Case report

Mixed pancreatic apudoma with symptoms of excess vasoactive intestinal polypeptide and insulin: improvement of diarrhoea with metoclopramide

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SUMMARY   A female patient is described with a single pancreatic tumour producing vasoactive intestinal polypeptide (VIP), insulin, and pancreatic polypeptide. The initial presentation was with diarrhoea and hypokalaemia and a raised plasma VIP was demonstrated. Her symptoms improved with metoclopramide administration and absolute concentrations of 28 aminoacid (peak IV) VIP were found to have fallen. She then developed hypoglycaemia with hyperinsulinism. All symptoms resolved after surgical excision. This case emphasises the potential of these tumours to contain more than one endocrine cell type synthesising different biologically active peptides.

Pancreatic endocrine tumours are associated with excessive secretion of insulin, gastrin, VIP,[1] glucagon,[2] somatostatin,[3] and pancreatic polypeptide.[4] In 1965 Law et al.[5] described a single metastatic islet cell tumour which was demonstrated to be producing gastrin, ACTH, and MSH.[2] Subsequently a number of case reports of single pancreatic endocrine tumours secreting multiple pancreatic polypeptide hormones have been described; these include combinations of gastrin and glucagon,[6] insulin and gastrin,[7] insulin and glucagon,[8] and gastrin and VIP.[9] A combination of VIP, glucagon, enteroglucagon, secretin, and serotonin is described.[10] In a series of 35 pancreatic endocrine tumours, Broder and Carter found four tumours secreting two pancreatic hormones (insulin and gastrin in three cases and insulin and glucagon in one case) and one tumour secreting three hormones (insulin, gastrin, and glucagon).[11] In a series of 24 tumours examined by immunocytochemistry seven stained for more than one of insulin, gastrin, glucagon, and ACTH.[12] More recently, fasting plasma pancreatic polypeptide concentrations have been

found raised in many patients, including 77% of VIP-secreting tumours, but this is unassociated with known significant metabolic effects.[13] Prostaglandin secretion[14 15] and calcitonin[15] secretion is also recognised.

In the above patients symptoms from one peptide hormone dominated but Hammar and Sale described an adult male with a more than 30 year history of the Zollinger-Ellison syndrome who then developed hypoglycaemia due to excessive insulin secretion by the tumour.[16] We report a lady with a tumour synthesising three peptides who initially presented with symptoms of VIP excess but who, after resolution of her diarrhoea, hypokalaemia, and plasma peak IV VIP immunoreactivity after metoclopramide administration, developed hypoglycaemia due to hyperinsulinism.

Methods

Plasma immunoreactive VIP,[17 18] insulin,[19] and pancreatic polypeptide[20] were measured by previously characterised specific radioimmunoassays. Fasting plasma was subjected to gel permeation chromatography. Samples (1 ml) were loaded onto a column of Sephadex G50 superfine (0·9 × 60 cm) and eluted at a flow rate of 3 ml/h, at

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4°C, with 0·06 mol/l sodium acetate pH 5·0 containing 0·2 mol/l sodium chloride. Fractions (0·5 ml) were collected and assayed at two different dilutions to determine immunoreactive VIP content. The column was precalibrated with Dextran Blue, cytochrome C (M wt 12384), pure porcine VIP (M wt 3326), and Na^{125}I (M wt 148) as molecular size markers. The coloured markers and Na^{125}I were also included with each sample chromatogram as internal markers.

Three portions of the freshly excised tumour were frozen at −20°C for subsequent radioimmunoassay. The portions were weighed, boiled in distilled water, homogenised, and centrifuged. The supernatants were separated and the pellets homogenised again in 0·1 M formic acid, centrifuged, and the supernatants separated. All supernatants were then assayed for VIP and pancreatic polypeptide in duplicate in serial dilution. The mean of the peptide concentration of the three portions, which were closely similar, was taken as the concentration of peptide in the tumour. The insulin content could not be accurately measured, as it is unstable in the acid extraction technique used to solubilise the VIP and pancreatic polypeptide.

For immunocytochemistry tissue was frozen in arcton (freon) at −156°C and subsequently freeze-dried and vapour fixed in benzoquinone.[21] Three μm serial paraffin sections were immunostained by the technique of indirect immunofluorescence using antibodies to insulin, VIP, and pancreatic polypeptide (for dilutions, see Table 1). For the simultaneous demonstration of two peptide hormones in the tissue, the double immunoperoxidase method using two different substrates, 4-Cl-1-napthol (blue product) and diamiobenzidine (brown product) was used.[22 23] Ultrastructural studies were carried out using tissue fixed in 3% glutaraldehyde in 0·1 M phosphate buffer pH 7·2.

Case report

A 48 year old white female secretary was admitted to the Hammersmith Hospital, London, in June 1979 with a 12 month history of profuse watery diarrhoea and the loss of 13 kg in weight to 44 kg. During the previous three months she had had about 20 bowel actions per 24 hours, waking almost hourly at night and with frequent faecal incontinence. For four weeks she had been anorexic and nauseated with intermittent vomiting and had complained of pain and weakness of the hands and calves.

She had a long history of extrinsic asthma and

Table 1 *Dilutions and absorption used in two immunocytochemistry techniques*

	Dilutions for immunocytochemistry		Absorption (nmol/ml diluted antiserum)
	Immunofluorescence	Peroxidase-antiperoxidase	
VIP	1:400	1:2000	0·1
Pancreatic polypeptide	1:3000	1:12 000	0·1
Insulin	1:800	1:800	1·0

on admission was taking beclomethasone, salbutamol, and disodium cromoglycate by inhaler as well as oral codeine phosphate 120 mg and Lomotil 3 tablets daily for her diarrhoea. She had undergone a simple left-sided mastectomy 18 months previously for a small intraduct carcinoma of the breast. There was no family history of endocrine adenomata.

Physical examination confirmed moderately severe asthma, no evidence of recurrence of her breast carcinoma, watery faeces per rectum, and no other abnormal findings. A pancreatic VIP-secreting tumour was diagnosed on the basis of repeatedly raised levels (n=11) of plasma VIP with a mean of 260±15 (SEM) pmol/l (normal <20 pmol/l) and an arteriographically and CAT scan proven mass in the tail of the pancreas. Plasma potassium was 2·2 mmol/l, bicarbonate 21 mmol/l, urea 7·0 mmol/l, and fasting blood glucose 3·8 mmol/l. Fasting plasma pancreatic polypeptide was also raised at 240 pmol/l (normal less than 100 pmol/l). Fasting plasma calcium, phosphate, gastrin, glucagon, and somatostatin concentrations were normal.

The stiffness and weakness of her hands and calves resolved on oral potassium replacement and her nausea and vomiting responded to metoclopramide 10 mg three times a day. Unexpectedly this drug also controlled her diarrhoea with reduction of the number of bowel actions to two per 24 hours. Withdrawal of the metoclopramide led to recurrence of severe diarrhoea and reintroduction of the drug again stopped it. This was confirmed on three separate occasions, with a mean daily faecal weight off metoclopramide of 1100 g and 260 g on metoclopramide.

With diarrhoea the concentration of total immunoreactive VIP was 343 pmol/l and without diarrhoea 230 pmol/l. Gel chromatography of this plasma showed the presence of five peaks (Fig. 1) and the percentage VIP immunoreactivity in each of the peaks is shown in Table 2. The recovery of total VIP-immunoreactivity loaded
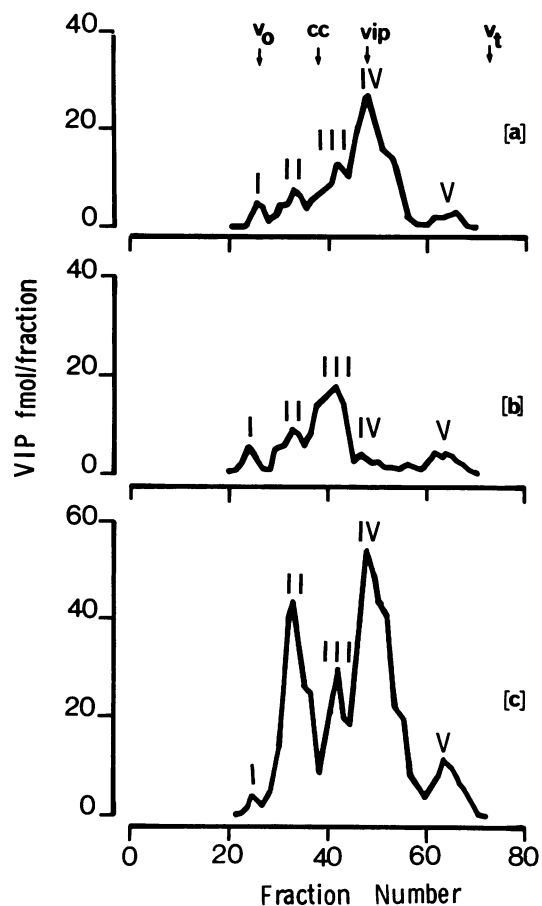
Fig. 1 *Immunoreactive VIP gel permeation chromatography profiles of plasma before (a) and after (b) metoclopramide treatment. Panel (c) shows the profile for the tumour extract. Molecular size markers are v_0: dextran blue; cc: cytochrome C; vip: pure porcine VIP; vt: sodium^{125}.*

Table 2 *Subdivision of VIP gel permeation chromatography profiles into five different immunoreactive peaks**

	Peaks				
	I	II	III	IV	V
KaV		0·15	0·35	0·48	0·80
Plasma pre-metoclopramide treatment (%)	3·5	11·7	21·4	59·8	3·5
Plasma post-metoclopramide treatment (%)	5·7	18·3	60·4	5·2	10·4
Tumour extract (%)	1·0	28·4	15·6	48·8	7·2

* The elution coefficients (KaV) for each peak and the percentage immunoreactivity in each of the five peaks are shown.

on the column was more than 95%. Before metoclopramide treatment the majority of VIP-immunoreactivity corresponded to the elution position of pure 28 aminoacid porcine in peak 4. After metoclopramide treatment, however, the majority eluted in peak 3, indicating a VIP-like molecule of greater molecular size. There was therefore an absolute fall in peak 4 immuno-reactivity from 205 pmol/l to 12 pmol/l and a rise in peak 3 from 73 pmol to 139 pmol/l.

She was discharged home to allow her general condition to improve before elective surgery. Her diarrhoea was still well controlled and she had gained 12 kg in weight by early August 1979. However, she then began to complain of faint-ness and tiredness before meals with rapid im-provement after eating. Fasting blood glucose levels were 0·7 mmol/l and 1·6 mmol/l with simultaneous insulin levels of 24 and 26 μunit/ml (normal <5 μunit/ml with this degree of hypo-glycaemia). A diagnosis of inappropriate insulin secretion was made and she was started on diaz-oxide 100 mg three times a day. She failed to respond to diazoxide even when the dose was increased to 200 mg four times a day and was admitted to hospital in hypoglycaemic coma (blood glucose 0·7 mmol/l; plasma insulin 32 μunit/ml). Subsequently she required four hourly high carbohydrate meals day and night to pre-vent hypoglycaemia.

At operation on 21 September 1979 (Professor LH Blumgart) a 7·5×6·0×4·0 cm encapsulated tumour was identified in the tail of the pancreas and a distal pancreatectomy was performed. There was no evidence of any metastases.

She had an uneventful postoperative course, was discharged home on 1 October, and returned to work at the end of the month. At nine month follow-up she had had no untoward symptoms and repeated blood glucose and plasma VIP, pancreatic polypeptide, and insulin estimations have been normal.

TUMOUR STUDIES
The cut surface of the tumour was firm, slightly lobulated, and greyish-white with areas of de-generation and haemorrhage. Conventional his-tological stainings (Fig. 2) confirmed the presence of an islet cell tumour composed of several dis-tinct cell types. One group of cells was polygonal in shape and arranged in ribbons separated by amyloid deposits; a second group was composed of more anaplastic cells separated by connective tissue, and the third group was more basophili-cally stained and had more regular nuclei. The tumour capsule was intact, there was no vascular
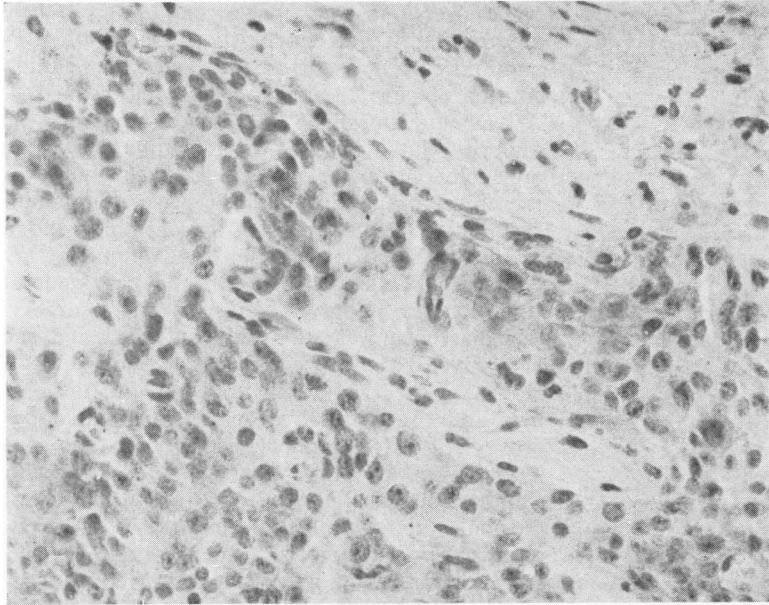
Fig. 2 *Conventional histology (haematoxylin and eosin) of the tumour. The clusters of tumour cells are separated by bands of connective tissue and amyloid deposits (×300).*

invasion in the tumour, and a lymph node and the spleen showed no evidence of tumour cells. The appearances were consistent with a pancreatic endocrine tumour with multiple cell types.

The mean tumour VIP, assessed by radioimmunoassay, was 869 pmol/g wet tissue. This can be compared with 33 ± 8 (SEM) pmol/g in histologically normal pancreatic tissue from seven human controls. Chromatographic analysis of VIP-immunoreactivity is shown in Fig. 1 and Table 2. Similar immunoreactive VIP peaks were seen in tumour extract as in plasma and the majority eluted in peaks 2 and 4. The mean tumour pancreatic polypeptide was 365 pmol/g wet tissue, which is in the normal range for human pancreas.

Tumour cells reacted with antibodies to VIP, insulin (Fig. 3), and pancreatic polypeptide, but cells staining for VIP and pancreatic polypeptide were relatively sparse. Both immunostaining of the serial sections and the double immunoperoxidase revealed a separate cellular origin for each of the three regulatory peptides. Ultrastructural studies showed the presence of an active and well-granulated tumour containing three types of secretory granules (Fig. 4): one large (200–220 nm) with a well-defined wide halo and core resembling insulin granules,[24] another rather small (100–130 nm in diameter) corresponding to VIP secretory granules similar to neurosecretory granules of other vipomas,[25][26] and a third of medium size with a closely attached membrane resembling PP granules (120–150 nm).[27]
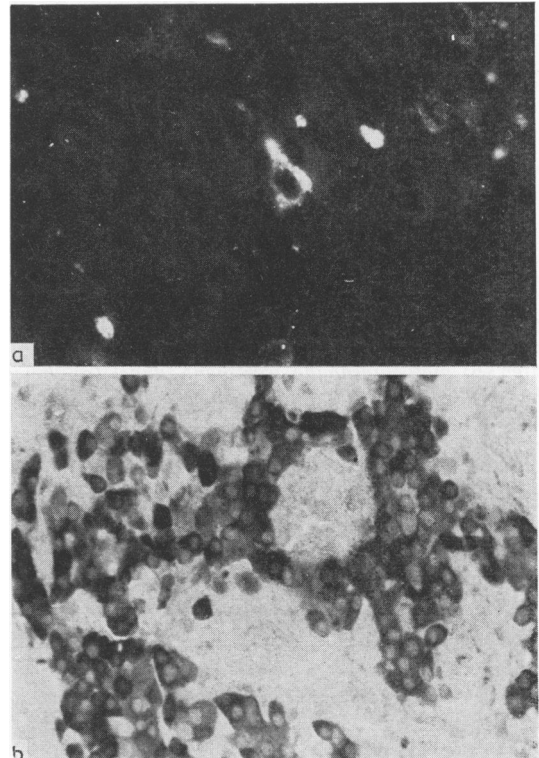

Fig 3 *(a) Scattered VIP-immunoreactive cells stained by the indirect immunofluorescent method (×550). (b) Clusters of insulin-containing cells immunostained using the immunoperoxidase method (×550).*
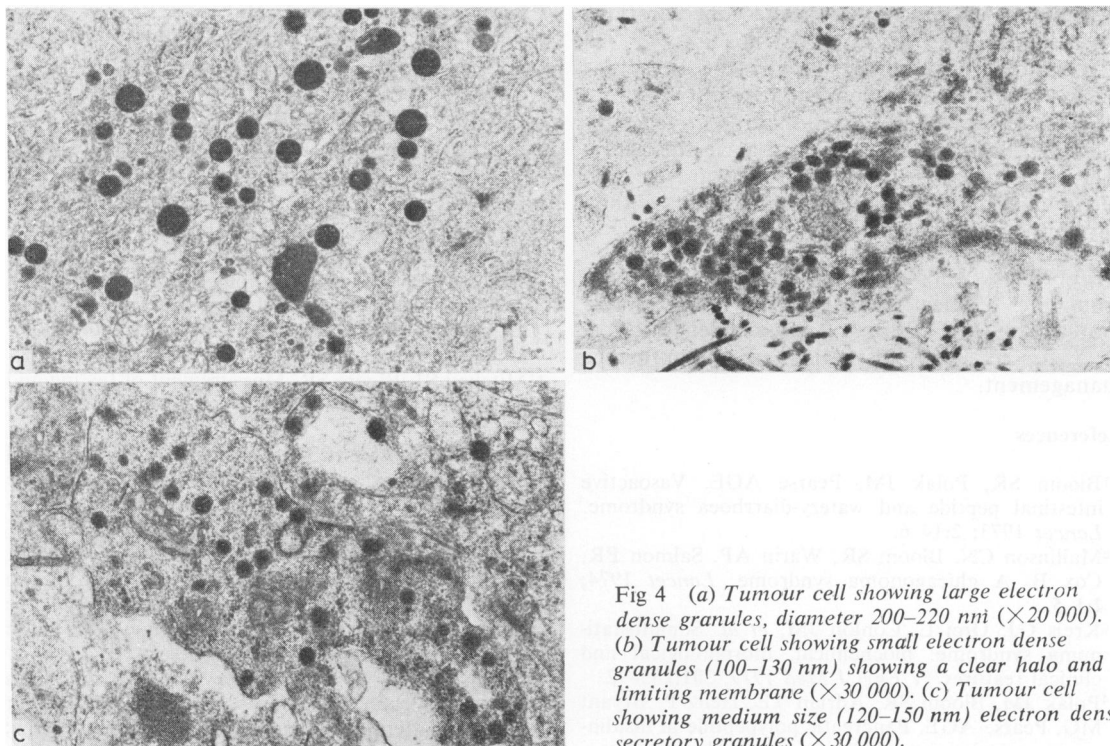
Fig 4 (a) *Tumour cell showing large electron dense granules, diameter 200–220 nm ($\times$20 000). (b) Tumour cell showing small electron dense granules (100–130 nm) showing a clear halo and a limiting membrane ($\times$30 000). (c) Tumour cell showing medium size (120–150 nm) electron dense secretory granules ($\times$30 000).*

Discussion

The clinical, biochemical, immunocytochemical, and ultrastructural evidence suggest that this tumour was secreting VIP, insulin, and pancreatic polypeptide. Pancreatic VIP secreting tumours have been previously described which secreted gastrin and pancreatic polypeptide, but these peptides did not cause specific symptoms. It is known that hypokalaemia causes a reduction in insulin secretion[28] and in this patient it seems possible that hypokalaemia prevented excessive insulin secretion when the diarrhoea was prominent. However, with resolution of the diarrhoea and normokalaemia, excessive insulin secretion with severe hypoglycaemia occurred. VIP is known to increase hepatic glucose mobilisation and the high 28 aminoacid VIP concentrations before metoclopramide treatment may also have played a role in preventing hypoglycaemia.

The list of drugs that may control the diarrhoea of the Verner-Morrison syndrome is growing. Corticosteroids are usually effective in the short-term. Somatostatin reduces intestinal juice secretion and plasma VIP concentrations in the short-term but its more prolonged effects on diarrhoea have yet to be assessed.[29] Indomethacin has been reported to reduce diarrhoea and probably acts by suppressing prostaglandin synthesis.[30][31] Lithium carbonate treatment reduced faecal output in one patient but did not abolish VIP secretion.[32] The nitrosurea cytotoxic drug streptozotocin can reduce tumour mass and cause long-term remissions.[33] In this patient metoclopramide stopped the diarrhoea, even though plasma total VIP concentrations were greatly raised. In volunteers intravenous VIP infusion to similar plasma concentrations of plasma VIP seen in this patient result in a failure of absorption in the jejunum of water, sodium, and chloride[34] and in the colon of water, sodium, chloride, and bicarbonate.[35] The most marked change in the chromatographic profiles after metoclopramide treatment was the fall in peak 4 immunoreactivity, which corresponds to pure 28 aminoacid porcine VIP, from 205 pmol/l to 12 pmol/l. These data show that peak 3 VIP, at least in the concentrations seen in this patient, are not apparently associated with diarrhoea and suggest that peak 2 VIP is also not biologically active. The possibility must exist that peaks 1–3 are biologically inactive precursor molecules. In contrast, peak 4 VIP appears to be most bio-

logically active and raised plasma concentrations are associated with profuse diarrhoea.

It is becoming increasingly recognised that single pancreatic apudomas frequently secrete more than one of the six tumour secreted peptides. This case demonstrates that if the symptoms due to one regulatory peptide are treated, the symptoms of another peptide may then develop.

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