Chapter 7
Gastrointestinal Hormones and Their Targets

Jens F. Rehfeld

Abstract Gastrointestinal hormones are peptides released from endocrine cells and neurons in the digestive tract. More than 30 hormone genes are currently known to be expressed in the gastrointestinal tract, which makes the gut the largest hormone producing organ in the body. Modern biology makes it feasible to conceive the hormones under five headings: The *structural homology* groups a majority of the hormones into nine families, each of which is assumed to originate from one ancestral gene. The individual hormone gene often has *multiple phenotypes* due to alternative splicing, tandem organization, or differentiated maturation of the prohormone. By a combination of these mechanisms, more than 100 different hormonally active peptides are released from the gut. Gut hormone genes are also *widely expressed* in cells outside the gut, some only in extraintestinal endocrine cells and neurons but others also in other cell types. The extraintestinal cells may synthesize different bioactive fragments of the same prohormone due to *cell-specific processing* pathways. Moreover, endocrine cells, neurons, cancer cells, and, for instance, spermatozoa *release the peptides differentially* (autocrine, endocrine, neurocrine, paracrine, spermiocrine secretion etc.), so the same peptide may act as a blood-borne hormone, a neurotransmitter, a local growth factor, or a fertility factor. The molecular targets of each bioactive peptide are specific G-protein coupled receptors expressed in the cell membranes of different target cells. Also the target cells of gut hormones occur widespread outside the digestive tract.

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Abbreviations

CCK	Cholecystokinin
CGRP	Calcitonin gene related peptide
EGF	Epidermal growth factor
G-cells	Gastrin-producing cells
GIP	Gastric inhibitory peptide (later renamed glucose-dependent insulinotropic polypeptide)
GLP-1 and -2	Glucagon-like peptide 1 and 2
IGF	Insulin-like growth factor
L-cells	GLP-producing cells
mRNA	Messenger ribonucleic acid
NPY	Neuropeptide Y
PP	Pancreatic polypeptide
PTHrP	Parathyroid Hormone-related Protein
PYY	Peptide YY
TGF-α (alpha) and -β (beta)	Transforming growth factor α (alpha) and -β (beta)
TSH	Thyroidea-stimulating hormone
VIP	Vasoactive intestinal polypeptide

Historical Introduction

The bloodborne regulation by specific messenger molecules was discovered in 1902 in London by Bayliss and Starling [1]. Following up on the observation that acidification in the upper small intestine, the duodenum, stimulated pancreatic secretion, Bayliss and Starling extracted from the duodenal mucosa a substance that released bicarbonate from the denervated pancreas when injected into blood. They gave this substance a very broad name, secretin. In 1905, John Edkins (also from London) suggested that extracts of the antral mucosa [2] contained an acid stimulatory messenger ("gastric secretin"—or simply gastrin). Hence, the first two bloodborne "chemical messengers" to be known in mammalian biology, secretin and gastrin, were both of gastrointestinal origin. Subsequently, also in 1905, Starling proposed the word *hormone* as a general designation for bloodborne messengers [3].

In the following decades, however, other types of hormone came into focus—steroids from the adrenals, ovaries, and testes; protein hormones from the pituitary gland; the thyronins from the thyroid gland; and insulin from the pancreas. The clinical implications and often life-saving effects of these discoveries made the interest for secretin and gastrin fade in the darkness of the bowels. Subsequently, only a small priesthood of physiologists continued to study the hormonal control of digestion. One of them was Andrew Ivy in Chicago, who with his assistant, Eric Oldberg, found a gallbladder emptying hormone [cholecystokinin (CCK)] in

extracts of the small intestine [4]. A stimulator of pancreatic enzyme secretion (pancreozymin) was discovered 15 years later by Harper and Raper in Newcastle [5]. But Jorpes and Mutt showed in the 1960s in Stockholm, however, that CCK and pancreozymin were one and the same peptide hormone [6] for which the acronym CCK is now used.

Secretin, gastrin, and CCK constitute the classical troika of gastrointestinal hormones, but since the early twentieth century, many more have been discovered (Fig. 7.1). In order not to lose overview, this chapter summarizes all the gut hormones, some of their targets, and their major biological activities in Tables 7.1, 7.2 and 7.3, but otherwise presents the general principles governing structure and biogenesis of gastrointestinal hormones and their receptors (see also [7, 8] for longer reviews). Readers interested in details about individual hormones, their targets, receptors, and their effects, should consequently consult multi-author volumes comprising the full range of gastrointestinal endocrinology [9–11]. Also, a shorter review on the history of gastrointestinal endocrinology has recently been published [12].

Comparative Aspects of the Development

Life in multicellular organisms began as a simple tube with only one opening. Take coelenterates, for instance (Fig. 7.2). They live in water that runs into their lumen, and from which nutrients are absorbed into the epithelial cell-lining. Coelenterates have a regulatory system of singular primitive neurons spread out in the wall. Apparently, these neurons release small regulatory peptides. Thus, multicellular life began as an isolated ‘gut’ whose function was controlled by regulatory or hormonal peptides. Consequently, viewing the phylo- and ontogenetical development of life, evolutionists could say that the specific organs and tissues in vertebrate organisms are derivatives of the primordial multicellular structure, the gut. Accordingly, the regulatory or hormonal peptides of the gastrointestinal tract are from the beginning essential caretakers of life; also human life and its disorders.

General Features of Gastrointestinal Hormones

The Structural Homology

Gastrointestinal endocrinology currently encompasses a large number of hormones, neuropeptides and growth factors. Not only have new hormones been found in gut extracts, but also peptides from the central nervous system and hormones first identified in other endocrine organs have been found in endocrine cells and/or neurons in the gastrointestinal tract. Moreover, peptides originally believed to be

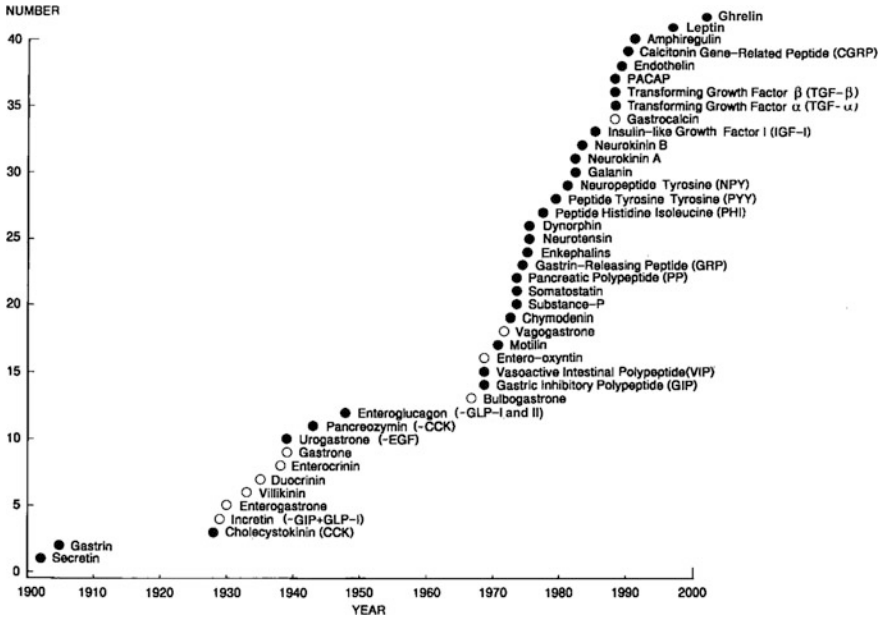

Fig. 7.1 Discovery and identification of regulatory peptides in the gastrointestinal tract 1900–2000. Discovery is indicated by year of first report. *Solid circles* indicated structural identification, and *open circles* indicated hormonal activities that still require structural identification. Some of the unidentified hormonal activities are explained by later identified hormones. For instance, the incretin activity is partly due to gastric inhibitory polypeptide (GIP) and glucagon-like peptide I (GLP-I). Commonly used acronyms are indicated in brackets after full name, except for PACAP, which is an acronym for pituitary adenylate cyclase-activating peptide

classical hormones but later shown to be neurotransmitters have been isolated from gut extracts. Finally, a number of growth factors have now been found in the gut—epidermal growth factor (EGF), originally isolated as the gut hormone, urogastrone, from urine; insulin-like growth factors (IGF) I and II; transforming growth factors (TGF)-α (alpha) and -β (beta); amphiregulin, and others.

The complexity is increased through individual genes for gut regulatory peptides encoding different peptides released in a cell-specific manner. Several principles for gene expression operate to provide such variety. Hence, alternative splicing of the calcitonin gene transcript to express CGRP is not the only example [13]. Also, the secretin gene is expressed in different molecular forms due to alternative splicing [14, 15].

Additional studies indicate that there are still hormonal activities in the gut that are not structurally identified. Perhaps some of the activities can be explained by already identified peptides. Hence, the hormonal stimulation of insulin secretion from the gut, originally called incretin, is today explained by at least two hormones, GIP and truncated GLP-1—probably in combination with other gut hormones, including gastrin and CCK peptides (for review, see [16]), whereas intestinal

Table 7.1 Peptide hormone, neuropeptide and growth factor families in the gastrointestinal tract and the pancreas

Families and members	Major regulatory activity
Secretin family	
Secretin	Stimulates pancreatic bicarbonate secretion
Glucagon	Increases glucose production and amino acid metabolism
Glucagon-like peptide 1 (GLP-1)	Stimulates insulin and inhibits glucagon secretion and gastric emptying
Glucagon-like peptide 2 (GLP-2)	Stimulates mucosal cell growth in intestinal crypts
Gastric inhibitory polypeptide (GIP)	Enhances glucose-stimulated insulin secretion and inhibits gastric secretion
Vasoactive intestinal polypeptide (VIP)	Inhibits gastrointestinal motility and stimulates fluid secretion
Peptide histidine isoleucine (PHI)	VIP-like actions
Growth hormone releasing hormone	Stimulates growth hormone secretion
Pituitary adenylyl cyclase-activating peptide (PACAP)	Contributes to the regulation of gastric acid secretion and gastrointestinal motor function
Gastrin family	
Gastrin	Stimulates gastric acid secretion and gastric mucosal cell growth
Cholecystokinin (CCK)	Stimulates pancreatic enzyme secretion, cell growth, and gallbladder emptying, but inhibits gastric acid secretion
Caerulein Cionin Not expressed in mammals	Cholecystokinin-like activities
Tachykinin family	
Substance P	Stimulates motility
Neurokinin A	Stimulates motility
Neurokinin B	Stimulates motility
Ghrelin family	
Ghrelin	Stimulates appetite and growth hormone secretion
Obestatin	Suppresses food intake (?)
Motilin	Contracts gastrointestinal smooth muscles to stimulate motility
PP-fold family	
Pancreatic polypeptide (PP)	Involved in feeding behavior (?)
Peptide YY (PYY)	Reduces gastric emptying, pancreatic exocrine secretion, and delays intestinal transit
Neuropeptide Y (NPY)	Modulates the contractility in smooth muscle cells
Somatostatin family	
Somatostatin	Inhibits gastric acid, gastrin secretion and other gut functions through endocrine, paracrine, and neurocrine release
Cortistatin	Somatostatin-like activities
Insulin family	
Insulin	Establishes energy resources in fat, liver and muscle cells
Insulin-like growth factor I (IGF-I)	Stimulates growth and differentiation in interaction with other growth factors

(continued)

Table 7.1 (continued)

Families and members	Major regulatory activity
Insulin-like growth factor II (IGF-II)	Stimulates growth and differentiation in interaction with other growth factors
Relaxin	Function in the gastrointestinal tract uncertain
EGF family	
Epidermal growth factor (EGF)	Stimulates growth of epithelial cells and inhibition of gastric acid secretion
Transforming growth factor α (TGFα)	EGF-like activities
Amphiregulin	Growth regulation of epithelial cells
Heparin-binding EGF-like growth factor	EGF-like activities
Opioid peptide family	
Enkephalins	Modulates transmitter activity from nerveplexes
β-endorphins	Modulates transmitter activity from nerveplexes
Dynorphins	Modulates transmitter activity from nerveplexes

Table 7.2 Singular peptide hormones, neuropeptides, and growth hormones in the gastrointestinal tract

Hormones and growth factors	Major regulatory activity
Apelin	Stimulates gastric mucosal growth and cholecystokinin secretion
Bradykinin	Contributes to control alkaline secretion in the duodenal mucosa
Calcitonin gene-related peptide (CGRP)	Modulates blood flow, secretion, and motility
Cocaine and amphetamine regulated transcript (CART)	Increases satiety
Galanin	Stimulates motility and luminal secretion
Gastrin-releasing peptide (GRP)	Stimulates antral gastrin secretion
Neurotensin	Increases the ileal brake
Orexin	Stimulates gut motility (?)
Transforming growth factor β (TGFβ)	Growth, differentiation, and inflammation
Thyrotropin-releasing hormone (TRH)	Releases TSH from epithelial cells in the gut

inhibitory effects on stomach secretion, the gastrone effects, may be explained by combinations of CCK, somatostatin, GIP, and EGF. However, the villikinin, duocrinin, enterocrinin, and the more recently suggested gastrocalcin [17] still await structural identification. At present there is, however, evidence that gastrocalcin may be PTHrP (the parathyroidhomone-related protein) known to be expressed as a paracrine regulator of differentiation and local intercellular signaling [18]. The multiplicity of gut hormones may jeopardize an overview of gut endocrinology. Structural identifications, however, have shown striking homologies between groups of peptides. Consequently, many of the biologically active peptides, hormones, neuropeptides, and growth factors in the gastrointestinal tract can be classified into nine families (Table 7.1). The expression of several hormone

Table 7.3 Receptors and receptor subtypes for some gastrointestinal hormones

Hormones	Receptors and subtypes
Atrial Natriuretic Peptide (ANP)	NP_A, NP_B, NP_C
Brain Natriuretic Peptide (BNP)	NP_A, NP_B, NP_C
C-type Natriuretic Peptide (CNP)	NP_A, NP_B, NP_C
Calcitonin	Calcitonin-R
Calcitonin Gene-Related Peptide (CGRP)	$CGRP_1$, $CGRP_2$
Cholecystokinin (CCK)	CCK_A, CCK_B
Gastric Inhibitory Polypeptide (GIP)	GIP-R
Gastrin	Gastrin/CCK_B
Gastrin-Releasing Peptide (GRP)	CGRP-R
Ghrelin	Ghrelin-R
Glucagon-Like Peptide-1 (GLP-1)	GLP-1-R
Motilin	Motilin-R
Neurotensin	NTR1, NTR2, NTR3
Parathyroid Hormone-related Protein (PTHrP)	PTH-R
Pituitary Adenylate Cyclase Activating Peptide (PACAP)	PAC_1
Peptide Tyrosyl Tyrosyl (PYY)	Y_1, Y_2, Y_3, Y_4, Y_5
Secretin	Secretin-R
Somatostatin	sst_1, sst_{2A}, sst_{2B}, sst_3, sst_4, sst_5
Substance P	NK_1, NK_2, NK_3
Vasoactive Intestinal Polypeptide (VIP)	$VPAC_1$, $VPAC_2$

genes both in the gut and pancreas reflects the intestinal origin of the pancreas. The nature of the homology varies. It may be an overall similarity in the primary structure as, for example, the PP-fold family. The similarity of the tertiary structure in this family is due to homologous residues necessary for stabilization of the three-dimensional structure [19].

Another type of homology is that of the gastrin family which, in addition to mammalian gastrin and CCK, also consists of the protochordean neuropeptide cionin [20] and frogskin peptide cerulein [21]. The decisive homology of this family is concentrated in the primary structure around the active site, the common C-terminal tetrapeptide amide sequence, -Trp-Met-Asp-Phe-NH_2. Comparison between propeptide and gene structures also reveals some similarity, but the family is still defined primarily by the conserved active site sequence and by neighboring O-sulfated tyrosyl residues.

The frequent occurrence of homology among hormones, neuropeptides, and growth factors is not specific for bioactive peptides in the gut. It is a common feature among all kinds of regulatory peptides, enzymes, and other proteins in the organism [22]. Each family is assumed to reflect the phylogenetic evolution by duplication and subsequent mutations of an ancestral gene.

The phylogenetic story shows that gastrointestinal hormones are indeed very old, several hundred million years [23]. So far, the data also support the idea that each hormone family has evolved from a single ancestor. An associated trait is that gastrointestinal hormones have, to a large degree, preserved their tissue-specific

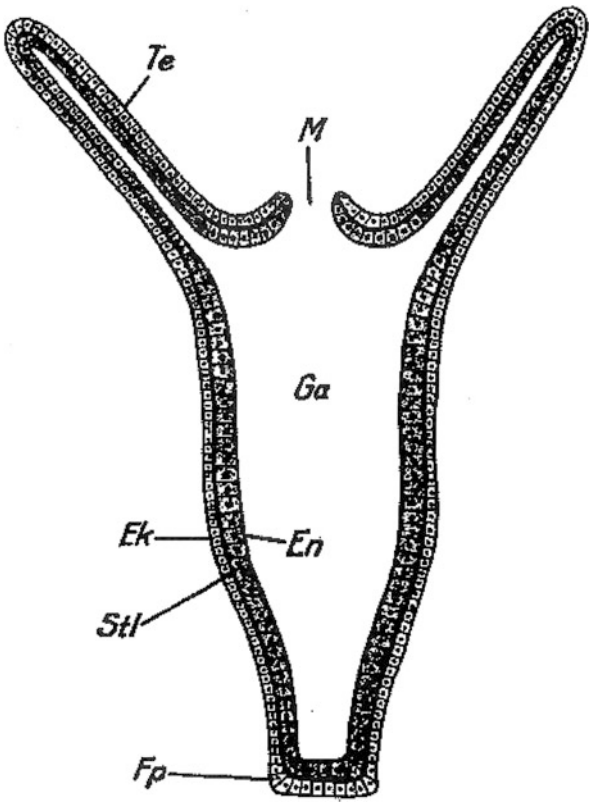

Fig. 7.2 Scheme of the structure of coelenterates with endoderm (*En*), ectoderm (*Ek*), footplate (*Fp*), gaster (stomach *Ga*), mouth (*M*), and tentacles (*Te*)

sites of expression during evolution, both in the primary and secondary sites [24]. Accordingly, the evolution emphasizes the general significance of gut hormones as intercellular messenger molecules.

At present, a few bioactive peptides in the gastrointestinal tract have no relatives or family (Table 7.2). Time will show whether gut peptides still awaiting discovery will show homologies with these peptides.

The Multiple Phenotypes

Three decades ago, one gene was believed to encode one hormonal polypeptide in accordance with what we have learned about the master hormone, insulin. However, more intricate dimensions were added when it became obvious that a single hormone gene often expresses several different bioactive peptides. Today, we know three ways in which a gut hormone gene can express different hormonal peptides.

Alternative Splicing of Transcripts

Alternative splicing was discovered when it was shown that calcitonin gene transcription generates mRNAs encoding either calcitonin peptides or calcitonin-gene related peptides (CGRPs) [13]. CGRPs are now known also to be abundantly expressed in intestinal neurons (see [25] for review). Moreover, a tachykinin gene transcript [26] and the transcript encoded by the secretin gene [14, 15] are also spliced alternatively in the gut. For many years, secretin was believed to exist only as a carboxyamidated peptide of 27 amino acid residues [27]. However, in the mid-1980s, two additional secretins with full bioactivity were identified in porcine gut extracts. One was the immediate precursor of amidated secretin-27, glycine-extended secretin-28, and the other was secretin-30 extended by a Lys-Arg sequence. The existence of glycine- and glycyl-lysyl-arginine-extended forms of secretin and the related VIP is not surprising. They are to be expected from what is known about the biosynthesis of carboxyamidated peptides. The discovery of secretin-71 [14], which contains the sequence of nonamidated secretin-27 N-terminally, followed by a Gly-Lys-Arg extension and a further C-terminal extension of 41 amino acid residues did, however, come unexpectedly (Fig. 7.3). With the exception of an arginine residue, the C-terminal sequence of secretin-71 is identical to the C-terminal 40-amino-acid residue fragment from porcine preprosecretin. Thus, the sequence that corresponds to secretin RNA encoding a 32-amino acid sequence has been spliced out from the primary secretin gene transcript. For reasons mentioned above, secretin-71 has full secretin bioactivity.

Multiple Products of Prohormones with One Active Sequence

The somatostatin and gastrin families represent peptide systems in which the gene encodes only one prohormone that contains only one active site, but where the prohormone is processed in a way to release peptides of different lengths with the same active C-terminus. Although the different bioactive products of the same precursor are bound to the same receptor, their varying clearances from plasma affect their hormonal significance considerably. Hence, it matters whether intestinal proCCK is processed mainly to CCK-58 or to CCK-8 (Fig. 7.3), or whether prosomatostatin is processed to somatostatin-28 or -14. So far, the biosynthesis of gastrin in antral G-cells has been examined particularly thoroughly. It is, therefore, a useful illustration of the second way in which one gastrointestinal hormone gene can encode different bioactive peptides (for reviews, see [7, 28]).

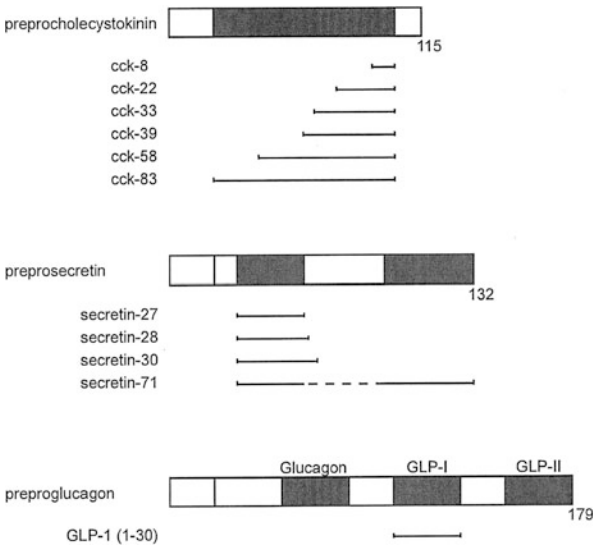

Fig. 7.3 Multiple phenotypes of three gut hormone genes. The cholecystokinin (CCK) gene encodes a prepropeptide which is processed to six CCK peptides varying in length from 83 to 8 amino acid residues through differentiated endoproteolytic cleavage. The six peptides have the same C-terminal bioactive octapeptide sequence. The secretin gene encodes a prepropeptide that through endoproteolytic cleavages and variable C-terminal trimming is processed to three bioactive secretin peptides of almost similar size (secretin-27, -28, and -30). In addition, bioactive secretion-71 is produced by splicing out RNA, encoding the midsequence of preprosecretin (i.e. broken line of secretin-71). The glucagon gene encodes a prepropeptide that through cell-specific endoproteolytic cleavages is processed to either genuine pancreatic glucagon (in pancreatic α-cells) or to glucagon-like peptides I and II (GLP-I, GLP-II) [7]

Differential Processing of Prohormones Containing Two or More Active Sequences

A third way in which one gene can express different bioactive peptides occurs when the gene encodes a propeptide containing different but often homologous peptide hormones or neuropeptides. Gastrointestinal hormones and neuropeptides comprise many examples of such genes of which the opioid-peptide genes, some of the tachykinin genes, the VIP gene, and the glucagon gene amply illustrate the phenomenon. Some of the genes not only encode a peptide precursor containing different bioactive peptides, which is then subjected to tissue-specific posttranslational processing, but the primary transcripts of these gene(s) may also undergo tissue-specific alternative splicing [26].

Proglucagon is an example of a poly-protein precursor that contains three similar but still different peptide sequences in mammals (Fig. 7.3). In pancreatic islet α-cells, proglucagon is processed to release the well-known pancreatic glucagon, whereas the C-terminal part of proglucagon remains silent in that neither GLP-I nor GLP-II is synthesized [29, 30]. The L-cells of the gut also express proglucagon but

process it in a different way to release GLP-I and GLP-II [29, 31]. Although glucagon and, for instance, GLP-I are highly homologous peptides, and both are glucoregulatory, they have separate activities and receptors. Proglucagon also tells another story of interest. Deduction of its structure from cloned cDNA provided the first evidence or suggestion of separate bioactive peptide moieties from the same precursor due to the homologies between the sequences 33–61, 72–107, and 126–158 [32]. Physiological studies, however, showed that the first deduced GLP-I (proglucagon 72–107), which is situated between two dibasic sites in the precursor, is a poorly active peptide. Instead, a truncated form of the original GLP-I, which corresponds to the proglucagon sequence 78–107, turned out to be a highly potent peptide [31, 33]. Thus, bioactive peptide structures cannot be predicted from cDNA and precursor sequences. This also requires exact identification of the released peptides accompanied by physiological studies of their activities.

Widespread Gene Expression

For gastrointestinal hormones, the expression cascade is elaborate and involves multiple processing enzymes with cleavages and derivatizations. Each step may control whether the initial gene transcription results in a bioactive peptide product. Transcription can occur without translation of the transcript, and lack of parallelism between mRNA, propeptide, and the mature bioactive peptide has been described (for review, see [34]). Hence, gene expression in "new" sites in the body requires specification of the sense in which the term expression is meant.

All gut hormones are widely expressed in tissues outside the gastrointestinal tract. For some, the extraintestinal expression is confined mainly to neurons and endocrine cells, especially neurons in the central and peripheral nervous systems. However, several gastrointestinal hormones are also expressed in other cell types and tissues. The literature on extraintestinal expression of gut hormones has become overwhelming. Therefore, the phenomenon will be described for a single hormonal system only (gastrin), which may serve as an example.

The gastrin gene is expressed in several other cell types than the antroduodenal G-cells. Quantitatively, these other cells release only little gastrin to blood in normal organisms since the extra-antral secretion seems to serve local purposes. Besides that, biosynthetic processing is often so different that bioactive gastrins may not even be synthesized. So far, extra-antral expression of progastrin and its products has been encountered in the distal small intestinal and colorectal mucosa [35], endocrine cells in the fetal and neonatal pancreas [36, 37], pituitary corticotrophs and melanotrophs [38, 39], hypothalamopituitary [40] and vagal neurons [41], and human spermatogenic cells [42].

The meaning of extraintestinal synthesis of gastrointestinal hormones is often unknown, but some suggestions can be offered. Local growth regulation is the first possibility. Secondly, it is possible that the low concentration of peptides is without significant function in the adult, but is a relic of a more comprehensive fetal

synthesis. A third possibility is that the low cellular concentration reflects constitutive secretion where the peptides are not stored in secretory granules.

Cell-Specific Prohormone Processing

Gastrointestinal hormone genes and prohormone structures are often so complex and the posttranslational processing so elaborate that the phenotypic result of gene transcription is unpredictable. Hence, the cellular equipment with processing enzymes and their necessary cofactors determine the structure of the particular prohormone product. This cell-specific processing of prohormones applies to all gastrointestinal hormones. But again, gastrin is also one of the most extensively studied gastrointestinal hormones with regard to cell-specific prohormone processing.

Almost every tissue in which progastrin is expressed has its own characteristic processing pattern. Four different patterns are shown in Fig. 7.4. For members of the gastrin family the processing varies with respect to endoproteolytic processing and with respect to amino-acid derivatizations such as tyrosyl sulfations and phenylalanyl amidations. In this context it is worth realizing that the different types of processing my influence each other, presumably by changing the affinity for the various processing intermediates as substrate for the processing enzymes. Thus, tyrosyl sulfation, the earliest posttranslational modification for the gastrin family of prohormones, increases endoproteolytic cleavage efficiency [43], and as endoproteolytic cleavage efficiency increases, so does C-terminal amidation process efficiency.

Cell-Specific Peptide Release

To understand the specific effects of the gastrointestinal peptides, it is necessary to realize that the different types of cells that express the respective genes also release the peptides in different ways. Secretion of gastrointestinal hormones was supposed to be endocrine only, until 30 years ago. But today, three alternative routes of secretion to neighboring cells and one to the secretory cell itself have been discovered (Fig. 7.5). Firstly, the peptides synthesized in neurons are released from synaptosomal vesicles in the nerve terminals to the receptors of adjacent target cells as neurotransmitters. In addition, it is possible that a spill-over of gut hormonal peptides released from peripheral neurons may be transported via blood, analogous to other extraintestinal neuropeptides. It is also possible that some peptidergic neurons expressing gut hormonal peptides, such as hypothalamo-pituitary neurons, release the peptides directly to blood vessels as neurocrine secretion. Secondly, it has been shown that there are specific paracrine cells that release, for instance, somatostatin in the gastrointestinal mucosa [44]. These cells

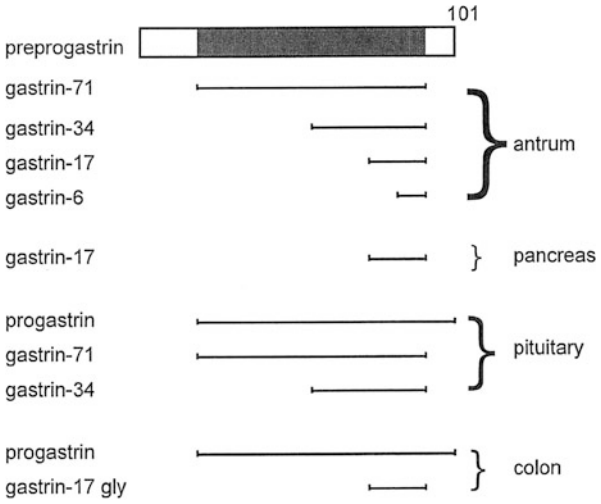

Fig. 7.4 Schematic illustration of cell-specific processing of preprogastrin in antral G-cells, G-cells in fetal and neonatal pancreas, in pituitary corticotrophic cells, and in unidentified cells in the colorectal mucosa [7]

carry peptidergic granules through cytoplasmic extensions to specific target cells in the neighborhood. Paracrine cells can be considered as hybrids of classical endocrine cells and neurons. It is, therefore, possible that a local spillover of peptides from paracrine cells may also reach the circulation.

Cells stimulate their own growth through autocrine secretion. Trophic peptides bind to specific receptors in the membranes of cells in which they are also synthesized (Fig. 7.5). Autocrine secretion is supposed to play a decisive role in tumor and cancer development [45–47]. There is, for instance, evidence to suggest that the growth of certain cultured bronchial carcinoma cells [48], pancreatic tumor cells [49] and gastric and colon cancer cells [50, 51] are stimulated by autocrine secretion of gastrin, and that growth of certain human pancreatic cancer cell lines is stimulated by gastrin and CCK peptides [52].

Cellular release of gastrointestinal peptides also occurs in a fifth way (Fig. 7.5). Spermatogenic cells in mammals express the gastrin, CCK, and PACAP genes [42, 53, 54]. The gastrin and CCK peptides are fully carboxyamidated and, like PACAP [55], concentrated in the acrosome. In accordance with the acrosomal reaction, the peptides are released from the spermatozoon by contact with the jelly-coat of the egg and subsequently bound to receptors in the egg membrane. Defects of the reproductive functions have now been found in PACAP-deficient mice [55].

Acrosomal release may prove an important mechanism of secretion for gut peptides if fertilization of the egg turns out to require such peptides. The release of bioactive peptides from acrosomal granules could be termed *spermiocrine* release (Fig. 7.5).

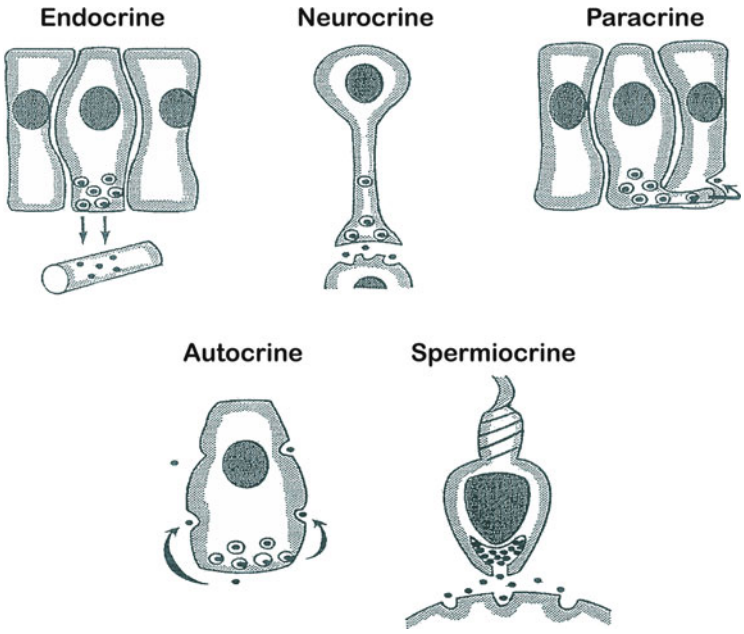

Fig. 7.5 Different types of cell-specific release of regulatory gut peptides: (1) endocrine release to capillaries from classic endocrine cells in the gastrointestinal mucosa; (2) neurotransmitter release from central or peripheral neurons to the synaptic cleft; (3) paracrine release to neighboring cells through short cellular processes; (4) autocrine release to receptors on membrane of same cell that synthesizes and releases the peptides; and (5) spermiocrine release from acrosomal granule of spermatozoa to receptors on egg cell membranes [7]

General Features of Gut Hormone Targets

Target Cells

The molecular targets of gastrointestinal hormones are specific G-protein coupled receptors expressed on a variety of cell membranes in the body. Many of the target cells are located in the gastrointestinal tract: Neurons (including the coordinating myenteric and submucosal nerveplexes); other endocrine gut cells; smooth muscles; secretory cells that release enzymes, amines, acid and bicarbonate etc. The hormonal control of intestinal target cells ensure that digestion, cellular growth turnover and motility of the gut occur in a coordinated manner in order to optimize the utilization of food and the subsequent energy delivery to the body. However, cells of many extraintestinal organs in the body also express receptors for gastrointestinal hormones, which teleologically may provide a reason for the occurrence of hormonal peptides from the gut in general circulation. These extraintestinal organs include for instance endocrine glands (the pituitary; thyroid C-cells; parathyroid glands; islets of Langerhans etc.); the liver; the gallbladder; the pancreas;

the cardiovascular system and the lungs. At low-level most other tissues in the body also express gut hormone receptors, the significance of which is still largely unknown. Finally, many gastrointestinal and extra-intestinal cancers express promiscuously the genes of both gut hormones and their receptors whereby these cancers are often equipped with local autocrine growth promoter mechanisms [56]. The known target functions of each gastrointestinal hormone are outlined in Tables 7.1 and 7.2.

Receptors

The receptors for gut hormones are as mentioned of the G-protein coupled or rhodopsin-like type with seven transmembrane loops. The amino acid chain is often heavily derivatized at for instance phosphorylation and glycosylation sites. Receptors structurally identified so far are listed in Table 7.3 in relation to their specific hormonal ligand. The relationship is often complex because a specific ligand may be bound to several receptors.

The detection of G-protein-coupled receptors in normal tissues is difficult since the number of receptors in normal target organs that is necessary to elicit a functional effect is small, compared, for instance, to the large amount of hormones synthesized in comparable sites. Therefore, the detection methods for receptors are limited and need to be critically evaluated. Several different in vitro techniques have been used to detect G-protein-coupled receptors: measurement of receptor mRNA by PCR techniques is a widely used way to assess receptors in normal and tumor tissue, with the limitations, however, that it is not the receptor protein that is detected and that he morphological correlate is missing (except for in vitro hybridization techniques). The lack of morphology and the high sensitivity of mRNA measurement by PCR imply that small amounts of normal cells expressing the receptors (blood vessel cells, immune cells, endocrine cells, connective tissue, and neurons etc.) may suggest receptor expression of the main target cells present in an organ. Since most tissue samples are highly heterogeneous from a cellular point of view, it is better to use a morphological method for receptor analysis. It is also preferable to detect the receptor protein itself, and if possible, the receptor-binding sites in these proteins, since the binding sites represent the functional molecular basis for peptide hormones [56]. A "gold standard" example is in vitro quantitative somatostatin receptor autoradiography on frozen tissue sections that combines morphology, binding site detection and receptor quantification. Because of limited cellular resolution, receptor autoradiography is optimal for the detection of receptors in larger cell groups. An attractive morphological alternative is immunohistochemical analysis of the receptors on formalin-fixed tissues [57–59] with the limitations that quantification is not possible and that an epitope that may be different from the binding site is identified. The existence of receptor subtypes for G-protein-coupled receptors has made the evaluation of the receptor profiles more complex.

In principle, all the mentioned methods are capable of detecting receptor subtypes. Unfortunately, antibodies raised against the known G-protein-coupled receptors and their subtypes rarely have the necessary reliability for immunohistochemical detection, i.e. the necessary specificity, affinity and titer. Nevertheless, adequate antibodies against the somatostatin receptor, the sst_2 and possibly also sst_5, are now available [59–61], and that is a major progress that eventually may occur also for antibodies to the other hormone receptors [62, 63].

Perspective

Gastrointestinal endocrinology has developed from an appendix of general endocrinology to a biological discipline of its own over the last 40 years. Today it comprises a multitude of more than 100 bioactive peptides expressed in a controlled cell-specific manner all over the body. The peptides participate in intercellular regulation from local control of growth and cell differentiation to acute systemic effects on metabolism all over the body. Thus, in the early 1970s, a revolution changed the fundamental concepts and opened wide perspectives for gastrointestinal hormones in physiology and pathophysiology.

Gastrointestinal peptide hormones must be viewed as evolutionarily conserved intercellular messengers of general significance. There are no obvious boundaries between their role in food intake and digestion and their function in other bodily regulations. Most regulatory peptides (hormones, neuropeptides, growth factors, and cytokines) are probably expressed in the gut, at least at some stage in the phylogenetic or ontogenetic development. Hence, the development of gastrointestinal endocrinology may continue its exponential growth with a broad definition of regulatory peptides. On the other hand, such extension almost deprives the concept of *gastrointestinal* endocrinology of its meaning. And that is exactly what this is all about: Gastrointestinal hormones should be viewed not only as local hormones of specific interest to digestive physiologists and clinical gastroenterologists. They are integrated chemical messengers in the coordination and regulation of many or most bodily functions in mammals. Thus, it is not surprising that today gut hormones are studied not only in physiology and cell biology, but also by microbiologists, psychiatrists, zoologists, cardiologists, diabetologists, and others.

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