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Endocrine cells producing regulatory peptides

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Summary. Recent data on the immunolocalization of regulatory peptides and related propeptide sequences in endocrine cells and tumours of the gastrointestinal tract, pancreas, lung, thyroid, pituitary (ACTH and opioids), adrenals and paraganglia have been revised and discussed. Gastrin, xenopsin, cholecystokinin (CCK), somatostatin, motilin, secretin, GIP (gastric inhibitory polypeptide), neurotensin, glicentin/glucagon-37 and PYY (peptide tyrosine tyrosine) are the main products of gastrointestinal endocrine cells; glucagon, CRF (corticotropin releasing factor), somatostatin, PP (pancreatic polypeptide) and GRF (growth hormone releasing factor), in addition to insulin, are produced in pancreatic islet cells; bombesin-related peptides are the main markers of pulmonary endocrine cells; calcitonin and CGRP (calcitonin gene-related peptide) occur in thyroid and extrathyroid C cells; ACTH and endorphins in anterior and intermediate lobe pituitary cells, α-MSH and CLIP (corticotropin-like intermediate lobe peptide) in intermediate lobe cells; met- and leu-enkephalins and related peptides in adrenal medullary and paraganglionic cells as well as in some gut (enterochromaffin) cells; NPY (neuropeptide Y) in adrenalin-type adrenal medullary cells, etc.. Both tissue-appropriate and tissue-inappropriate regulatory peptides are produced by endocrine tumours, with inappropriate peptides mostly produced by malignant tumours.
Key words. Bombesin; substance P; CRF; ACTH; opioids; calcitonin; somatostatin; PP; glucagon; GRF; secretin; GIP; gastrin; CCK; motilin; neurotensin; endocrine cells; endocrine tumours.

Introduction

Endocrine cells producing regulatory peptides are specialized epithelial cells characterized by their secretory granules of variable size, shape, density and inner structure enveloped by a unit membrane. The granules are formed at the trans side of the Golgi complex, from condensing vacuoles whose contents, simultaneously with the process of controlled proteolysis of prohormones, undergo progressive densification to form clathrin-coated 'immature' progranules and then 'mature' secretory granules storing the active hormones[107]. Besides hormonal peptides and related prohormone fragments, secretory granules store hormone-unrelated proteins like chromogranins, monoamines, such as catecholamines and serotonin, polyamines and metal cations[122,178].

In addition to secretory granules, like those storing peptides and chromogranins, and the large, dense-cored vesicles of nerves, a population of small clear vesicles, closely resembling the small synaptic vesicles which store classic neurotransmitters, has been described in some endocrine cells, such as paraganglionic, adrenal medullary and pulmonary endocrine cells[28,78,98]. Recently these small vesicles of nerves and endocrine cells (including adrenal medullary and pituitary cells) have been found to be selectively marked by a Ca^{2+}-binding membrane glycoprotein, the synaptophysin or P38 protein[102]. P38 protein immunoreactivity has been detected in adrenal medullary and paraganglionic cells, pancreatic islets, adenohypophysis and thyroid C cells as well as in pulmonary and gastric endocrine cells and related growths, while no reactivity has been observed in intestinal endocrine cells, cardiac atrial cells producing natriuretic hormone and parathyroids[24,102,177]. Cholinergic[33,173], aminergic[110] and GABAergic[62] mechanisms have been found to operate in at least some of the P38-positive cells. Two other vesicle membrane proteins[21,97], neuron specific enolase[10], three distinct chromogranin proteins[122,125,143,178] and a number of regulatory peptides and amines are now known to be common markers of nerves and endocrine cells.

Thus, although the proposed neural crest origin of endocrine cells[110] has been confirmed only for adrenal medulla, carotid body and thyroid C cells[89], the ability of many (not all) endocrine cells, independently from their neural crest origin, to express morphologic and functional patterns characteristic of nerve cells is widely documented and may justify the designation of such cells as 'neuroendocrine' cells[111]. It seems interesting that during phylogenesis nerve cells first develop as peptidergic elements scattered in both the ectoderm and endoderm of coelenterates, partly as elongated 'sensory' cells contacting the epithelial surface, with processes at their basal part[56], a pattern resembling some paracrine cells of mammalian endodermal derivatives[137,141].

As a rule, in different endocrine cell types distinct genes are expressed coding for different propeptides. However, alternative processing of the same m-RNA may result in two distinct propeptides leading to different regulatory peptides, as in the case of calcitonin and calcitonin gene-related peptide (CGRP), coded by the same gene through different propeptides showing tissue specific, though partly overlapping, distributions[126,158]. More often, two or more active peptides, showing the same cellular distribution, may be the products of the same propeptide, coded by a single gene, as in the case

Table 1: Classification of peptide-characterized endocrine cells forming the DES and some endocrine glands

Tissue	Cell type	Main peptides	Amines
Carotid body	Type I	Enkephalins	NA, DA, 5HT
Sympathetic paraganglia	Main cell	Enkephalins	NA, DA
Sympathetic ganglia	SIF cell	Enkephalins	DA, 5HT
Adrenal medulla	A type	Enkephalins; NPY	A
	NA type	Dynorphins; bombesin	NA
(cat)	III type	Neurotensin	NA
Pituitary:			
anterior lobe	'ACTH' cell	ACTH, β-endorphin	
intermediate lobe	Main Cell	α-MSH; CLIP; β-endorphin (1–27)	
Skin	Merkel cell	Enkephalin (rodents); VIP-like (other mammals)	
Thyroid	C cell	Calcitonin, PDN-21; CGRP; somatostatin	5HT
Lung	P cell	Bombesin; calcitonin	5HT
Pancreas and gut	EC_1 cell	Substancse P and K	5HT
	EC_2 cell	Enkephalin	5HT
	D cell	Somatostatin	
	B cell	Insulin	
	A cell	Glucagon; CRF	
	L cell	Glicentin; PYY	
	PP cell	PP; GRF	
	G cell	Gastrin	
	CCK cell	Cholecystokinin	
	M cell	Motilin	
	S cell	Secretin	
	GIP cell	GIP	
	N cell	Neurotensin	

of ACTH and endorphins, both arising from proopiomelanocortin (POMC)[101]. Alternative posttranslational processing of the same prohormone in separate cells, due to different proteolytic cleavage, may also result in different regulatory peptides, as in the case of α-MSH and CLIP (corticotropin-like intermediate lobe peptide) produced in pituitary intermediate lobe cells, but not in ACTH/β-endorphin cells of the anterior pituitary, from further cleavage of ACTH[128]. Similarly, peptides of various molecular size enclosing the same biologically active sequence may result from different processing of the same prohormone in separate cell types (as for proglucagon in pancreatic A cells or intestinal L cells) or in the same cell type of different tissues (as for progastrin in duodenal or pyloric gastrin cells), or in many tumour cells in respect to normal parent cells.

Development of another enzyme activity, cleaving preferentially at Lys-Lys sites, in addition to the Lys-Arg specific enzyme[99] acting in cells of both the anterior and intermediate lobes of the pituitary might explain further cleavage of ACTH, β-endorphin (to β-endorphin 1–27) and δ-endorphin (to release β-MSH) in intermediate lobe cells[132]. Conversely, in pancreatic A cells, where only Lys-Arg sites seem to be cleaved consistently, unmasking of two such sites (blocked in intestinal L cells), possibly due to changes of interacting chromogranins[122], might promote further cleavage of glicentin (uncleaved in L cells) to glucagon.

Careful identification of hormonal and prohormonal peptides, including their cryptic peptides, is required as a basis for classification of the endocrine cells forming glands or scattered in various epithelia as components of the so-called diffuse endocrine system (DES, table). Structural characterization at light and electron microscopical levels, topography, response to functional stimuli, detection of specific receptors and localization of biogenic amines, individual enzymes or structural proteins are also important tools for precise cell characterization at morphological and functional levels[137].

Regulatory peptides produced in the DES, adrenal medulla and paraganglionic cells as well as in POMC-producing pituitary cells will be dealt with in the following sections.

Bombesin, gastrin-releasing peptide (GRP) and related peptides

Endocrine cells reacting with antibodies against the amphibian peptide bombesin or its mammalian equivalent GRP have been detected in amphibian[88] and avian[157] stomach and in mammalian lung[174]. Ultrastructurally, bombesin/GRP immunoreactivity has been localized to cells with small, round, thin-haloed granules of P type[28,157]. In both human lung[175] and chicken proventriculus[166], co-localization of bombesin and 5HT immunoreactivities has been reported. In the lung, bombesin/GRP-immunoreactive cells may form small, intraepithelial bodies, the so-called neuroepithelial bodies, showing prominent innervation, including synapses between epithelial cells and nerves, possibly to be interpreted as an hypoxia-sensitive chemoreceptor apparatus[86,93].

No bombesin/GRP immunoreactivity has been demonstrated in P-type cells of the human gut and pancreas, the staining unequivocally reported in early studies[114] being possibly due to crossreactivity of bombesin antibodies with substance P and substance K, which are both stored in gut EC cells and share with bombesin the C-terminus sequence Leu-Met-NH_2, or with chromogranin B, known to react with N-terminally-directed bombesin antibodies[49].

Immunoreactivity against neuromedin B, the mammalian counterpart of the bombesin-like amphibian peptides ranatensin and litorin, has been shown in pituitary TSH (rat) and gonadotrophic (human) cells[181]. Bombesin/GRP and neuromedin B (a bombesin-related peptide), immunoreactivities have been reported in noradrenalin-producing cells of the adrenal medulla[90]. Bombesin/GRP immunoreactivity has been reported in a number of 'neuroendocrine' tumours, including pulmonary tumourlets, carcinoids and small cell carcinomas[27,54,100,135,153] and thyroid medullary carcinoma[73]. In both human lung tissue and related tumours, C-terminus GRP fragments 14–27 and 18–27 seem to represent the major bombesin-related hormonal species[180]. However, antibodies directed against the C-flanking peptide of human pro-GRP[144] proved to be much more efficient than bombesin/GRP antibodies in staining small cell carcinomas arising in both pulmonary and extrapulmonary sites[58], suggesting preferential preservation of this peptide during posttranslational processing of pro-GRP in tumour cells.

Due to the CRF-potentiating effect of bombesin-like peptides on ACTH release from pituitary cells, a Cushing syndrome has been reported in association with a thyroid medullary carcinoma producing both calcitonin and bombesin-like peptide(s) but neither ACTH nor CRF[68].

Substance P and related tachykinins

Substance P has been found to be co-localized with serotonin and chromogranin A in the argentaffin granules of a subpopulation of gut EC cells, called EC_1 cells[65,122]. Substance P producing EC_1 cells are a major component of midgut (ileum, jejunum, appendix, caecum), rectal, ovarian, testicular and pulmonary argentaffin carcinoids[57,96,116,135]. Other tachykinins, like substance K (or neurokinin-A) and neuropeptide K, known to be produced simultaneously with substance P during posttranslational processing of β-protachykinin[103], have been also detected in non-tumour intestine and serotonin-producing EC cell carcinoids[35,104]. A major role for tachykinins in the genesis of the 'carcinoid syndrome',

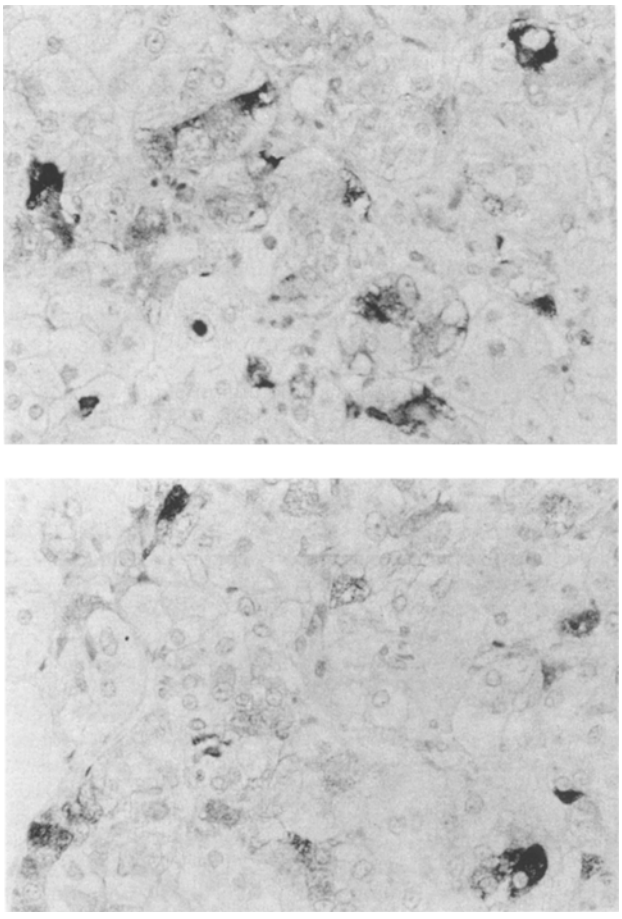

Figure 1. NPY (*a*, serum B48, Milab, Malmö, Sweden) and ACTH (*b*, serum 596010, Ortho Diagnostic Systems, Raritan, N.J.) immunoreactive cells scattered in the human adrenal medulla. Immunoperoxidase, × 240.

with special reference to flushing and diarrhoea, seems likely. Substance P has also been found in the adrenal medulla and some phaeochromocytomas[52] as well as in some paraganglionic cells of the carotid body and cervical, coeliac and mesenteric ganglia[66].

ACTH- and/or opioid-related peptides (endorphins, enkephalins, dynorphins)

Two distinct types of pituitary cells have been shown to produce ACTH and opioid peptides from posttranslational processing of proopiomelanocortin (POMC). In the anterior lobe ACTH/β-endorphin cells, cleavage of the precursor at Lys-Arg sites produces ACTH (POMC 106–144), β-lipotropin (POMC 147–239), further cleaved to δ-lipotropin (POMC 147–206) and β-endorphin (POMC 209–239), as well as pro-δ-MSH (POMC 1–103). In intermediate lobe cells further cleavage at basic residues, with special reference to Lys-Lys sites, may produce, in addition, α-MSH (ACTH 1–13), CLIP (ACTH 17–38), β-MSH (lipotropin 43–60) and biologically inactive β-endorphin (1–27)[128,132]. Although in man intermediate lobe cells are poorly represented in foetal pituitary and either lacking or rudimentary in the adult gland, ACTH-secreting intermediate lobe tumours have been identified in some cases of Cushing's disease[80]. Both anterior lobe and intermediate lobe type of processing, including production of ACTH, β-endorphin, δ-MSH, β- and δ-lipotropins, α-MSH and CLIP, have been found to operate in extrapituitary tumours producing 'ectopic' hormones[3,120].

Cells reacting with β-endorphin, β-lipotropin and/or pro-δ-MSH antisera, though apparently lacking ACTH and α-MSH immunoreactivity, have been identified in the human intestine, especially in the small intestine[131]. ACTH-like and/or β-endorphin immunoreactivities have been detected in various endocrine cells of mammalian gut and pancreas[81,149,171], a finding supported by immunochemical studies on tissue extracts[46,108,154]. Part of this material might be co-localized with gastrin in the G cells, although a separate intestinal and pancreatic cell type seems also involved[149] resembling in its morphology and distribution the intestinal 'VL cell' identified ultrastructurally[138]. Whether authentic ACTH or some ACTH-related peptide is produced remains to be ascertained. Endocrine tumours producing ACTH and β-endorphin have been reported in the gut and pancreas, occasionally coupled with Cushing's disease.

ACTH and β-endorphin immunoreactivities have also been detected in occasional cells of normal human adrenal medulla as well as in some adrenal phaeochromocytomas[30,91] (fig. 1). Apart from the pituitary, adrenals and gut, tumours producing ACTH, α-MSH, β-endorphin, pro-δ-MSH and other POMC-derived peptides have been observed in sites as the lung, thymus, thyroid and prostate, where related immunoreactivities are normally lacking, although they may appear in hyperplastic or dysplastic lesions[3,31]. POMC of extrapituitary tumours may differ from pituitary POMC in the length of its mRNA and type of posttranslational processing[3,145].

Enkephalin immunoreactivity has been detected in a variety of cells, including adrenal medullary cells[94], cells of chromaffin and non-chromaffin paraganglia[176] (fig. 2), Merkel cells of rodent's skin[60], small intensely fluorescent (SIF) cells of sympathetic paraganglia[75], some endocrine cells of the human lung[37], a fraction of argentaffin EC cells of some mammals[4] and rat pituitary GH cells[172]. Human phaeochromocytomas, paragangliomas and some pulmonary carcinoids have also been shown to produce enkephalins and related peptides[30,54,63,94]. In the adrenal medulla, enkephalin-related peptides have been shown to originate from a specific precursor molecule, proenkephalin, containing six met-enkephalin and one leu-enkephalin sequences, which are only partly released as free enkephalins, the rest being secreted as a mixture of larger enkephalin-containing peptides[160].

A second enkephalin precursor, called proenkephalin-B or prodynorphin, containing three leu-enkephalin and no met-enkephalin sequences as part of larger opioid peptides (dynorphins and neo-endorphins) has been identified in the

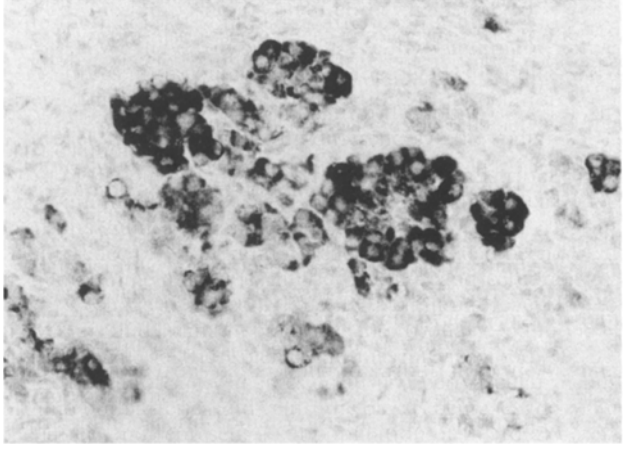

Figure 2. Type I cells forming 'Zell ballen' in the human carotid body, stained with anti-enkephalin serum B15 (Milab). Immunoperoxidase, × 230.

pig hypothalamus and posterior pituitary[71]. Dynorphin immunoreactivity has been detected in noradrenalin cells of bovine adrenal medulla[42]. The contribution, if any, of this propeptide to the genesis of the 'leu-enkephalin' immunoreactivity co-localized with serotonin in some gut EC cells, lung endocrine cells and SIF cells remains to be investigated.

Corticotropin-releasing factor (CRF)

CRF-like immunoreactivity has been detected in glucagon-producing A cells of vertebrate pancreas[113], in endocrine cells of the cat, monkey and rat pyloric mucosa and small intestine distinct from gastrin- and glucagon-immunoreactive cells[113], and in the lung and adrenal tissue[148]. Only part of the CRF antisera staining hypothalamic CRF have been found to react with the peptide stored in extraneural tissues, suggesting that the latter peptide has some difference in structure or molecular species in respect to hypothalamic CRF.

CRF-like immunoreactivity has been detected in endocrine tumours of the pancreas, bronchial carcinoids and pheochromocytomas as well as in small cell carcinomas of the lung[113,147]. A case of Cushing syndrome apparently due to inappropriate CRF secretion from metastatic carcinoma of the prostate, producing marked pituitary ACTH cell hyperplasia, has been reported[32].

Bioactive CRF-like peptides have been detected in a number of extrapituitary tumours producing 'ectopic' ACTH[9,161], a finding of interest in explaining the progressive growth and potential malignancy of such tumours. Besides CRF, vasopressin- and bombesin-like peptides might also contribute to CRF-like bioactivity[68] and tumour growth.

Thyrotropin-releasing hormone (TRH)

TRH-like immunoreactivity has been detected in pancreatic insulin-producing B cells and part of glucagon-producing A cells of the rat developing pancreas[76]. Immunochemical studies suggest identity of the pancreatic peptide with the hypothalamic tripeptide pGlu-His-ProNH_2.

Calcitonin and calcitonin gene-related peptide (CGRP)

In thyroid C cells, calcitonin has been shown to be co-localized with its C-terminal flanking peptide (PDN-21 or katacalcin) and CGRP[2,126,158]. Moreover, co-localization with somatostatin has been observed in majority (rabbit) and minority (rat, human) subsets of C cells[23,72,168].

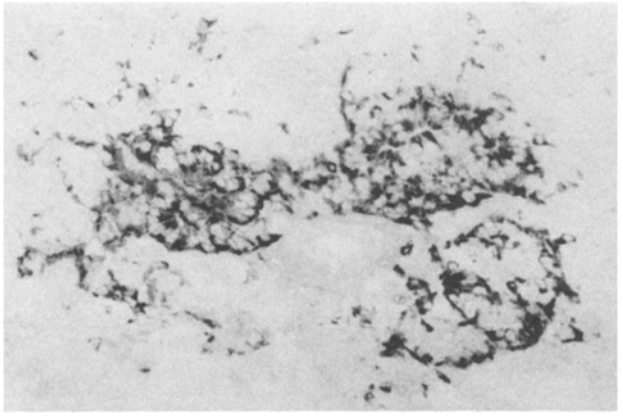

Figure 3. PP cells of PP-rich irregular islets in the posterior head of human pancreas, stained with rabbit anti-canine pro-PP icosapeptide serum 3204 (from T. W. Schwartz, Dept. Clinical Chemistry, Rigshospitalet, Copenhagen, Denmark). Immunoperoxidase, × 70.

Ultrastructurally, thyroid C cells are characterized by round, solid, medium-sized granules storing, besides neuroendocrine peptides, chromogranins A and C. Apart from the thyroid, C cells also occur in the ultimobranchial body, the 'upper' or 'inner' parathyroid (deriving from the fourth branchial pouch) and thymus IV. Calcitonin-[7] and CGRP-immunoreactive cells[34] have been detected in the human lung; however, their failure to react with some of the anti-calcitonin sera staining thyroid C cells, their frequent co-localization of bombesin/GRP immunoreactivity[159] and the smaller size of their secretory granules[28] distinguish them from thyroid C cells.

Calcitonin, PDN-21 and CGRP have been detected regularly in thyroid medullary carcinoma[130] and, less frequently, in tumours from the lung, pancreas, adrenal medulla and other sites[29,41,63]. In many cases of thyroid medullary carcinomas, calcitonin, PDN-21 and CGRP have been found to coexist, in the same or distinct cells, with somatostatin, GRP, PP, ACTH and neurotensin[26,40,73,106,130].

Somatostatin

Somatostatin D cells have been observed in the pancreas and along the whole gastrointestinal tract, from cardia to rectum[5]; some cells have also been detected in chicken thymus[152]. Many of these cells show long cell processes contacting other endocrine and exocrine cells, a possible morphologic basis for a local paracrine function. Secretory granules of D cells are round, homogeneous, poorly dense and unreactive or poorly reactive with silver techniques, with the only exception of Davenport's alcoholic silver, and antisera to chromogranins, while reacting with lead-haematoxylin[133,137,141].

A case of extreme somatostatin cell hyperplasia of the gastroduodenal mucosa causing dwarfism, obesity and goitre has been described[67]. Somatostatin-producing D cell tumours have been observed in the pancreas (with or without associated 'somatostatinoma' syndrome: diabetes, diarrhoea, steatorrhoea, hypo- or achlorhydria, anaemia and gallstones), duodenum, stomach, jejunum, ileum and rectum[39,79,140].

Somatostatin has been found to be co-localized with calcitonin in a large (rabbit) or minor (rat and human) proportion of thyroid C cells and in several thyroid medullary carcinomas[23,26], as well as with catecholamines in a fraction of human adrenal medullary cells and pheochromocytomas[94].

Peptides of glucagon and PP families

a) *Glucagon/glicentin, GLP_1/GLP_2; VIP/PHI; GRF.* By using C-terminally directed specific antibodies *glucagon* has been localized to A cells of the pancreas and gastric oxyntic and cardiac mucosa, whose secretory granules (α-granules) are characterized ultrastructurally by a solid, glucagon-storing core surrounded by an argyrophil halo containing chromogranin A and glicentin-related pancreatic peptide (GRPP) immunoreactivity[25,117,141]. Glicentin C-terminus hexapeptide, MPGF (major proglucagon fragment), containing glucagon-like peptides GLP_1 and GLP_2, and chromogranins C and B are also localized in α-granules[122,142,169]. Glucagon C-terminus immunoreactivity has also been reported in a few intestinal cells lacking the characteristic α granules of A cells and corresponding to a minor fraction (about 20%) of intestinal L cells[48,77,131]. The latter cells, which are scattered in both the large and small bowel, show solid, homogeneous granules with scarce or variable argyrophilia and chromogranin A or B immunoreactivity, consistent chromogranin C immunoreactivity and reactivity with antibodies directed against the proglucagon-related peptides *glicentin,* GRPP, *glucagon-37* (oxyntomodulin), GLP_1 and GLP_2[55,118,142,169].

Growth hormone releasing factor (GRF) immunoreactivity has been detected immunohistochemically in PP cells of the human and rat pancreas[16]. It has been extracted from the human pancreas and characterized immunochemically as an N-terminally extended molecule[129]. A possible relationship of GRF immunoreactivity to small-granulated P-type and mixed P/D_1 type cells occurring in human pancreas, especially during foetal life[28], remains to be investigated.
Although *VIP* immunoreactivities reported in endocrine cells of mammalian gut and pancreas are likely due to cross-reacting chemically-related peptides, especially of L cells (GLP_1 or GLP_2 ?) and D_1/PP cells (GRF ?), VIP-storing cells seem to occur in the intestine of other vertebrates[83,121]. VIP has been found to be co-localized with enkephalins in adrenal chromaffin granules of the frog[87] while VIP- and met-enkephalin-immunoreactivities occur in Merkel cells of distinct species[61].

b) *PP, PYY and NPY.* Most L cells, besides storing glucagon-related peptides, also store *PYY* (peptide with N-terminal tyrosine and C-terminal tyrosine) in the same granules[17,22,135]. Moreover, a minority of L cells also store a PP-like peptide distinct from PYY and accounting for their reactivity with some PP antisera (as Chance's hPP serum) lacking PYY crossreactivity, as well as with antisera directed against the icosapeptide fragment of proPP[48].

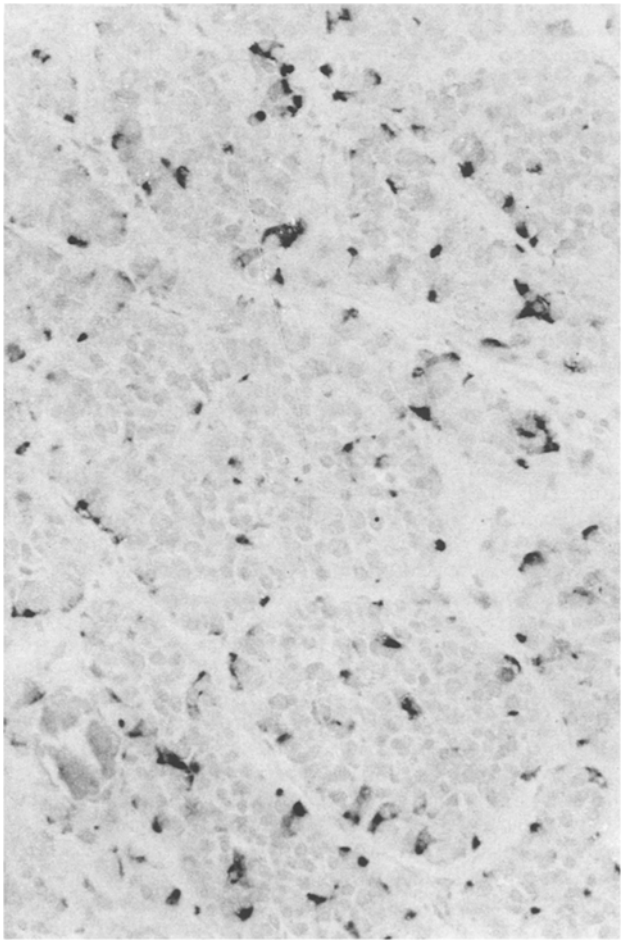

Figure 4. Rectal carcinoid stained with rabbit serum PP221 (from M.M.T.O'Hare, Dept. Medicine, Queen's University of Belfast, U.K.) directed against C-terminus PP hexapeptide, reacting with both PP and PYY. Note scattered immunoreactive tumour cells. Immunoperoxidase, × 235.

Cells producing both PP and PYY as well as chromogranin C, but lacking glucagon-related peptides and chromogranin A, occur in the pyloric mucosa of the dog and cat and, rarely, in other mammals[133,142].
As a rule pancreatic PP cells store *PP* as well as the icosapeptide (fig. 3); however, uncleaved pro-PP, lacking C-terminus icosapeptide immunoreactivity, seems to be produced in a minority of these cells[142,151]. PYY has been detected in a minority of pancreatic PP cells[142], while coexistence of PP-related and glucagon-related peptides occurs rarely in the pancreas, apart from during foetal life[1]. So far, no difference of peptide immunoreactivity has been identified in the two ultrastructural subtypes of human PP cells, the F and D_1 subtype[139].
The various associations of glucagon-related and PP-related peptides or propeptides in the gut and pancreas suggest that intestinal L cells may represent the phylogenetic and ontogenetic ancestor cells of both glucagon A cells and PP cells. ProPP immunoreactive or C-terminus glucagon reactive subsets of intestinal L cells, pyloric and pancreatic cells producing both PP and PYY, immature A cells of human early foetal pancreas showing glicentin immunoreactivity while lacking glucagon C-terminus immunoreactivity[146] and/or showing co-localized PYY immunoreactivity[1], pancreatic cells storing both PP and GRF[16], are all findings suggesting a continuous spectrum of cells (and peptides) evolving from typical intestinal L cells (storing PYY and glicentin/glucagon-37/GLP_1/GLP_2) to classical pancreatic glucagon A cells (producing glucagon-29, GRPP, glicentin C-terminus hexapeptide and MPGF) or to PP cells (producing PP and pro-PP icosapeptide together with GRF).
Neuropeptide Y (NPY) immunoreactivity has been detected in adrenal chromaffin cells of adrenalin-producing type (fig. 1) and pheochromocytomas[30,95].

c) *Endocrine tumours* producing glucagon-related and/or PP-related hormones have been found in the pancreas, intestine (fig. 4), ovary, kidney and thyroid[14,15,47,64,135]. PYY, PP, pro-PP-icosapeptide, glicentin/glucagon-37, GLP_1, GLP_2 and occasionally even glucagon-29 immunoreactivities have been detected in L cell tumours of the rectum, colon, appendix, ileum, ovary and kidney, in the same or separate cellular subsets and with or without associated EC cells producing serotonin and substance P[48]. The only L cell tumour apparently producing an hyperfunctional syndrome was a kidney tumour associated with decreased intestinal motility and absorption as well as hypertrophy of intestinal mucosa, all of which disappeared after tumour resection[12,53].
Both mature A cells with typical α-granules and immature, foetal-type A cells with solid, L-like granules lacking glucagon C-terminal immunoreactivity, have been detected in pancreatic glucagonomas, some of which seem to recapitulate A cell ontogenesis as observed in early foetal pancreas[14,142]. PP cells are often found to coexist in such tumours while pure PP cell tumours are observed rather rarely in the pancreas[15,64]. In most cases tumour PP cells resemble ultrastructurally the D_1 subtype, although the F subtype, normally prevailing in the PP-rich tissue originating from the ventral pouch, has also been observed sometimes[139]. PP immunoreactive cells have also been detected in some duodenal tumours, including so-called 'gangliocytic paragangliomas' or 'neurocarcinoids'[140] and in some thyroid medullary carcinomas, especially of familiar type[106]. The usefulness of PP as a marker of multiple endocrine neoplasia families has been stressed[50].
VIP-producing tumours (vipomas) of the pancreas and intestine are epithelial endocrine tumours, mainly of low grade malignancy, associated with watery diarrhoea, hypokalaemia and achlorhydria (WDHA) syndrome[13,29]. As expected, besides VIP, tumour cells produce and secrete PHI

(peptide histidine isoleucine), a VIP-like peptide encoded by the same gene as VIP itself, as an integral part of pro-VIP[11]. PP cells have been observed in 11 out of 27 pancreatic vipomas investigated[29]; in some cases, PP- and VIP-immunoreactivity coexisted in the same tumour cell[74]. These findings further support a possible relationship of vipomas with cells of PP, A and L lines[142]. Glucagon, somatostatin, neurotensin, and calcitonin have been also detected in several of these tumours[29,43]. VIP and PHI production has been also observed in adrenal phaeochromocytoma[63] and ganglioneuroblastoma, sometimes coupled with the 'vipoma' syndrome[11,92] and, occasionally, in thyroid medullary carcinoma and lung small cell carcinoma[127].

VIP has been localized immunocytochemically to very small, round, thin-haloed (P-type) granules stored in pancreatic and intestinal tumour cells[29]. Small, round, thin-haloed granules resembling those of vipoma cells have been also detected in endocrine tumours of the lung, pancreas and gut (with or without associated acromegaly) producing GRF[6,38,135], a peptide known to display consistent homology with PHI and VIP[69,123].

Secretin and GIP

To obtain specific detection of secretin or GIP in immunohistochemical and immunocytochemical tests special care must be taken to avoid crossreactivity of pertinent antisera with chemically related hormone and prohormone sequences (such as glucagon, glicentin, GLP_1, GLP_2, VIP, PHI and GRF). In all mammals so far investigated both secretin and GIP cells proved to be exclusive to the small intestine, usually with preference for the duodenum and upper jejunum. Only in the rat and mouse are these cells about as numerous in the ileum as in the upper small intestine. As a rule, secretin cells occur preferentially in the villi and upper crypts while GIP cells are more deeply situated in the crypts[13,131,165]. Most secretin cells show cytoplasmic granules with intense argyrophilia and chromogranin A immunoreactivity while GIP cell granules react poorly to both these tests[133,163].

Ultrastructurally, secretin cells show considerable pleomorphism of secretory granules, ranging from solid, round to ovoid and thin-haloed patterns to target-like granules with a round, osmiophilic core storing secretin surrounded by a more or less dense argyrophil matrix, likely to contain chromogranin A[163]. Target-like granules are prominent in the dog and human secretin cells (fig. 5).

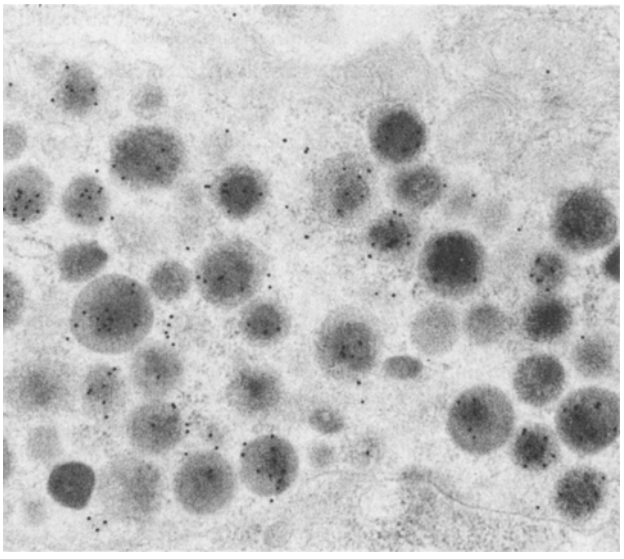

Figure 5. Electron immunocytochemistry of secretin in an S cell of the dog duodenum using rabbit anti-porcine secretin serum R.7875-02-2 (Milab) free of GIP, glucagon, glicentin, GLP_1 and GLP_2 crossreactivity. Note selective deposition of gold particles over target-like secretory granules, with preference for their dense, osmiophilic core. Protein A-immunogold technique, uranyl counterstaining. × 28,000.

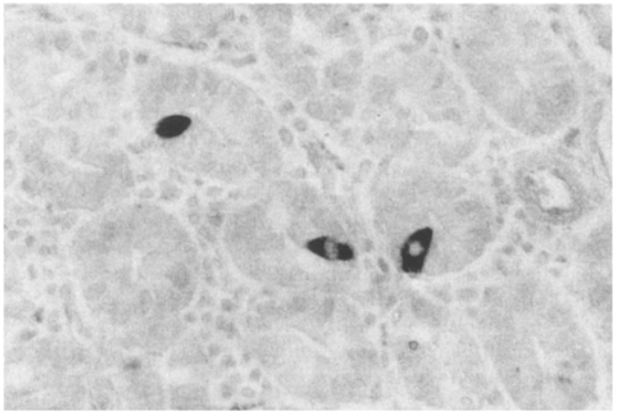

Figure 6. CCK cells of the dog duodenal mucosa stained with rabbit anti-porcine CCK-33 serum AB01 (CRB, Cambridge, U.K.), with middle to N-terminus specificity and no gastrin crossreactivity. Immunoperoxidase, × 350.

GIP cells, whose specific detection, free of crossreacting A and L cells, has been obtained with monoclonal antibodies devoid of any peptide crossreactivity[18], are characterized ultrastructurally by solid, relatively small granules showing uniform reactivity with GIP antibodies and differing clearly from secretin cell granules[165].

So far, neither secretin nor GIP cells have been convincingly detected in endocrine tumours. Nutrient-mediated regulation of exocrine (secretin) and endocrine (GIP) secretions of the pancreas seems to be the main function of secretin and GIP cells.

Gastrin, CCK and C-terminus gastrin/CCK

Gastrin G cells are medium-sized, ovoid to bottle- or pear-shaped cells concentrated in the deep neck and upper body of pyloric glands. Mammalian G cells react with antibodies directed against all sequences of gastrin-17, gastrin-34, C-terminally extended gastrin and progastrin, including its N-terminal and C-terminal cryptic peptides[59,70]. Many of their moderately argyrophil granules storing chromogranins A and B together with gastrin-17 and progastrin fragments, are characterized ultrastructurally by a typical vesicular pattern with floccular content[141]. More solid granules, apparently storing an increased proportion of large gastrin molecules, are also present in G cells, especially in the Golgi area[141,170]. Xenopsin, a neurotensin-like octapeptide, has also been detected in mammalian G cells[124], while neurotensin itself seems present in chicken pyloric G cells[119].

In the human duodenum, but not in dog or cat duodenum, few gastrin cells resembling G cells are scattered in Brunners glands and deep crypts. A few cells with small, round, solid granules reacting with both C-terminal and N-terminal gastrin-34 antibodies (so-called intestinal gastrin or IG cells) have also been observed in the human duodenum[19,167]. In the pancreas of some species (rat, cat), but not of man, gastrin G cells have been detected during foetal and neonatal life[85].

An abundant population of cells reacting intensely with antibodies directed against the gastrin C-terminal tetrapeptide but lacking reactivity with other gastrin-progastrin antibodies (TG cells) has been detected in the duodenum, jejunum and ileum of man and other mammals. Ultrastructurally, these cells show large, round to irregular secretory granules, sometimes with inner dense bodies, occasionally giving tar-

get-like patterns[82,167]. Their possible relationship with pancreastatin, a new peptide with partial structural similarity with gastrin and vasopressin at its C-terminus[155], remains to be investigated.

Cholecystokinin (CCK)-producing cells have been characterized with non-C terminal reactive antibodies, lacking histochemical crossreactivity with gastrin (fig. 6). They are scattered in the crypts and villi of the duodenum and jejunum. Their secretory granules are small, round and thin-haloed[164,167]. In the human duodenum (but not in the duodenum of the dog and other mammals or in the human jejunum) a large fraction of these cells, besides reacting with non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also show immunoreactivity with C-terminus gastrin-34 antibodies, co-localized with CCK in a variable proportion of secretory granules[167]. CCK/gastrin cells have been also reported in human foetal duodenum and newborn rat duodenum[82]. These 'mixed CCK/gastrin cells', resemble more CCK cells than gastrin G cells both histologically and ultrastructurally, and seem to be considered as CCK cells developing (or retaining from foetal life) partial gastrin coexpression, a mode of behaviour possibly reminiscent of a common phylogenetic origin of CCK and gastrin cells[84].

Hyperplasia and hyperfunction of pyloric gastrin cells has been reported in patients with peptic ulcer disease, hyperchlorhydria and food-stimulated hypergastrinaemia or in achlorhydric patients due to type A chronic atrophic gastritis and secondary hypergastrinaemia[115,141]. Gastrin cell tumours, with and without associated hypergastrinaemia, hyperchlorhydria and peptic ulcer disease (Zollinger-Ellison syndrome) have seldom been found in the stomach, jejunum and biliary tree or liver, more frequently in the duodenum and, especially, in the pancreas[134]. In tumour cells secretory granules were often fewer than in normal cells, more solid and smaller in size, thus resembling progranules of normal cells, in keeping with the higher proportions of gastrin-34 and progastrin they produce[36,109]. Cells with large, often irregularly shaped granules resembling those of C-terminal gastrin immunoreactive cells (TG/VL cells) have also been observed in pancreatic and intestinal gastrinomas[8,134]. So far, CCK-immunoreactive tumour cells have only been observed as a minority population of a single duodenal gastrinoma[140].

Motilin

Motilin cells, like secretin and GIP cells, are exclusive to the small intestine, particularly to its upper part[112]. Ultrastructurally, motilin-immunoreactive cells are characterized by small, round, solid and fairly osmiophilic granules and abundant microfilaments[162]. They seem to play an important part in the modulation of gut motility, especially in the interdigestive phase. Motilin cells have been found only occasionally in intestinal endocrine tumours[179].

Neurotensin and xenopsin

In mammals, neurotensin N cells are mostly confined to the small intestine, especially the ileum and lower jejunum; only occasional cells have been detected in the large bowel[177]. Ultrastructurally, they correspond to a cell with large, solid, dense granules[150] (fig. 7) of consistent argyrophilia and chromogranin A immunoreactivity[133]. In the chicken antrum, neurotensin-immunoreactive cells have been observed[150] which may correspond to a fraction of G cells storing both gastrin- and neurotensin-like peptides[119]. While neutrotensin immunoreactivity has never been detected in mammalian stomach, the presence of xenopsin (a neurotensin-like octapeptide first identified in the skin of *Xenopus laevis*) in mammalian pyloric G cells has been ascertained[124]. Neurotensin cells have also been detected in chicken thymus[152] and in a subpopulation of noradrenalin-containing cells (the so-called III type cells) of the cat adrenal medulla[156].

Neurotensin cells have been repeatedly reported in pancreatic tumours, with special reference to those associated with the watery diarrhoea syndrome, sometimes as an overwhelming cellular component, usually with concomitant VIP secretion[29,44]. Concomitant production of xenopsin and gastrin by the same tumour cells[45] and of neurotensin and gastrin by the same pancreatic tumour[43] has been reported, a finding in keeping with the coexistence of gastrin and neurotensin-like peptides in normal pyloric G cells.

Neurotensin cells have occasionally been found also in endocrine tumours of the appendix and rectum[105,179]. Generalized pruritus and dermatographism have been observed in a patient showing an inoperable rectal tumour associated with very high plasma levels of neurotensin. The powerful histamine-releasing action of neurotensin on mast cells might be involved in causing these symptoms[20].

Concluding remarks

Despite the impressive progress made during the past few years, functional characterization of the manifold population of peptide-producing endocrine cells is far from being fully achieved. The products of some ultrastructurally-characterized cells in the gastrointestinal mucosa, skin and urethra remain to be ascertained[135,137], the exact precursor molecules of several regulatory peptides localized to endocrine cells are still to be identified, the intragranular enzymes involved in their posttranslational processing, as well as pertinent regulatory mechanisms, are largely unknown, while specific receptors and intracellular mediators involved in endocrine cell activation remain mostly to be characterized. Clarification of these points is essential not only for the understanding of many endocrine functions but also for appropriate interpretation of pertinent pathological processes, with special reference to tumour pathology. At present, it seems clear that endocrine tumours produce more frequently those peptides (or related propeptides) that are normally expressed by their tissue of origin. Examples include bombesin/GRP in lung, calcitonin in thyroid, substance P in midgut, glicentin in rectal and enkephalins in adrenal medulla and paraganglionic tumours. However, tumour cells show an increased plasticity of peptide expression, especially in malignant tumours, leading to production of several inappropriate peptides (among which ACTH and calcitonin are those more frequently reported), sometimes with clear-cut site-dependent preference, as for gastrin and VIP expression in pancreatic tumours[136]. A tendency for tumour cells to release an increased proportion of larger molecular forms or

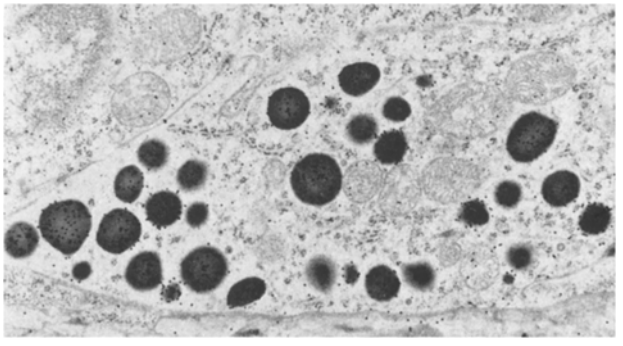

Figure 7. Electron immunocytochemistry of neurotensin in large, solid secretory granules of an N cell in the dog ileum, using anti-neurotensin serum 122/3 (from Dr G. E. Feurle, Meidzinische Poliklinik, University of Heidelberg, FGR). Protein A-immunogold technique, uranyl counterstaining; × 14,500.

even the entire, uncleaved propeptide, has also been noted in many tumours and is likely to be due to defective intragranular prohormone storage and/or processing. Clarification of molecular and cellular mechanisms underlying these phenomena may help in understanding the biology and clinical behaviour of such growths.

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