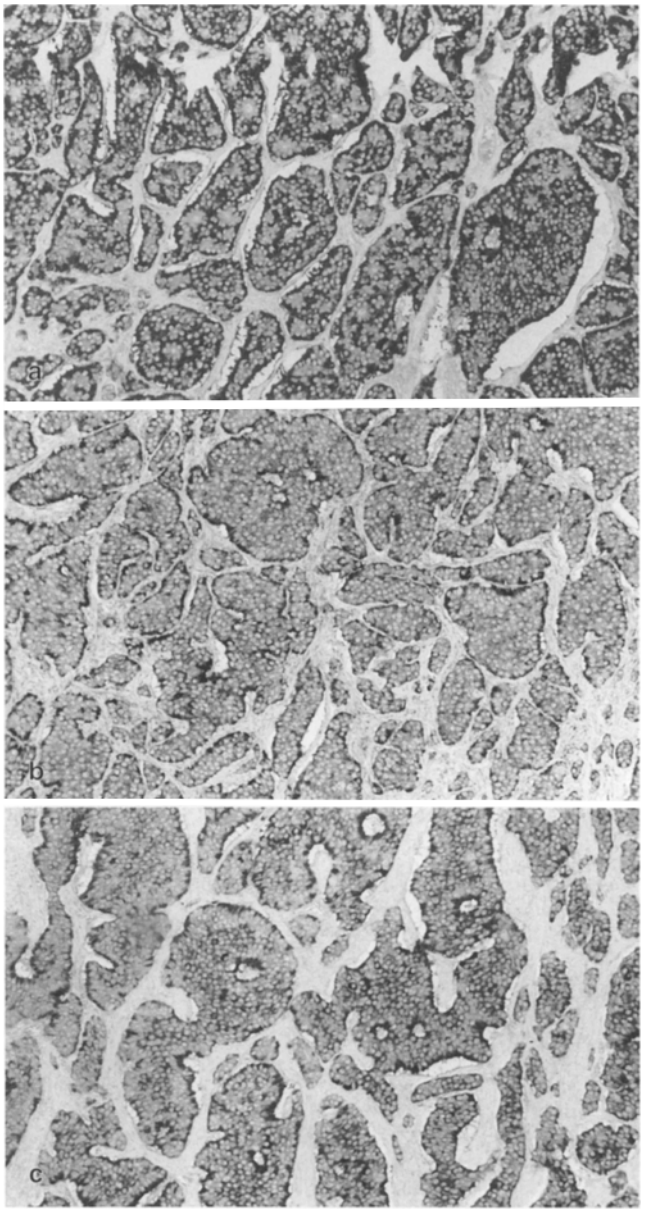

Fig. 2a–c Classical carcinoid of the ileum. Strong chromogranin A immunoreactivity (**a**), whereas chromogranin B (**b**) and 5HT (**c**) are mainly immunolocalized in palisading tumour cells at the periphery of tumour islets. (APAAP technique, semi-adjacent sections of the same case, ×40)

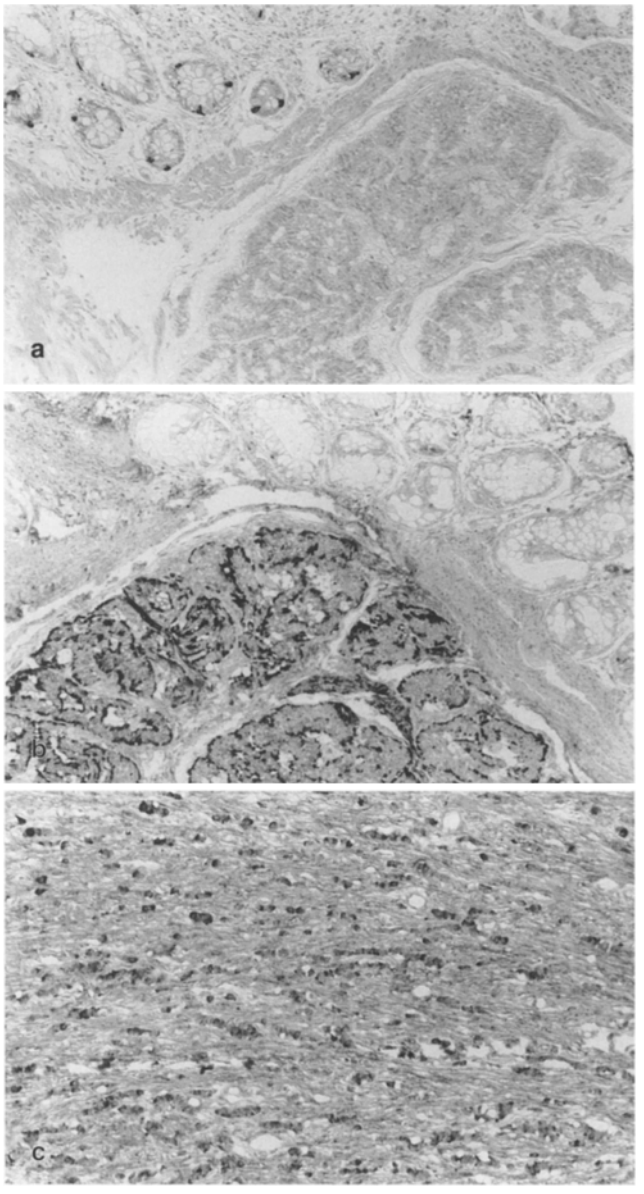

Fig. 3a–c Immunoreactivity of different granins in carcinoids of the rectum. **a** Chromogranin A-negative rectal carcinoid with trabecular/glandular pattern. Note positive neuroendocrine cells in the normal mucosa. (APAAP technique, ×40). **b** Same case demonstrating chromogranin B immunoreactivity. (APAAP technique, ×40). **c** Secretogranin II positivity in a 'ribbon-looped' rectal carcinoid. (APAAP technique, ×40)

cretogranin II immunoreactivity was detectable either in a few scattered cells or in tumour cell clusters; completely secretogranin II-negative small intestinal carcinoids, as in argentaffin appendiceal carcinoids, were not observed. Substance P was focally demonstrated in 21 of the 23 tumours.

Caecal carcinoids

Chromogranin A and B, 5HT and secretogranin II showed similar distribution patterns to those seen in ileal carcinoids. Substance P immunoreactivity was found in 3 tumours.

Rectal carcinoids

Only 6 tumours showed the typical 'looped ribbon' pattern (Fig. 3c [6]); the others displayed irregular trabeculae with occasional rosettes, intermingled in some cases with nest components. A moderate to strong chromogranin B (Fig. 3b) and secretogranin II (Fig. 3c) immunore-

Table 2 Distribution of chromogranins (chromogranin A, chromogranin B, and secretogranin II) in various neuroendocrine tumours of the gastrointestinal tract (- negative, + few scattered positive tumour cells, ++ focal positivity, +++ majority of tumour cells positive; *parentheses* weak to moderate intensity of immunostaining)

Tumour	CGA	CGB	SII
Well differentiated			
Gastric carcinoid	25/25 +++	25/25 (++)	25/25 (++)
Gastrin producing tumour	2/17–	4/17–	5/17–
	4/17+	5/17+	6/17+
	11/17++	8/17++	6/17++
Somatostatin producing tumour	4/5+	2/5+	2/5–
	1/5++	3/5+++	3/5+
'Gangliocytic paraganglioma'	1/4–	2/4–	2/4–
	3/4++	2/4++	2/4++
Appendiceal EC carcinoid	49/49+++	49/49+++	17/49–
			32/49+
Appendiceal L cell carcinoids	1/2–	2/2+++	2/2+++
	1/2+		
Carcinoid of the ileum	23/23+++	23/23+++	14/23+
			9/23++
Carcinoid of the ceacum	4/4+++	4/4+++	2/4+
			2/4 (++)
Retal L cell carcinoid	12/17–	17/17+++	4/17++
	5/17+		13/17+++
Poorly differentiated			
Neuroendocrine carcinoma of the stomach	2/2++	1/2–	2/2–
Neuroendocrine carcinoma of the rectum	3/3–	3/3++	1/3–
	3/3–	3/3++	1/3–
			2/3++

activity was demonstrable in the majority of tumour cells. Chromogranin A was completely negative in 12 tumours (Fig. 3a); a few scattered cells were demonstrated in 5 tumours. In 8 tumours moderate to strong focal immunoreactivity for prostatic acid phosphatase was shown.

Poorly differentiated neuroendocrine carcinomas

of the 5 poorly differentiated endocrine carcinomas, 2 were in the stomach and 3 in the rectum. All these tumours were large and had metastasised. None of the tumours was associated with a clinically overt endocrine syndrome. Chromogranin A was immunohistochemically focally demonstrated in both gastric tumours, chromogranin B in 1 gastric and in all 3 rectal tumours, and secretogranin II in 2 rectal tumours. Two rectal tumours expressed prostatic acid phosphatase immunohistochemically.

Discussion

The occurrence of chromogranin A and B and of secretogranin II has been described both in normal neuroendocrine cells [12, 21–23, 26, 28, 35, 37, 39] and in neuroendocrine tumours of the gastrointestinal tract [8, 20, 23, 25, 36, 37, 39]. Chromogranin A, chromogranin B, and secretogranin II were found in the vast majority of gastrointestinal carcinoids. However, in most of these studies no quantitative analysis of granins expression was performed. Using oligonucleotide probes for the demonstration of chromogranin A and B mRNAs, Funa et al. [13] recently pointed out that foregut, midgut, and rectal carcinoid tumours are different in their endocrine properties regarding the expression of chromogranin A and B.

Our results demonstrate various distribution patterns of chromogranin A, chromogranin B, and secretogranin II in neuroendocrine tumours of the gastrointestinal tract. Although, owing to their location and histological features, most of these tumours are easily identified, immunohistochemical proof of their neuroendocrine nature and hormone production has become mandatory. In particular, since chromogranin A is widely used in diagnostic histopathology as a general neuroendocrine marker, it is of importance to recognise its presence or absence in all varieties of neuroendocrine tumours.

In our series chromogranin A was consistently found in most tumour cells of gastric carcinoids and serotonin-producing carcinoids of the appendix, small intestine, and large caecum. In all other tumour types we observed a wide range of chromogranin A immunoreactivity. Gastrin-producing tumours, 'gangliocytic paragangliomas', poorly differentiated neuroendocrine carcinomas, and appendiceal and rectal L cell carcinoids may lack chromogranin A completely. However, there seems to be no intestinal neuroendocrine tumour type that consistently fails to contain chromogranin A-positive cells.

Chromogranin B was consistently detectable in carcinoids of the stomach, the appendix, the small and large intestine, and the rectum. Amongst endocrine tumours of the stomach and the upper small intestine a complete lack of chromogranin B immunoreactivity was noted in some gastrin tumours and 'gangliocytic paragangliomas'. Although consistently found in argyrophil gastric (ECL) carcinoids, chromogranin B immunoreactivity

was considerably weaker than chromogranin A immunoreactivity.

Secretogranin II immunoreactivity was demonstrated in the majority of the tumour cells of appendiceal and rectal L cell carcinoids. Serotonin-producing ileal and caecal carcinoids contained a few secretogranin II-positive cells, whereas some gastrin- or somatostatin-producing tumours, 'gangliocytic paragangliomas', appendiceal carcinoids, and poorly differentiated neuroendocrine carcinomas showed a complete lack of secretogranin II immunoreactivity. In gastric carcinoids secretogranin II was found in a similar distribution pattern to chromogranin B.

A gastric carcinoid displaying a strong chromogranin A immunoreactivity in the absence of gastrin- and somatostatin-immunoreactive cells is highly suggestive of an ECL cell tumour. From our results this tumour type shows considerably weaker and focally distributed chromogranin B and secretogranin II immunoreactivity. The clear demonstration of chromogranin B immunoreactivity in gastric carcinoids contrasts with observations recorded in a study by Bordi et al. [2], who were unable to detect chromogranin B in these tumours. A strong chromogranin A immunoreactivity is also present in fundic neuroendocrine cell hyperplasia associated with atrophic gastritis; chromogranin B- and/or secretogranin II-positive hyperplastic cells were only occasionally detected. In gastrin-producing tumours chromogranin/secretogranin immunoreactivities were characteristically related to immunohistochemical demonstration of gastrin. Five duodenal tumours associated with ZES and showing very few gastrin-positive cells also lacked or contained only a few scattered chromogranin-/secretogranin-positive cells. Somatostatin-producing tumours [3, 7, 16] are characterized by a glandular pattern and the frequent occurrence of psammoma bodies. The somatostatin cell tumours in the present series usually showed only a few chromogranin A- and secretogranin II-positive cells; on serial sections these cells expressed various peptides (such as gastrin, insulin, PP). A somatostatin-rich appendiceal carcinoid with psammoma bodies has recently been described [4]. In contrast to paragangliomas in other locations [31], the majority of 'gangliocytic paragangliomas' completely lack chromogranin A.

Serotonin-producing appendiceal and ileal carcinoids showed similar distribution patterns of chromogranin A and B. In agreement with reports in the literature [4, 8, 14, 24, 27, 37], chromogranin A immunoreactivity was found in almost all tumour cells, whereas weaker chromogranin B expression was mainly found in tumour cell islets with peripheral palisading. Both chromogranin A and B mRNA has been found in midgut carcinoids [13]. In classical intestinal carcinoids secretogranin II was present in single cells or small cell clusters; however, secretogranin II was completely absent from 17 of 49 serotonin-producing appendiceal carcinoids. It is uncertain whether these immunohistochemical findings can be used to support the concept that intestinal carcinoids are histogenetically related to the epithelial cells of the intestinal mucosa, while the appendiceal carcinoids may represent a distinct type of intestinal paraganglioma [38].

Both rectal and appendiceal L cell carcinoids showed a distinct and consistently different immunohistochemical expression of granins from serotonin-producing carcinoids. Chromogranin B and secretogranin II were demonstrated in the majority of tumour cells in all 19 cases. Chromogranin A, however, was either completely absent or was immunolocalised only focally in tumours with a predominant trabecular pattern; this finding agrees with previous reports [10, 27]. Prostatic acid phosphatase has been found in 8 of 17 rectal carcinoids and thus seems to be a regular finding in tumours originating from the hindgut [10]. However, prostatic acid phosphatase has recently been demonstrated in both strumal and primary renal carcinoids without any embryological relationship to the hindgut [15, 32]. Both appendiceal L cell carcinoids in the present series contained PP-positive cells, an unusual feature in midgut carcinoids [1, 4].

Poorly differentiated endocrine tumours are also referred to as atypical carcinoids. The granins were detected only focally within these tumours. Both gastric tumours expressed chromogranin A, while chromogranin B was found in all three rectal tumours; thus these tumours are similar in the distribution of chromogranin A and B to well-differentiated carcinoids of the corresponding sites.

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