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Human Pancreatic Polypeptide (HPP) Immunoreactivity in an Infiltrating Endocrine Tumour of the Papilla of Vater with Unusual Morphology *

Report of a Case

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Summary. Histological, histochemical and clinical features of an endocrine duodenal tumour situated in the papillary region were studied. The tumour had a remarkable mixed histological growth pattern, consisting of epithelial glandular structures which showed a gradual transition into a spindle-cell tumour, resembling a neurogenic tumour. The neoplasm was considered malignant since it had infiltrated into the muscular layer of the duodenal wall.

The tumour was non-argentaffin and non-argyrophil. No serotonin could be demonstrated histochemically. Immunoreactive pancreatic polypeptide (HPP) was detected by indirect immunofluorescence in the majority of tumour cells of the epithelial glandular structures, whereas areas with a spindle-cell pattern were almost unreactive to the HPP-antiserum. No reaction was found with antibodies against gastrin, insulin, glucagon, vasoactive intestinal polypeptide or somatostatin.

The patient had no endocrine symptoms that could be ascribed to the production of HPP by the neoplasm. Twenty-four months postoperatively, the patient's serum HPP concentration had begun to rise, suggesting recurrence of the tumour.

Key words: Duodenal endocrine tumour – Papilla of Vater – HPP-immunoreactive tumour cells – Neurogenic component

Introduction

Endocrine tumours of the duodenum, most often occurring in its upper part or in the region of the papilla of Vater, are generally included within the

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group of carcinoids which are referred to as carcinoids of the foregut type (Williams and Sandler 1963). Weichert et al. (1971) have described a pattern in the duodenal endocrine tumours which they have called "carcinoid-islets cell type", claiming that these tumours have morphological and functional features both of carcinoids and pancreatic islet cell tumours. Although they usually do not have the features associated with the production of serotonin, there seems to be a functional relationship with respect to the production of certain polypeptide hormones.

Human pancreatic polypeptide (HPP) is a recently identified pancreatic hormone (Floyd et al. 1975), produced by endocrine cells which occur both in the islets and the exocrine pancreatic parenchyma (Larsson et al. 1976). This peptide has so far been found in a large proportion of endocrine tumours of the pancreas and in a fairly large proportion of rectal carcinoids (Heitz et al. 1976; Alumets et al. 1981). It therefore seemed appropriate to report here the occurrence of immunoreactive HPP cells in a duodenal endocrine tumour which lends further support to the assumption of a biological relationship with endocrine tumours of other parts of the gastrointestinal tract as well as of the pancreas.

Case Report

A 50-year-old obese man was admitted to the hospital because of repeated gastrointestinal bleeding. He had had a history of previous alcohol abuse but had stopped drinking 8 years prior to admission. A mild diabetes had been diagnosed a year earlier; he was on oral hypoglycemic agents. He also had a moderate arterial hypertension.

During the last 4 years, he had suffered from recurrent melenas, requiring massive blood transfusions on several occasions. The source of the bleeding had not been found, despite repeated investigations including X-ray of the upper gastrointestinal tract, gastroscopy, colonoscopy and coeliac artery angiography which had been carried out at another hospital. The patient was referred to our hospital for further investigation. On admission, he had no gastrointestinal complaints and was feeling well. Except for the mild diabetes, he presented no signs of endocrinopathy. Physical examination as well as routine laboratory investigations were normal. Analyses concerning blood levels of gastrointestinal hormones were not carried out at this point.

A benign tubular adenoma was removed from rectum during proctoscopy, which was otherwise normal. Endoscopy of the upper gastrointestinal tract revealed two erosions in the antral mucosa. A tumour, 1×2 cm, was disclosed in the region of the papilla of Vater. It was oval in shape, appeared well demarcated and showed an irregular, linear ulceration on the top. The papillary orifice could not be visualized. Repeated endoscopic biopsies failed to show any tumorous tissue.

At operation the tumour was found to enclose the papilla of Vater. Peroperative pancreatography showed a normal pancreatic duct. The bile duct could not be visualized, however laboratory tests disclosed no signs of biliary obstruction. The tumour, including the papilla, was excised and the bile duct and the pancreatic duct were resutured to the duodenum.

The postoperative course was uneventful. During the following 23 months the patient was subjected to repeated examinations including endoscopy, ERCP, hepatic scintigraphy and angiography. Laboratory tests, including plasma assays for gastrin, CCK, vasoactive intestinal polypeptide, HPP, insulin, C peptide, calcitonin, cortisol, serotonin and determination of urinary excretion of 5-hydroxyindoleacetic acid were all within normal limits. However, at a recent check-up (November 1980), 24 months after the operation, the patient's serum HPP values were found to have increased (highest value being 2.2 ng/ml[1]) indicating a recurrence of the tumour. So far, the patient has remained well and there have been no signs of gastrointestinal bleeding.

1 Serum HPP was assayed by Dr. G. Lundqvist, Akademiska Sjukhuset, Uppsala, Sweden (Normal value = <0.04 ng/ml)

Materials and Methods

The tissue specimen was fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin and van Gieson stain, some were also studied with the method of Masson-Hamperl (Romeis 1948) and the diazocoupling method (Pearse 1960) for the demonstration of argentaffin material and serotonin respectively. Grimelius' silver nitrate procedure (Grimelius 1968) was used for demonstrating argyrophilia. The presence of mucin was evaluated with the PAS stain.

Sections used for immunofluorescence were refixed in Bouin's solution for 3 h and thoroughly rinsed in buffer overnight. The indirect immunofluorescence method was employed (Coons et al., 1955). The sections were incubated overnight with antisera against the following polypeptides: gastrin (No 4542, J.F. Rehfeld, Århus Univ., Århus, Denmark; dilution 1/100), glucagon (J. Thorell, General Hospital, Malmö, Sweden; dilution 1/50), insulin (J. Thorell, General Hospital, Malmö, Sweden; dilution 1/200), HPP (Anti-HPP_{24-36}, 734/3, J.F. Rehfeld, Århus Univ., Århus, Denmark; dilution 1/50), vasoactive intestinal polypeptide (J. Fahrenkrug, Copenhagen County Hospital, Glostrup, Denmark; dilution 1/20) and somatostatin (J.F. Rehfeld, Århus Univ., Århus, Denmark; dilution 1/50). Controls were used as recommended by Goldman (1968).

Results

Gross Pathology. The surgical specimen[2] consisted of a part of the duodenum. A lobulated tumour, 1×2 cm, was found in the posterior wall in the region of the papilla. It was covered by mucosa showing a patchy erosion. On cut sections the tumour tissue showed expansive growth within the submucosal and muscular layers of the duodenal wall, the surface of which appeared faintly yellowish-white. It reached the margins of the surgical excision. No pancreatic tissue was involved in the tumour.

Light Microscopy. Histologically the tumour was found to involve the submucosal layer and the muscularis propria (Fig. 1a). It was devoid of a capsule and showed a polycyclic delineation which was broken on several places by tumour nodules infiltrating into the surrounding muscle bundles of the muscularis propria. In its deeper parts, the tumour was built up of epithelial cells, which were polyhedral, sometimes cylindrical with round or elongated nuclei and rather distinct cell boundaries. In some places, these cells were arranged in a palisade-like fashion and had formed glandular or trabecular structures as well as rounded solid nests, which clearly stood out in contrast to the connective tissue stroma (Fig. 1b). The tumour cells showed little atypia and mitotic figures were rare. Their cytoplasm contained weakly eosinophil, granular material. In the superficial part of the tumour, there was a gradual transition towards a spindle cell pattern where elongated tumour cells were arranged in whorls or fascicle-like structures, intermingled with connective tissue elements, the contrast between the two components being barely noticeable (Fig. 1c). This part of the tumour resembled a neurogenic tumour such as neurofibroma or neurolemmoma (Fig. 1d).

The tumor cells were non-argentaffin and non-argyrophil. The PAS reaction was negative and the diazo-coupling method failed to reveal any serotonin in the tumour cells.

2 Thanks are due to Dr. Lars-Bertil Schnürer, Director of the Department of Pathology, Central County Hospital, Örebro, Sweden, for providing us with pathological material in this case

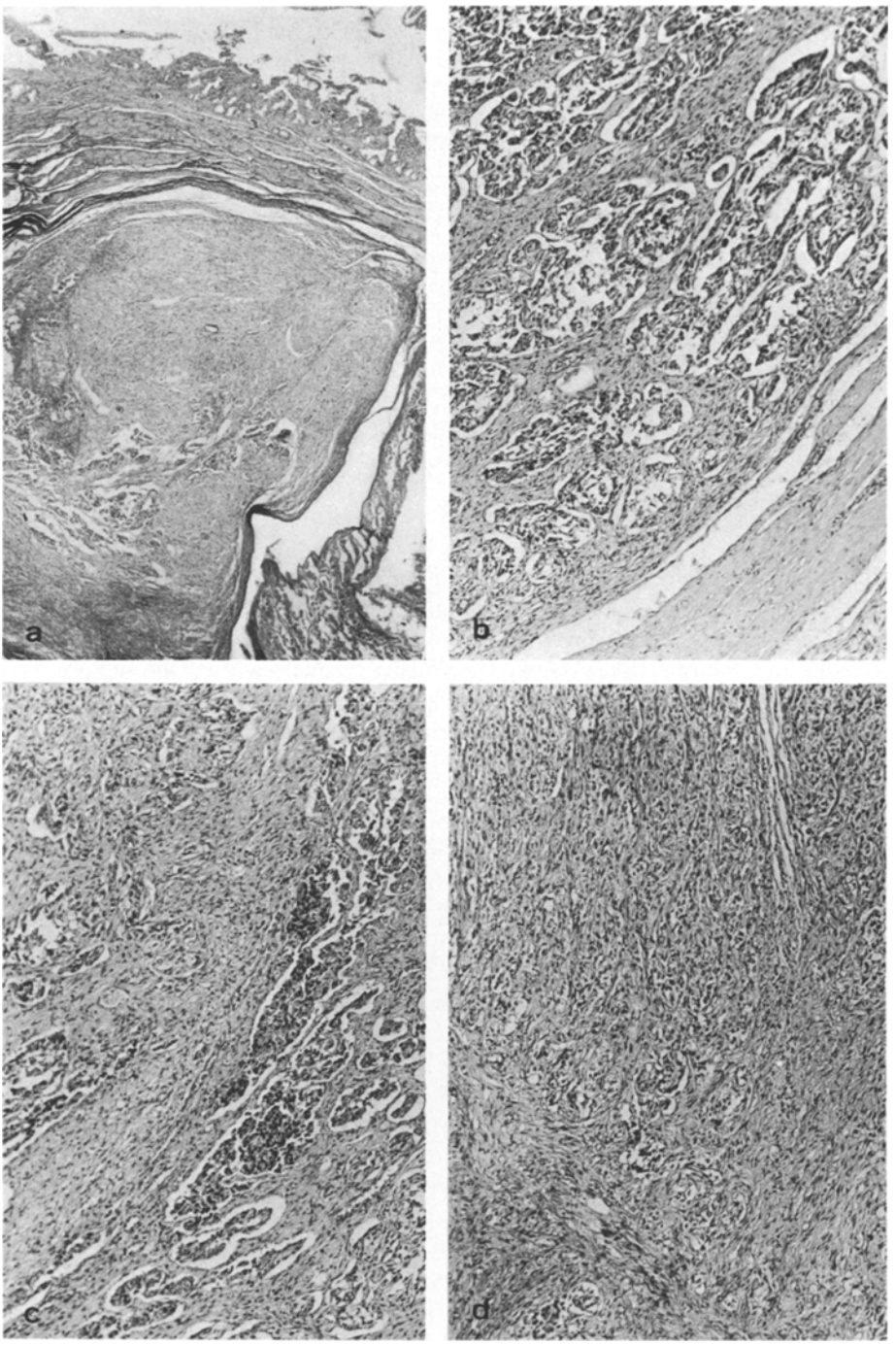

Fig. 1.a Low power photomicrograph of the lobulated tumour growing in the submucosal and muscle layer of the duodenal wall (H-e, ×17). **b** Area from the deeper part of the tumour showing epithelial glandular or solid formations, clearly distinguishable from the stroma. (H-e, ×68). **c** Area with an intermediate type of growth pattern showing epithelial structures merging into a fibro-cellular component with numerous spindle-cells. (H-e, ×68). **d** Superficial part of the tumour which is predominantly built up of spindle-cells. The cells are arranged in streaks and curves running in various directions. This architecture resembles a neurogenic tumour. (H-e, ×68)

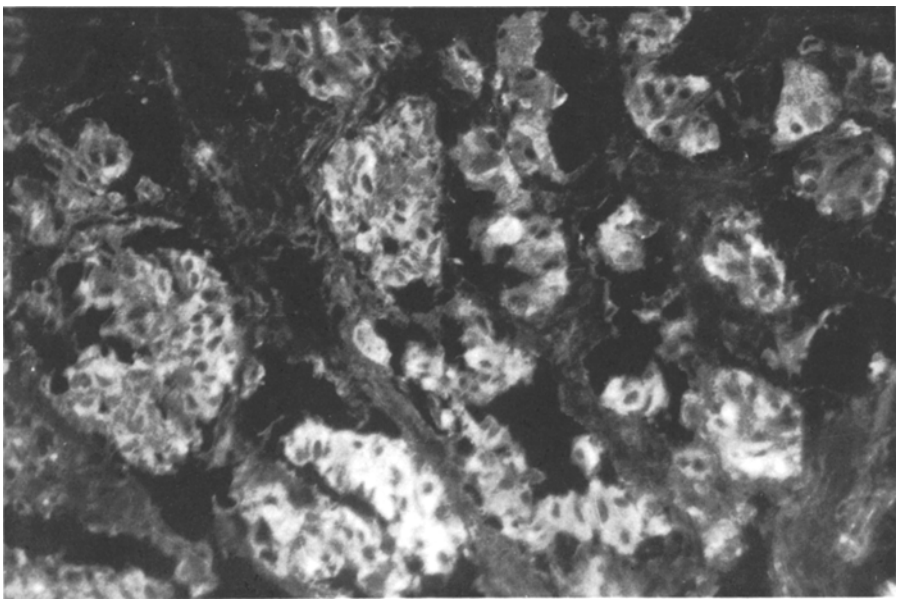

Fig. 2. Epithelial glandular and solid formations with numerous immunoreactive HPP-cells. (Indirect immunofluorescence, ×260)

Immunofluorescence. The deeper part of the tumour, containing epithelial structures displayed HPP immunoreactivity of varying intensity which was present in the major proportion of the tumour cell population (Fig. 2). In the part showing spindle cell pattern, only occasional tumour cells reacted with the antiserum against HPP. No immunoreaction could be demonstrated with the other antisera used.

Discussion

Gastrin has been found in duodenal endocrine tumours (Creutzfeldt et al. 1975) as well as in pancreatic islet cell tumours where it is a rather common finding, occurring either alone or together with other hormones (Larsson 1978). Substance P has also been reported to occur in some duodenal carcinoids (Wilander et al. 1979a), whereas this neuropeptide seems to be present more frequently in carcinoids of the midgut type (Wilander et al. 1979b). HPP has, so far, not been found in tumours outside the pancreas or rectum. The present case represents an example of an intestinal endocrine tumour of the foregut type, containing a large number of HPP immunoreactive cells.

As far as the origin of the tumour is concerned, we may speculate that it may have arisen from HPP cells in ectopic pancreatic tissue in the duodenal wall. HPP storing cells are reported to be particularly numerous in the uncinate process and pancreatic tissue adjacent to the duodenum (Tsung-Min Lin 1980). However, no pancreatic structures could be identified within the tumour or in its vicinity. Nevertheless, occasional HPP cells have been reported to occur

normally in the duodenal mucosa of mammals (Paulin and Dubois 1978). Such cells may have served as progenitor cells of the tumour. Alternatively, the tumour cells may have originated from multipotent, immature endocrine cells present in the duodenal mucosa, which have, during their development into neoplastic cells, retained their capacity to produce certain polypeptides such as HPP.

Since the physiological actions of HPP are still largely unknown, nothing definite can be said about possible effects of HPP secretion by tumours. It has been suggested as a causative factor in the WDAH-syndrome (Larsson et al. 1976; Lundqvist et al. 1978), but VIP is generally considered to be the major agent responsible (Ebeid et al. 1978; Modlin et al. 1978; Wu et al. 1979). In spite of the fact that the HPP cells constituted a considerable part of the tumour cell population in our case, the patient did not suffer from diarrhoea or any other symptoms which are associated with the known hormonal syndromes caused by endocrine tumours in the pancreas or gastrointestinal tract. Similarly, a remarkable lack of clinical endocrine symptoms was reported in a series of 25 patients with rectal carcinoids (Alumets et al. 1981). In 19 of these rectal carcinoid tumours, various immunoreactive polypeptide hormones were found, HPP and glucagon being the most common. This lack of symptoms suggests that the peptides may be secreted in an inactive form, largely retained, or rapidly inactivated after release from the cells. In our case, where HPP was the only peptide found with the antisera used, the possibility may also be considered that HPP in excess is not capable of producing any apparent clinical symptoms. Since our patient lacked endocrine symptoms no specific hormone analysis was performed preoperatively.

A remarkable feature in the tumour was the presence of a component which structurally resembled a neurogenic tumour. Tumours of mixed carcinoid and neurofibroma type with a gradual transition between the two components have been recognized in the ileum where they have been combined with multiple separate, pure neurofibromas (Arnesjö et al. 1973). Pure carcinoids of the duodenum have also been reported to occur in association with separate neurofibromas (Lee and Garber 1970). This association suggests a close biological relationship to neural structures. The tumour in our case has to be included within the group of neoplasms which have been named "apudomas". These tumours develop from peripheral endocrine cells which have been defined by Pearse (1966) as the APUD-series, based on common histochemical and biochemical properties, such as the ability to concentrate and decarboxylate amine precursors. The APUD-system largely corresponds to the peripheral endocrine (paracrine) system of Feyrter (cf. Feyrter 1953).

According to the concept of Pearse (1969) these endocrine cells are of neuroectodermal origin. The combination of endocrine epithelial cells and of neurogenic structures within one tumour, as in the present case, would be in agreement with this suggestion. However, such an argument presupposes that the two components in the present case share a common histogenesis. This has not been shown.

Another possibility may be that the two components of the tumour represent a simultaneous neoplastic proliferation of two histogenetically distinct but topo-

graphically and functionally closely related structures (i.e., endocrine epithelial cells and neural elements). Such a close topographic relationship has in fact been described already by Masson (1924) and Feyrter (cf. Feyrter 1953) under normal conditions in the gut and other areas where members of this endocrine system occur. Furthermore, Auböck (1977) has reported the presence of Schwann cells in close proximity to tumour cells as well as nerve fibres within tumour cells in rectal carcinoids.

The clinical feature of gastrointestinal haemorrhage is an infrequent presenting symptom in patients with neoplastic lesions in the papillary region (Knudson et al. 1964). Obstruction of the common bile and/or pancreatic ducts is a common finding, but in our case this feature was absent.

The observation that the postoperative serum HPP values, after having been within normal limits, have tended to rise (at latest check-up by November 1980, 24 months after the operation), suggests a recurrence of the neoplasm. This also indicates the value of HPP as a tumour marker which may be used in the evaluation of the prognosis and course of tumours of this kind.

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