Virchows Arch [Pathol Anat] (1985) 405:311–323

Virchows Archiv A

Pancreatic endocrine tumours associated with WDHA syndrome

An Immunohistochemical and electron microscopic study*

Akishi Ooi[1, 5], **Toru Kameya**[2], **Masaru Tsumuraya**[1], **Ken Yamaguchi**[3], **Kaoru Abe**[3], **Yukio Shimosato**[1], **and Noboru Yanaihara**[4]

[1] Pathology Division, National Cancer Center Research Institute, Tokyo
[2] Department of Pathology, Kitasato University, School of Medicine, Kanagawa
[3] Endocrinology Division, National Cancer Center Research Institute, Tokyo
[4] Laboratory of Bioorganic Chemistry, Shizuoka College of Pharmacy, Shizuoka
[5] Department of Pathology, School of Medicine, Kanazawa University, Kanazawa

Summary. Nine pancreatic endocrine tumours of patients with watery diarrhoea hypokalaemia achlorhydria (WDHA) syndrome were examined by immunohistochemistry and electron microscopy. All cases revealed neoplastic proliferation of VIP (vasoactive intestinal peptide)-immunoreactive (IR) cells. Immunoreactivity to a novel peptide hormone PHM-27, which is processed from a common big precursor peptide of VIP (prepro VIP/PHM-27), was identified in VIP-IR cells of 8 tumours. VIP-PHM-IR cells had secretory granules measuring about 130 to 220 nm in diameter. Radioimmunoassay of tumour tissue extracts showed high VIP and PHM contents in proportional amounts in most cases. According to the results of immunostaining, the 8 tumours fell into two large groups; 5 with PP (pancreatic polypeptide)-IR cells and 3 with CT (calcitonin)-IR cells. The former group demonstrated VIP cells and PP cells intermingled in various proportions, including one tumour in which coexistence of PP-IR and VIP-IR in the same cells was demonstrated. Cell heterogeneity of the tumours and possible relationships of VIP, PP and CT cells were discussed.

Key words: Endocrine pancreatic tumours – WDHA syndrome – Peptide hormones – Immunostaining

Introduction

Panreatic endocrine tumours associated with watery diarrhoea hypokalaemia achlorhydria (WDHA)syndrome are relatively rare and few comprehensive studies employing immunohistochemistry, electron microscopy and radio-

* This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare and from the Ministry of Education, Science and Culture, Japan

Offprint requests to: T. Kameya at the above address

immunoassay have been performed previously (Capella et al. 1983; Jaffe et al. 1977; Rambaud et al. 1975). The weight of the evidences has indicated that the causative agent of this syndrome is vasoactive intestinal polypeptide (VIP) (Kane et al. 1983; Bloodworth et al. 1982; Long et al. 1981). However, there still remains the possibility that other peptide hormones contribute to or modify this syndrome. Among these, the most plausible candidates are pancreatic polypeptide (PP) (Lundqvist et al. 1978) and calcitonin (CT) (Öberg et al. 1981; Gutniak et al. 1980; Cox et al. 1979). The reason for the disagreement probably derives from the multiplicity of hormones the tumour produces. Recent advances in endocrinological biochemistry clarified not only the primary structure of VIP, PP and CT, but also the coding m-RNAs and the precursor big peptides of VIP and CT (Itoh et al. 1983; Amara et al. 1982).

A peptide (P) with amino terminal histidine (H) and carboxyl terminal methionine (M) with 27 amino acid residues (PHM-27) is a newly discovered peptide sharing with VIP a common big precursor, prepro VIP/PHM-27 (Itoh et al. 1983). It differs by only two amino acids from the porcine counterpart, PHI-27 (Tatemoto et al. 1981). Coexistence of VIP and PHI-27 was demonstrated in nerves in normal tissues and in some VIPoma cells (Bloom et al. 1983; Christofides et al. 1982).

The purpose of this investigation is to examine the coexistence of immunoreactive (IR) VIP and PHM in the same cells of tumours with WDHA syndrome and to clarify the distribution of VIP, PP and CT cells in these tumours.

Materials and Methods

Nine tumours associated with WDHA syndrome (Yamaguchi et al. 1984) out of 79 cases of endocrine pancreatic tumours available to us were studied morphologically. Tissues were routinely fixed in formalin, 4% buffered paraformaldehyde and/or Bouin's solution, and dehydrated 5 μ serial sections were used for routine haematoxylin-eosin and immunostainings. When fresh tumour was available for electron microscopy, tissues were fixed in cold phosphate-buffered 2.5% glutaraldehyde (2 h) followed by 1% OsO_4 (1 h), dehydrated and embedded in Epon 812. When necessary, a semithin section cut from Epon-embedded tissue was placed on a glass microscopic slide coated with 1% gelatin. After removal of the plastic with saturated potassium hydroxide, the section was submitted to immunostaining. Cellular localization of the hormones was detected by the avidin biotin complex method (Hsu et al. 1981). Primary antisera to VIP (R-501) (Yamaguchi et al. 1980a), PHM-27 (R-8502) (Itoh et al. 1983), glucagon (GA-10), insulin (NY-1), and somatostatin (OAL 272) (Yamaguchi et al. 1981) were prepared and characterized by one of us (N.Y.). Anti-CT (25H2T) was purchased from Immuno Nuclear Corporation, Stillwater, Minn., USA. Anti-bovine PP (615-R-110-146-17) was a gift from Dr. R.E. Chance, Lilly Research Laboratories, Indianapolis, Ind., USA. Working dilutions of the respective antisera were from 1,000 to 20,000. Specificity was checked by immunostaining of normal tissues of known localization and an absorption test with an excess of the respective antigen (1–10 μg/ml) for each antiserum. In immunohistochemical analysis of serial sections for simultaneous localization of two hormones, each antiserum of working dilution containing excess counterpart antigen (more than 10 μg/ml diluted antiserum) was used to ensure the absence of possible cross-reaction with each other. After observation of an immunohistochemically stained semithin section of plastic embedded material, an adjacent ultrathin section was made and stained by uranium and lead. Identical cells definitely positive for specific hormones were examined by electron microscopy. Polypeptide hormones in the

tumour extracts were determined by radioimmunoassays (RIAs) specific for VIP (Yamaguchi et al. 1980a), PHM, PP (Chance et al. 1979), and CT (Abe et al. 1977) according to the method previously described. In order to know the molecular size heterogeneity of IR-VIP and IR-PHM, one extract of the tumour (case 5) was examined by Sephadex G-50 superfine gel filtration (1.0 × 45 cm), which was equilibrated and eluted with 1 N acetic acid. The sample was fortified with ^{125}I-human albumin and Na^{125}I as internal standards, applied to the column, and eluted by means of fraction collector-pump control system (Yamaguchi et al. 1983). Fractions of 0.8 ml each were collected, lyophilized and reconstituted by the standard diluent for RIA when assayed. The column was also calibrated with synthetic VIP and PHM.

Results

Routine histological examination demonstrated a remarkable variety of cytological and histological patterns and did not reveal a predominant histological type. Three cases of multiple endocrine neoplasia (MEN) type 1 showed combinations of hyperplasia, adenomas and carcinomas of endocrine cells. Cases 1 and 9 are described in greater detail elsewhere (Yamaguchi et al. 1980b). Results of immunostaining are summarized in Table 1. VIP-IR cells were detected in all cases. Serial sections of nine cases excluding case 1 in which additional sections were not available, were examined. Most tumour cells immunoreactive to anti-VIP were also reactive to anti-PHM in all the cases examined (Fig. 1a, b). The numbers of immunoreactive cells varied in each case. Tumour cells not reactive to either antiserum were also present. Electron microscopic examinations were performed in four cases (cases 3, 5, 8 and 9). The tumour cells had a well developed endoplasmic reticulum. The majority had secretory granules, but their populations varied in individual cells. The secretory granules were characterized by a round electron dense core separated from the limiting membrane by less electron dense haloes (Fig. 2). The mean diameters of the secretory granules were 224 ± 56 ($n=119$), 192 ± 38 ($n=224$), 156 ± 38 ($n=267$) and 134 ± 38 ($n=431$) nm in cases 3, 5, 8 and 9 respectively. The results of RIA of tissue hormones are shown in Table 2. All except two cases without PHM data showed high VIP and PHM values. There was significant paral-

Table 1. Immunohistochemistry of 9 pancreatic endocrine tumours with WDHA syndrome

Case No.	VIP	PHM	PP	CT	GL	SS	INS
1 (MEN-1)	+	NT	+	−	+	+	+
2	+	+*	+	−	−	−	−
3	+	+*	+	−	−	−	−
4 (MEN-1)	+	+*	+	−	+	−	−
5	+	+*	+	−	+	+	−
6	+	+*	−	+	−	−	−
7	+	+*	−	−	−	−	−
8	+	+*	−	+	−	−	−
9 (MEN-1)	+	+*	−	+	−	−	−

MEN-1 = multiple endocrine neoplasia-Type 1; + = a few immunostained cells; + = numerous immunostained cells; − = no immunostained cells; * = immunoreactive to both VIP and PHM

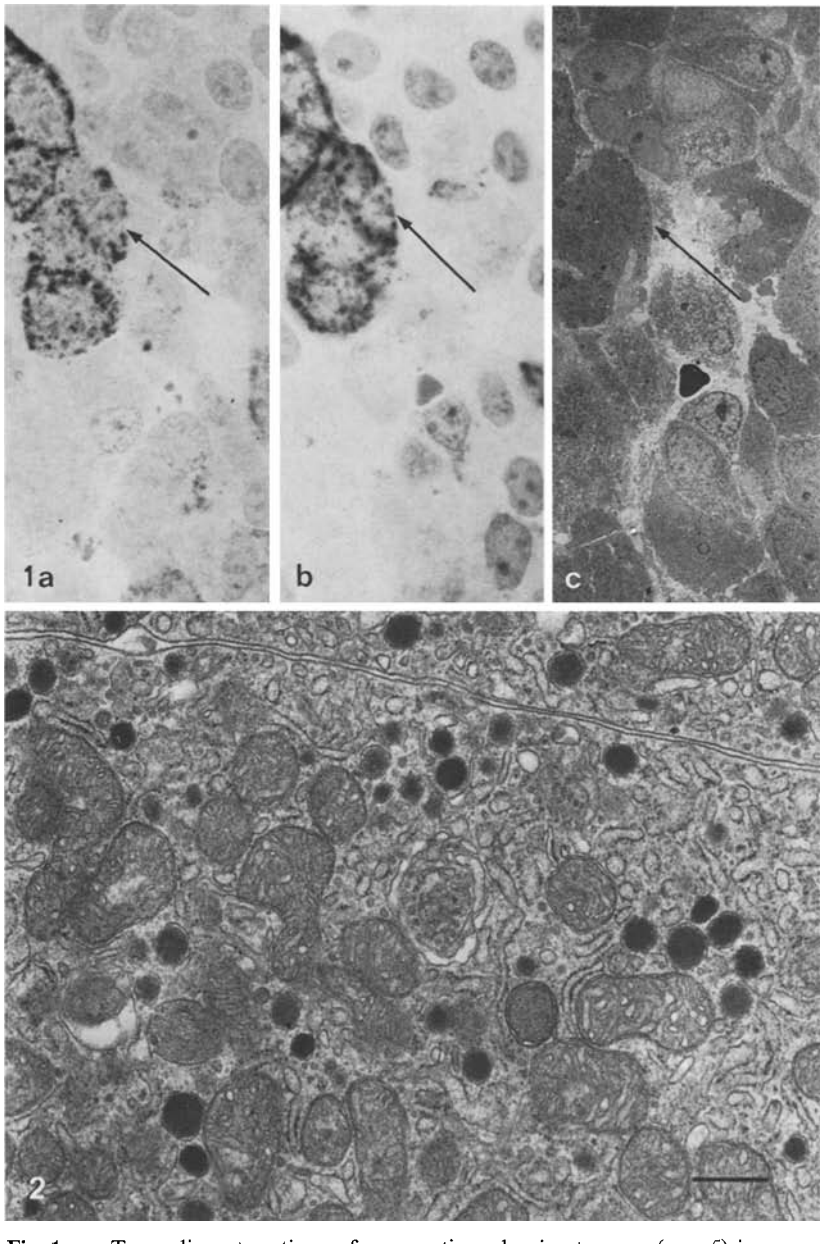

Fig. 1a–c. Two adjacent sections of pancreatic endocrine tumour (case 5) immunostained for VIP (**a**) and PHM (**b**). At least five cells are positive for both hormones. ×1,000. **c** Electron micrograph of the identical area to **a** and **b**. ×1,000 (An identical cell is indicated by an arrow in **a, b** and **c**)

Fig. 2. High magnification of the cell indicated by an arrow in Fig. 1c. Note the numerous mitochondria and electron dense secretory granules. ×20,000 (scale indicates 0.5 μ)

Table 2. Peptide hormones in pancreatic endocrine tumours with WDHA syndrome (RIA)

Case No.	Hormone content (ng/g wet tissue)			
	VIP	PHM	PP	CT
1	53,000	23,000	630,000	UD
2	53,000	29,000	89,000	7.5
3	12,000	5,800	240,000	15
4	1,700	NT	160,000	UD
5	32,000	2,100	430	UD
6	14,000	1,700	UD	2,800
7	7,400	3,000	UD	800
8	2,500	2,600	UD	750
9	9,000	NT	UD	77
normal pancreas	190 ± 120 $n=15$	63 ± 29 $n=3$	750 ± 530 $n=15$	ud $n=15$

NT = not tested; UD = undetectable

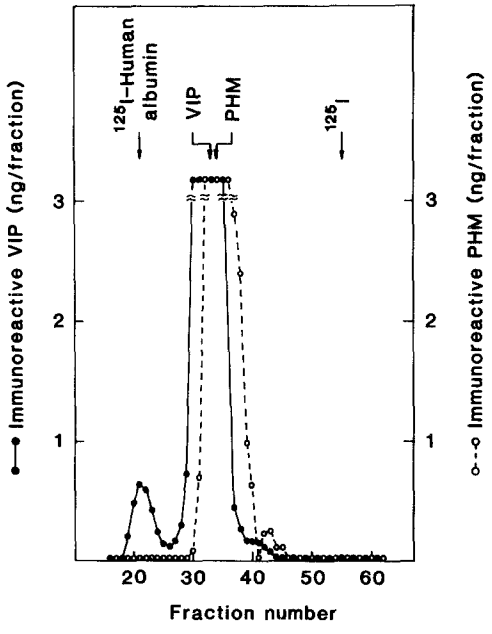

Fig. 3. Gel filtration patterns of extracts prepared from the tumour of case 5. Substances used for markers are shown at top

lelism between both hormones in 6 cases but not case 6. Gel filtration studies revealed that IR-VIP in the tumour extract of case 5 was composed of 2 peaks (Fig. 3). A small but definite peak was eluted at a position of ^{125}I-human albumin. In addition, the major IR-VIP peak was eluted at the position of synthetic VIP. As for IR-PHM, almost all immunoreactive PHM was eluted at the position corresponding to that of synthetic PHM. As indicated in Table 2, 4 cases (cases 1 to 4) exhibited high PP content and 3 cases (cases 6 to 8) high CT content. According to the results of

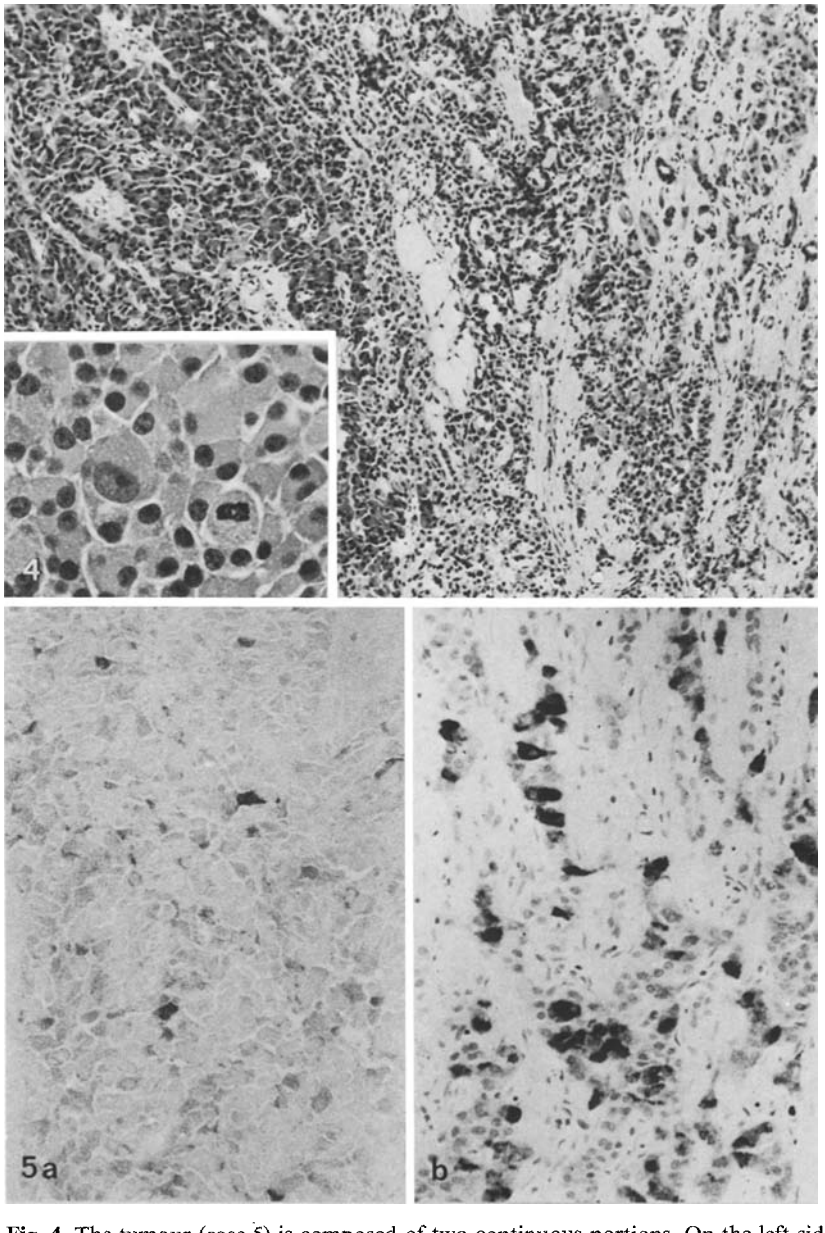

Fig. 4. The tumour (case 5) is composed of two continuous portions. On the left side tumours cells proliferate in solid pattern. On the right, tumour cells are arranged in thin cords in fibrous stroma. Small glandular patterns are identified at upper right corner. ×85. Insert figure demonstrates high magnification of solid portion. ×340

Fig. 5a, b. Two representative portions of the tumours (case 5) immunostained for VIP (**a**) and PP (**b**). ×150

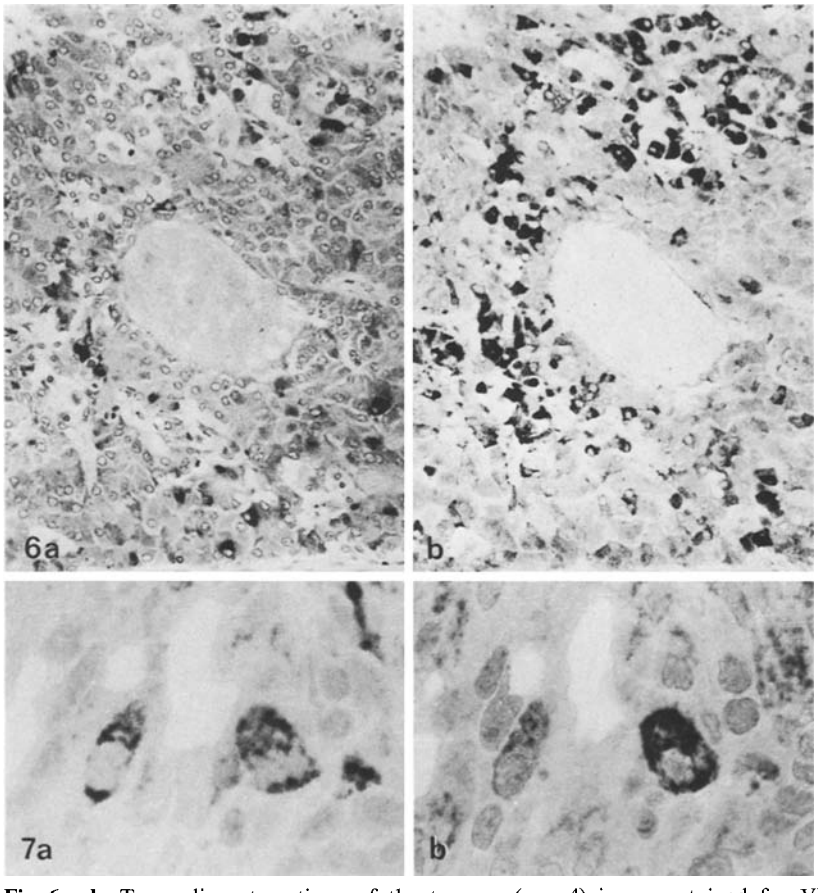

Fig. 6a, b. Two adjacent sections of the tumour (case 4) immunostained for VIP (**a**) and PP (**b**). VIP-IR cells and PP-IR cells are intermingled. ×150

Fig. 7a, b. Two adjacent sections of the tumour (case 3) immunostained for VIP (**a**) and PP (**b**). At least two cells are positive for both hormones. ×600

immunohistochemistry 8 cases (apart from case 7) were divided into two groups: five cases (cases 1 to 5) with VIP cells and PP cells; 3 cases (cases 6, 8 and 9) with VIP cells and CT cells. In the former group VIP-IR cells and PP-IR cells were intermingled in various proportions and distribution. In case 3, the coexistence of both hormones was demonstrated in some tumour cells. In case 5 VIP-IR cell tumour was circumscribed by PP-IR cells. In three cases with high tissue CT value, CT-IR and VIP-IR cells were mixed. Aspects of cases 3 and 4 were presented briefly elsewhere (Kameya et al. 1982). Representative cases with several cell populations are described in detail below.

Case 5. A large tumour measuring 4.5 cm in diameter was located in the pancreatic body. It consisted of two distinct histological patterns, which

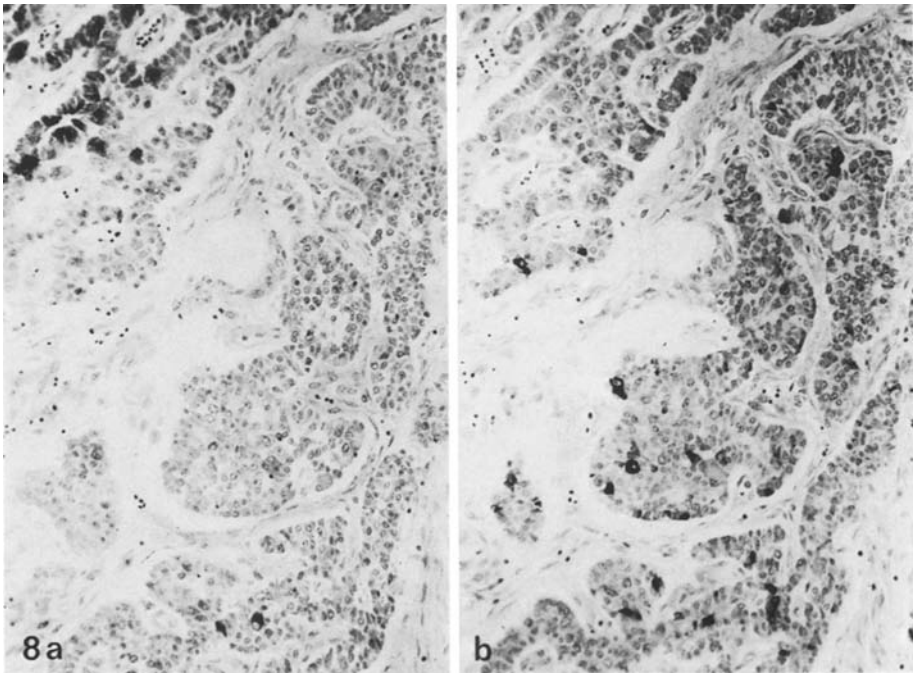

Fig. 8a, b. Two adjacent sections of the tumour (case 8) immunostained for VIP (**a**) and CT (**b**). Two types of cells occupy different parts of the tumour. × 200

were connected by an apparently transitional area (Fig. 4). The central area of the tumour was composed of a solid proliferation of cells, which had round nuclei and abundant eosinophilic cytoplasm. Many mitotic figures were found (Fig. 4 insert). VIP and PHM reactivities were identified in these cells (Fig. 5a). This solid tumour was surrounded with less eosinophilic cuboidal cells arranged in cords or clusters. They were immunohistochemically found to be positive for PP or glucagon (GL) (Fig. 5b), but there were no positive cells for insulin (INS) in the field. Although small ducts were often encountered in the immediate vicinity of PP cell clusters, no evident connection with ductal epithelium could be found.

Case 4. This was a case of multiple endocrine neoplasia (MEN) type 1. Multiple tumour nodules up to 2.0 cm in diameter were scattered in the head and neck of the pancreas. The largest was a well encapsulated and solid one, showing a partly glandular pattern. VIP positive cells and PP positive cells were intermingled (Fig. 6). However, no single cells with both immunoactivities were identified by close examination of adjacent serial sections alternately stained for VIP and PP. Marked PP cell hyperplasia of islets and an extra-insular region with distorted acino-islet ductal system was noted around this tumour. This case was diagnosed as adenoma, consisting of VIP cells and PP cells accompanied with PP cell hyperplasia.

Case 3. The tumour was well demarcated by dense fibrous tissue from surrounding pancreatic tissue. Proliferating tumour cells with large pleomorphic nuclei approximately twice the size of acinar cells, showed partly tubular formation. Mitotic figures were frequent. PP-IR cells and slightly fewer VIP-IR cells were identified. It was noteworthy that PP and VIP immunoreactivities were demonstrated simultaneously in the same tumour cells (Fig. 7). Cells containing only VIP and PP were also present. Electron microscopy revealed that cells immunoreactive to anti-VIP alone or to anti-PP alone possessed granules of 228 ± 53 ($n=94$) and 240 ± 60 ($n=180$) nm in diameter, respectively. They had virtually identical ultrastructural features to those of VIP-PP cells.

Case 8. The tumour cells comprised a network of cords with well vascularized stroma. Immunostaining revealed that cell cords of VIP cells and groups of CT cells were mixed (Fig. 8). The two types of cells were recognized only by immunostaining.

Discussion

Neoplastic proliferation of VIP-IR cells was confirmed in all tumours of the WDHA syndrome cases. In 8 cases available for serial sections, some tumour cells were confirmed to react to both anti-VIP and anti-PHM sera simultaneously. PHM-27 is a newly discovered peptide hormone synthesized from a common big precursor protein to VIP, prepro VIP/PHM-27 (Itoh et al. 1983). In 1981 Tatemoto et al. discovered a new peptide, PHI, from porcine gut. PHM is a human counterpart of PHI, which differed from porcine PHI in only two amino acid residues. Bloom et al. and Christofides et al. reported the coexistence of both hormones in nerves in normal tissues and in some VIPoma cells, respectively (1982; 1983). The present study further demonstrated the coexistence of PHM immunoactivity in most VIP-IR cells of pancreatic endocrine tumours.

Due to the small size of the tumour cells it was difficult to find identical cells in adjacent sections in some cases, but in such cases significant correlation was found between the distributions of both positive cell groups. It is safe to conclude that both immunoreactivities exist simulatneously in most tumour cells. Cells non-reactive to both antisera also may represent either a functional state of the tumour cells, which do not store appreciable amounts of immunoreactive substances, or another distinct population.

Conventional fixation and epoxy resin polymerization for EM was found to be inappropriate for good preservation of VIP immunoreactivity (Capella et al. 1983). Immunoelectron microscopy using protein A gold complex method (Roth et al. 1978) on epoxy resin embedded thin sections was far from satisfactory for not only VIP but also PHM in our tumour cases. The ultrastructure of secretory granules of VIP-PHM immunoreactive cells were essentially similar in shape, size and electron density in each case but there were differences in the mean diameters of granules among the 4 cases examined. As both hormones are translated from common m-RNA,

they should be produced in an equimolar ratio. Most cases show significant correlation between the contents of both hormones on RIAs. Although a gel filtration study was done only in case 5, it was shown that most IR-VIP had the same molecular size of authentic VIP, and that most of IR-PHM had the same molecular size of authentic PHM. As far as IR-VIP is concerned, we have reported that extracts of 6 VIP-producing tumours had gel filtration patterns similar to case 5 (Yamaguchi et al. 1980a). These results indicated that almost all IR-peptides detected by immunohistochemistry should be identical or very similar to authentic VIP and PHM.

Most pancreatic endocrine tumours have a multiplicity of cell types (Mukai et al. 1982; Heitz et al. 1982), and a frequent combination of VIP cells and PP cells was recognized (Larsson et al. 1976; Lundqvist et al. 1978). PP cells were concluded to be an evident component of the tumours because of their existence in metastatic foci of lymph nodes and/or liver as well as in the primary lesion (Heitz et al. 1976; Polak et al. 1976). In the present study, three different patterns of distribution of VIP cells and PP cells were demonstrated. PP cells were positive in five cases of which cases 4 and 5 were typical. While both cells were evently mixed in case 4, PP cells made a mantle of the VIPoma in case 5. The cytological appearance of both cells in case 5 was distinctly different but histologically both parts were continuous. Therefore, the neoplastic nature of the PP cells was most probable but the evidence was not conclusive. As the tumour was located in the body of the pancreas in case 5, it was unlikely that the assembly of PP-IR cells surrounding VIPoma represented a PP-rich region (Orci et al.1978) invaded by the tumour.

In case 3, PP-IR and VIP-IR were demonstrated simultaneously in the same cells. To the best of our knowledge this has not been reported by other investigators. As immunoreactivity was abolished by prior absorption of serum by the corresponding antigen and the serum contained an overdose of the counter antigen, cross reaction was most unlikely. The two peptides show no similar amino acid sequences (Blundell et al. 1980; Itoh et al. 1983), and it is known that VIP precursor has no sequence homologous with PP. Yet little is known about the precursor molecule of PP. Capella et al. (1983) reported 32 cases of VIP producing pancreatic endocrine tumours. Among them 11 cases were accompanied with PP-IR cells, but no case was found in which both immunoreactivities were identified in single cells.

Evidence is accumulating that some endocrine cells produce more than two hormones (Larsson 1980). However, most reports were exclusively concerned with those having common precursors of such as combinations of adrenocorticotropic hormone and γ-melanocyte stimulating hormone (Nakanishi et al. 1979; Kameya et al. 1983a), and VIP and PHM as discussed before. There are some examples outside this category; CT and SS in pancreatic endocrine tumours (Galmiche et al. 1980); gastrin-releasing peptide and CT in medullary thyroid carcinoma (Kameya et al. 1983b) and bronchial endocrine cell (Tsutsumi et al. 1983). CT is a common product of the APUD tumours (Abe et al. 1977) and in the present study CT-IR cells were demonstrated in three tumours out of four with high tissue CT-IR. In contrast

Capella's recent series of VIPomas contained no case with CT-IR cells (1983). This disparity may be caused by the immunoreactivity of antisera used or methods of sampling the tumour. As CT-IR cells were located in a small portion of a large tumour in some cases, it is possible to miss the cells.

In case 5, many PP-IR cells were demonstrated but the tissue content of PP was within a normal range. Case 7 the tumour which showed a high CT tissue content had no CT-IR cells. The discrepancies between the plasma level, tissue content and demonstrable IR cells can be explained in several ways. First, RIA is so highly sensitive that it can detect trace amounts which immunohistochemistry cannot detect. Secondly, as mentioned above, changes due to methods of sampling the tumour and processing such as fixation and embedding for immunostaining may be involved. Thirdly, some tumours capable of production and secretion of hormones may not have storage capacity at the immunohistochemical and/or RIA levels.

References

Abe K, Adachi I, Miyakawa S, Tanaka M, Yamaguchi K, Tanaka N, Kameya T, Shimosato Y (1977) Production of calcionin, adrenocorticotropic hormone, and β-melanocyte-stimulating hormone in tumors derived from amine precursor uptake and decarboxylation cells. Cancer Res 37:4190–4194

Amara SG, Jonas V, Rosenfeld MG (1982) Alternative RNA processing in calcitionin gene expression generates mRNAs encoding different polypeptide products. Nature 298:240–244

Bloodworth JMBJ, Greider M (1982) Endocrine pancreas and diabetes mellitus. In: Bloodworth JMBJ (ed). Endocrine pathology. Williams and Wilkins, Baltimore, London, pp 556–631

Bloom SR, Christofides ND, Delamaster J, Buell G, Kawashima E (1983) Diarrhoea in vipoma patients associated with cosecretion of a second active peptide (peptide histidine isoleucine) explained by single coding gene. Lancet ii:1163–1165

Blundell TL, Humbell RE (1980) Hormone families: pancreatic hormones and homologous growth factor. Nature 287:781–787

Capella C, Polak JM, Buffa R, Tapia FJ, Usellini L, Bloom SR, Solcia E (1983) Morphologic patterns and diagnostic criteria of VIP-producing endocrie tumours. Cancer 52:1860–1874

Chance RE, Moon NE, Johnson MG (1979) Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). In: Jaffe BM, Behman HR (eds). Methods of Hormone Radioimmunoassay. Academic Press, London, pp 657–672

Christofides ND, Yiangou Y, Blank MA, Tatemoto K, Polak JM, Bloom SR (1982) Are peptide histidine isoleucine and vasoactive intestinal peptide co-synthesized in the same pro-hormone? Lancet ii:1398

Cox TM, Fagen EA, Hillyard CJ, Allison DJ, Chadwick VS (1979) Role of calcitonin in diarrhoea associated with medullary carcinoma of the thyroid. Gut 20:629–633

Galmiche JP, Chayvialle JA, Dubois PM, David L, Descos F, Paulin C, Ducastelle T, Colin R (1980) Carcitonin-producing pancreatic somatostatinoma. Gastroenterology 78:1577–1583

Gutniak M, Rosenqvist U, Grimelius L, Lundberg JM, Hökfelt T, Rökaeus A, Rosell S, Lundqvist G, Fahrenkrug J, Sundblad R,Gutniak E (1980) Report on a patient with watery diarrhoea syndrome caused by a pancreatic tumour containing neurotensin, enkephalin and calcitonin. Acta Med Scand 208:95–100

Heitz P, Polak JM, Bloom SR, Adrian TE, Pearse AGE (1976) Cellular origin of human pancreatic polypeptide (HPP) in endocrine tumours of the pancreas. Virchows Arch [Cell Pathol] 21:259–265

Heitz PU, Kaspar M, Polak JM, Klöppel G (1982) Pancreatic endocrine tumours. Immunohistochemical analysis of 125 tumours. Hum Pathol 13:263–271

Hsu SM, Raine L, Fanger H (1981) A comparative study of the PAP method and avidin-biotin-complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 75:734–738

Itoh N, Obata K, Yanaihara N, Okamoto H (1983) Human preprovasoactive intestinal polypeptide, PHM-27. Nature 304:547–549

Jaffe BM, Kopen DF, DeSchryver-Kecskemeti K, Gingerich RL, Greider M (1977) Indomethacin-responsive pancreatic cholera. New Engl J Med 297:817–821

Kameya T, Yamaguchi K, Tsumuraya M, Abe K (1982) Morphological features in multiple hormone production by pancreatic endocrine tumours. In: Miyoshi A (ed). Gut peptide and ulcer. Proceedings of Hiroshima Symposium on Gut Peptide and Ulcer. Biomedical Research Foundation, Tokyo, pp 207–214

Kameya T, Shimosato Y, Kodama T, Tsumuraya M, Koide T, Yamaguchi K, Abe K (1983a) Peptide hormone production by adenocarcinomas of the lungs; Its morphologic basis and histogenetic consideration. Virchows Arch [Pathol Anat] 400:245–257

Kameya T, Bessho T, Tsumuraya M, Yamaguchi K, Abe K, Shimosato Y, Yanaihara N (1983b) Production of gastrin releasing peptide by medullary carcinoma of the thyroid. Virchows Arch [Pathol Anat] 401:99–108

Kane MG, O'Dorisio TM, Krejs GJ (1983) Production of secretory diarrhea by intravenous infusions of vasoactive intestinal polypeptide. New Engl J Med 309:1482–1485

Larsson LI (1980) On the possible existence of multiple endocrine, paracrine and neurocrine messengers in secretory cell system. Invest Cell Pathol 3:73–85

Larsson LI, Schwartz T, Lundqvist G, Chance RE, Sundler F, Rehfeld JF, Grimelius L, Fahrenkrug J, Schaffalitzky de Muckadell O, Moon N (1976) Occurrence of human pancreatic polypeptide in pancreatic endocrine tumours. Am J Pathol 85:675–684

Long RG, Bryant MG, Mitchell SJ, Adrian TE, Polak JM, Bloom SR (1981) Clinicopathological study of pancreatic and ganglioneuroblastoma tumours secreting vasoactive intestinal polypeptide (vipomas). Br Med J 282:1767–1770

Lundqvist G, Krause U, Larsson LI, Grimelius L, Schaffalitzky de Muckadell O, Fahrenkrug J, Johnson M, Chance RE (1978) A pancreatic-polypeptide-producing tumour associated with the WDHA syndrome. Scand J Gastroenterology 13:715–718

Mukai K, Greider MH, Grotting JC, Rosai J (1982) Retrospective study of 77 pancreatic endocrine tumours using immunoperoxidase method. Am J Surg Pathol 6:387–399

Nakanishi S, Inoue A, Kita T, Nakamura M, Chang ACY, Cohen SN, Numa S (1979) Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. Nature 278:423–427

Öberg K, Lööf L, Boström H, Grimelius L, Fahrenkrug J, Lundqvist G (1981) Hypersecretion of calcitonin in patients with the Verner-Morrison syndrome. Scand J Gastroent 16:135–144

Orci L, Malaisse-Lagae F, Baetens D, Perrelet A (1978) Pancreatic-polypeptide-rich regions in human pancreas. Lancet ii:1200–1201

Polak JM, Bloom SR, Adrian TE, Heitz PH, Bryant MG, Pearse AGE (1976) Pancreatic polypeptide in insulinomas, gastrinomas, vipomas and glucagonomas. Lancet i:328–330

Rambaud JC, Galian A, Scotto J (1975) Pancreatic cholera (WDHA syndrome): Histochemical and ultrastructural studies. Virchows Arch [Pathol Anat] 367:35–45

Roth J, Bendayan M, Orci L (1978) Ultrastructural localization of intracellular antigens by the use of protein A-gold complex. J Histochem Cytochem 26:1074–1081

Tatemoto K, Mutt V (1981) Isolation and characterization of the intestinal peptide porcine PHI (PHI-27), a new member of the glucagon-secretion family. Proc Natl Acad Sci USA 78:6603–6607

Tsutsumi Y, Osamura Y, Watanabe K, Yanaihara N (1983) Simultaneous immunohistochemical localization of gastrin releasing peptide (GRP) and calcitonin (CT) in human bronchial endocrine type cells. Virchows Arch [Pathol Anat] 400:163–171

Yamaguchi K, Abe K, Miyakawa S, Ohnami S, Sakagami M, Yanaihara N (1980a) The presence of macromolecular vasoactive intestinal polypeptide (VIP) in VIP-producing tumours. Gastroenterology 79:687–694

Yamaguchi K, Kameya T, Abe K (1980b) Multiple endocrine neoplasia type 1. Clin Endocrinol Metabol 9:261–284

Yamaguchi K, Adachi I, Miyakawa S, Abe K, Yanaihara N (1981) Macromolecular forms of somatostatin-producing tumours. In: Japanese Society of Gut hormone (ed), Igakutoshoshuppan Tokyo, pp 228–235

Yamaguchi K, Abe K, Kameya T, Adachi I, Taguchi S, Otsubo K, Yanihara N (1983) Production and molecular size heterogeneity of immunoreactive gastrin-releasing peptide in fetal and adult lungs and primary lung tumours. Cancer Res 43:3932–3939

Yamaguchi K, Abe K, Otsubo K, Hanieuda C, Suzuki M, Shimada A, Kimura S, Adachi I, Kameya T, Yanaihara N (1984) The WDHA syndrome: clinical and laboratory data on 28 Japanese cases. Peptides 5:415–421

Accepted November 6, 1984