Review

The gut as the largest endocrine organ in the body

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Summary

Secretin, gastrin and cholecystokinin were the first discovered gut hormones. Today we recognize more than 30 gut hormone genes and a multitude of bioactive peptides, which make the gut the largest endocrine organ in the body. Due to structural homologies gut peptide hormones/growth factors have been divided into separate families. It has been emphasized that those peptides are widely distributed, but have a specific expression in different cell types. The intestine can also be regarded as a sensory organ operating via neurons, endocrine cells and immune cells with gut peptides as signalling substances. Expression studies of peptide receptors in gut neuroendocrine tumours in combination with tailored peptide analogs have been helpful in developing new diagnostic and therapeutic strategies. New fields of research will relate to gut peptides associated with deficiency diseases and as potential growth factors in malignancies. Enterochromaffin cells, interspersed throughout the entire gastrointestinal mucosa, form the largest endocrine cell system. The physiological role of hormonal messengers, peptide receptors and amine transporters is currently under investigation as well as their potential involvement in disease, e.g. the secretory diarrhea associated with midgut carcinoid tumours.

Key words: gastrointestinal tract, monoamines, neuroendocrine tumours, peptide hormones

Introduction

Secretin, gastrin and cholecystokinin were the first gastrointestinal hormones discovered [1-3] and also the first to be structurally identified. Up to the 1970s several investigators claimed that these hormones were the three dominant regulators of digestion (Trinity doctrine). At the time they were even proposed to act via the same receptor [4]. With the rapid progress of peptide chemistry from radioimmunoassays, chromatography, HPLC to sequencing and peptide synthesis, physiological studies related to the bioactivity of peptides have been upgraded to high specificity. Immunocytochemical studies using monoclonal antibodies have reduced the number of previously non-classified gut endocrine cells to be fewer than 10% (Table 1). Molecular biological techniques like RNA/DNA isolation and sequencing have been helpful in identifying the primary translation product and low stringency hybridization in identification of genes and related mRNA. Prohormones, or bioactive peptide fragments, present in minute amounts in tissue samples can be amplified and their target action studied with guidance of cloning/expression studies of peptide receptors. With the synthesis of hormone analogues new drugs have been developed with high affinity binding to peptide receptors. Such pharmacological tools have reinforced studies on the role of peptides in gastrointestinal diseases. Today we recognize more than 30 peptide hormone genes, expressing more than 100 bioactive peptides, besides other hormonal messengers, e.g. monoamines and eicosanoids [5]. These facts truly make the gut the largest endocrine organ in the body.

Distribution of gut endocrine cells

The distribution of endocrine cells in the gastrointestinal tract differs from that of the classical endocrine glands, e.g. islets are spread in the exocrine pancreas and endocrine cells are interspersed between gut mucosal cells. Knowledge about the physiology and anatomic distribution of the gut endocrine system is most helpful for the clinician to understand the pathophysiology of certain diseases, e.g. patients with excess hormone production from gut endocrine tumours (Table 1) [6].

The peptide families

Based on structural homologies, e.g. similar primary structure or common active sites, gut peptides can be divided into six hormone families: The secretin family (secretin, glucagon and glucagon-like peptides, vasoactive intestinal polypeptide and peptide histidine isoleucin, growth hormone releasing hormone and pituitary adenyl cyclase-activating peptide), the insulin family (insulin, insulin-like growth factor I & II and relaxin), the EGF family (epidermal growth factor, transforming growth factor-α and amphiregulin), the...
Table 1. Gastrointestinal endocrine cell types, their localization and relation to hormonal syndromes.

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Cell type</th>
<th>Main product</th>
<th>Main localization</th>
<th>Possible syndrome</th>
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<td>Glucagon</td>
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<td>Somatostatin</td>
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<td>Unknown</td>
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<td>S</td>
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<td>Neurotensin</td>
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<td>L</td>
<td>Enteroglucagon/PYY</td>
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Abbreviations: s/i — small-intermediate-sized cells; EC — enterochromaffin; ECL — enterochromaffin-like cell; PP — pancreatic polypeptide, VIP — vasoactive intestinal polypeptide; CCK — cholecystokinin; GIP — gastric inhibitory polypeptide; PYY — PP-like peptide with N-terminal tyrosine amide; PHH — persistent hyperinsulinemic hypoglycemia; ZES — Zollinger–Ellison syndrome.

Modified after Rindi et al. (1999) [6].

gastrin family (gastrin and cholecystokinin), the PP-fold family (pancreatic polypeptide, peptide YY and neuropeptide Y), the tachykinin family (substance P and neurokinins) and the somatostatin family (somatostatin and corticotatin).

Gut hormones as general messengers

In a recent review Rehfeld [5] emphasized that gut hormones should be regarded as general intercellular messengers in the body, not only regulators of the gastrointestinal tract. This concept was based on the following observations:

1. **Hormone families** can be assembled with regard to structural homologies. This may in turn indicate common ancestral genes, e.g. gastrin in mammals, cerulium in amphibians and cionin in protochordates all share the common C-terminal tetrapeptide amide, which is essential for bioactivity.

2. The **gut hormone genes are widely expressed**, not only in endocrine cells, but also in central and peripheral neurons and certain tumour cells.

3. The **hormone-producing cells can release their products by endocrine, paracrine, neurocrine or autocrine secretion**. Peptides may thus act as true blood-borne hormones as well as local growth factors.

4. The **hormone genes can express multiple bioactive peptides** due to mechanisms like alternative splicing or differential processing of the translation product, e.g. glycine-extended intermediates of gastrin-17 may act as growth promoters with only minor effects on gastric acid secretion in comparison with gastrin-17.

5. The same hormone gene can have a **cell-specific expression** in different cell types, e.g. antral G-cells express several small and large molecular forms of gastrin, while the pituitary expresses progastrin and large molecular gastrin and colonic.
The gut endocrine-nervous-immune system

The intestinal mucosa can also be regarded as a large sensory organ with complex interaction between neurons, endocrine cells and the immune system. The enteric neurons system consists of 100 million neurons, the endocrine system utilizes some 100 identified messengers and the gut immune system harbour more than 70% of the immune cells in the body [7]. This complex system is responsible for nutrient tasting and elicits stimulus-adequate responses in terms of motility, secretion, perfusion and tissue defense via both intrinsic and extrinsic nervous reflexes. On their mucosal surface gut endocrine cells have microvilli, which may serve to taste the intraluminal milieu [8]. For instance, after a meal cholecystokinin is released from duodenal endocrine cells, which in turn liberate digestive enzymes from the pancreas, but also activate neurons of the gallbladder wall with subsequent emptying of bile for breakdown of fats and proteins in the meal. Via vagal afferents cholecystokinin can also inhibit gastric emptying and initiate a satiety response. Another example of an integrated network action is motilin, which after release from duodenal endocrine cells activates a motor program, which causes coordinated propulsion via migrating myoelectric complexes.

The interactions in inflamed and damaged tissues are yet rather poorly understood. These tissues produce cytokines, prostaglandins and tachykinins, all with neuronal actions, e.g. bradykinins can activate visceral afferents leading to pain and tachykinins liberated from axonal reflexes may sensitize afferent neurons. Changed neuronal sensitivity may be involved in common gastrointestinal diseases like the irritable bowel syndrome. Tachykinins can also cause local vasodilation and extravasation of immune cells and may thus play a role in restricting tissue damage [7].

The endocrine gut in disease

The most obvious endocrine gut disorders are related to excess production of hormones by neuroendocrine tumours. Since several of these tumour types express high numbers of somatostatin receptors (sstR), new diagnostic and therapeutic strategies have been developed. Somatostatin analogues reduce secretion via inhibitory G-proteins and therefore marked symptom relief from hormonal symptoms can be achieved. By using radio-labelled somatostatin analogues, tumours can be localized scintigraphically or during surgery with the assistance of scintillation detection (radioguided surgery). Residual tumours may be treated by radionuclide therapy, since radionuclides are internalized into the tumour cells after sstR binding [9]. New fields of research may relate to gut peptides associated with deficiency states. Such involvement seems evident for many motility disorders, e.g. congenital or acquired aganglionosis and biliary dyskinesia. Clinical results from treatment with peptides have been reported for hypomotility in scleroderma using somatostatin analogues, for achalasia using vasoactive intestinal polypeptide and for gastric dysrhythmia using erythromycin to activate motilin receptors [10].

The enterochromaffin cell system

The enterochromaffin (EC) cells constitute the largest endocrine cell population in the gastrointestinal tract and were also the first gut endocrine cells to be identified. They were shown to bind chromium salts and were therefore called ‘enterochromaffin cells’ [11]. Later, their capacity to bind and reduce silver ions was demonstrated and they were also named ‘argentaffin cells’ [12]. Their function was unknown for a long period. However, an endocrine function was early suggested by Feyrter, who proposed a ‘diffuse neuroendocrine system’ in the gut [13]. With the introduction of the formaldehyde-induced fluorescence technique a.m. Hillarp-Falck, gut endocrine cells were shown to be able to synthesize monoamines, a capacity recognized as APUD (Amine Precursor Uptake and Decarboxylation). The APUD cells shared many properties with neurons and were thought to be derived from the neural crest [14]. Later studies have suggested an origin in the gut stem cells [15]. EC cells are widely distributed in the gastrointestinal tract and are found in the mucosa of the gastric antrum, duodenum, jejunum, ileum, appendix, colon and rectum. They have an almost exclusive intraepithelial location, resting on the basal lamina and projecting into the gut lumen with their apical portion. The cytoplasm of the EC cells is occupied by a large number of secretory granules, which are the storage sites of the secretory products [16]. The size, shape and electron density of the secretory granules are characteristic for each endocrine cell type. The appearance of the secretory granules actually formed the basis for classification of gut endocrine cells before the introduction of immunocytochemistry. The main secretory product of EC cells is serotonin and the EC cells account for more than 90% of all serotonin synthesized in the body. Minor amounts of peptide hormones, e.g. tachykinins, enkephalins and motilin, may also be synthesized in subsets of EC cells [17]. Serotonin is synthesized from the amino acid tryptophan by hydroxylation and decarboxylation in the cytoplasm of EC cells and subsequently transported into the secretory granules by an active transport mechanism (vesicular monoamine transporter, VMAT). Upon specific stimulation, granules are translocated to the cell membrane and the granule contents are released by exocytosis. Secreted serotonin may influence adjacent cells by a paracrine action or reach distant cells via the circulation. Inactivation of serotonin is accom-
plished by reuptake of the amine by EC cells, neurons and platelets, or by enzymatic degradation (monoamine oxidase, MAO) in the liver and lung, followed by excretion in the urine as the main metabolite 5-hydroxyindoleacetic acid. EC cells have close contacts with nerve elements of both afferent and efferent type adjacent to the basal lamina of the mucosa (neuroendocrine complexes) and true synapses have been identified [18]. Physiologically both serotonin and tachykinins have similar effects on the gastrointestinal tract, i.e. smooth muscle contraction, local vasodilation and secretion of water and electrolytes. Certain stimuli from the lumen (acid pH, hypertonic glucose, amino acids and noxious stimuli) can cause release of serotonin, which in turn activates afferent nerve endings, which via intrinsic reflexes evoke adequate mucosal responses like hyperemia, secretion and peristalsis [19].

### Enterochromaffin cell tumours

Tumour transformation of gut endocrine cells may give rise to tumours that are characterized by their neuroendocrine phenotype. Such tumours were first described by Lubarsch [20]. Oberndorfer [21] coined the term 'carcinoid' to separate this tumour entity from the common adenocarcinoma. Specific hormone production in gut neuroendocrine tumours was first reported by Lembeck [212] in 1953 with the biochemical demonstration of serotonin in a carcinoid tumour. Today, a large number of different amine and peptide hormones have been demonstrated in gut neuroendocrine tumours and their association with hormonal syndromes have been described in detail [23]. With the identification of specific hormones in tumours and their related syndromes it became evident that gut neuroendocrine tumours do not represent a single entity, but rather a group of tumours sharing a common origin and phenotype. The neuroendocrine tumours have been classified according to their embryological origin as foregut (stomach, duodenum, pancreas, lung, thymus), midgut (jejunum, ileum, appendix, ascending colon) and hindgut (transverse, descending and sigmoid colon, rectum) derivatives [24].

In the recent WHO classification [25], four categories of tumours are recognised: well-differentiated endocrine tumours ('carcinoids'), well-differentiated endocrine carcinomas ('malignant carcinoids'), poorly differentiated endocrine carcinomas and mixed exocrine-endocrine tumours. Gut neuroendocrine tumours are rare with an incidence of approximately 2/100,000 population and year [26]. Carcinoid tumours of the ileum account for 20% of all gastrointestinal carcinoids and represent the most frequent malignant gut neuroendocrine tumour. Ileal carcinoids are thought to originate from EC cells due to the phenotypic similarities between EC cells and tumour cells, e.g. ultrastructural features, presence of vesicular proteins and hormonal profile. Patients with ileal carcinoids frequently present with a classical carcinoid syndrome due to metastatic disease with excessive hormone production. Control of hormonal symptoms can be achieved in most patients by interventional and medical treatment, but definite cure is difficult to obtain. Detailed knowledge of the mechanisms controlling proliferation and hormone secretion from carcinoid tumours is therefore necessary to obtain adequate control of the disease.

### Amine handling properties of enterochromaffin cell carcinoids

*In vitro* studies on ileal carcinoids in primary culture have demonstrated a number of similarities between the amine-handling properties of carcinoid tumours and monoaminergic neurons [27]. Carcinoid tumour cells in *vitro* synthesize and secrete serotonin into the culture medium at a constant rate. Some of the secreted amine is taken up in the tumour cell by an imipramine sensitive mechanism (membrane-pump), followed either by sequestration into the secretory granules or degradation by MAO. Treatment of cultures with reserpine depletes tumour cells of their amine content. Secretion of serotonin from tumour cells can be enhanced by $\beta$-adrenoceptor stimulation (isoprenaline), while stimulation of somatostatin receptors (octreotide) inhibits the secretion (Figure 1). Dexamethasone also inhibits the secretion of serotonin from cultured tumour cells, most likely via a stabilizing effect on the cell membrane. These studies reveal complex mechanisms for the amine handling of tumour cells and suggest alternative ways of interfering with the secretory process. Recently, a novel aspect of the amine handling of carcinoid tumours was highlighted, when the expression of VMAT was demonstrated in gut endocrine cells and carcinoid tumours [28, 29]. VMAT is an integral membrane protein with 12 transmembrane domains that exists in two isoforms, VMAT1 and VMAT2. Both VMAT1&2 are located to...
the membrane of secretory granules and vesicles and transport amines from the cytoplasm into the secretory granule. Both transporters utilise the proton gradient across the vesicle membrane for transport and are sensitive to reserpine. However, they differ in tissue distribution and substrate affinity. Ileal (EC cell) carcinoids predominantly express VMAT1, while gastric (ECL cell) carcinoids express VMAT2 and rectal carcinoids lack both VMAT1 and VMAT2 (Figure 2). VMAT expression may therefore become a useful marker in the classification of gut neuroendocrine tumours, but the findings may also have clinical implications. The synthetic noradrenaline analogue metaiodobenzylguanidine (MIBG), which is used to visualize and treat neuroendocrine tumours, has high affinity for chromaffin granules and VMAT. One may therefore hypothesize that MIBG uptake in neuroendocrine tumours is mediated by VMAT and that the level of VMAT expression in tumours will predict the efficacy of MIBG visualization and treatment.

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References

25. Solcia E, Klöppel G, Sobin LH. World Health Organization,


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