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The New Biology of Gastrointestinal Hormones

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Rehfeld, Jens F. The New Biology of Gastrointestinal Hormones. *Physiol. Rev.* 78: 1087–1108, 1998.—The classic concept of gastrointestinal endocrinology is that of a few peptides released to the circulation from endocrine cells, which are interspersed among other mucosal cells in the upper gastrointestinal tract. Today more than 30 peptide hormone genes are known to be expressed throughout the digestive tract, which makes the gut the largest endocrine organ in the body. Moreover, development in cell and molecular biology now makes it feasible to describe a new biology for gastrointestinal hormones based on five characteristics. *1*) The structural homology groups the hormones into families, each of which is assumed to originate from a common ancestral gene. *2*) The individual hormone gene is often expressed in multiple bioactive peptides due to tandem genes encoding different hormonal peptides, alternative splicing of the primary transcript, or differentiated processing of the primary translation product. By these mechanisms, more than 100 different hormonally active peptides are produced in the gastrointestinal tract. *3*) In addition, gut hormone genes are widely expressed, also outside the gut. Some are expressed only in neuroendocrine cells, whereas others are expressed in a multitude of different cells, including cancer cells. *4*) The different cell types often express different products of the same gene, "cell-specific expression." *5*) Finally, gastrointestinal hormone-producing cells release the peptides in different ways, so the same peptide may act as an acute blood-borne hormone, as a local growth factor, as a neurotransmitter, and as a fertility factor. The new biology suggests that gastrointestinal hormones should be conceived as intercellular messengers of general physiological impact rather than as local regulators of the upper digestive tract.

I. INTRODUCTION

Gastrointestinal hormones are by all criteria ordinary hormones. Nevertheless, they have never been fully accepted in endocrinology. Endocrinology textbooks and general endocrinology meetings witness how gastrointestinal hormones often occupy only little room in today's concept of endocrinology. The restrained acceptance is a paradox in three ways. *1*) Endocrinology was conceptually put on a firm scientific footing in the gut with the discovery of secretin (12, 13) and with the subsequent introduction of the word *hormone* (227). *2*) The gut is the largest hormone-producing organ in the body, both in terms of number of endocrine cells and number of hormones (for comprehensive reviews, see Refs. 211 and 251). *3*) The widespread expression of gastrointestinal hormone genes outside the gastrointestinal tract makes the hormones multifunctional regulators of general physiological interest. Hence, gastrointestinal hormones may at the same time act as acute metabolic hormones, as neurotransmitters, as local growth factors, and as fertility factors. This widespread gene expression with tissue-specific prohormone processing combined with differential functions constitutes essential parts of what may be conceived as a new and fascinating biology, which should appeal also to general endocrinology and physiology.

The basic concept of endocrinology, blood-borne regulation by specific messenger molecules, was discovered in 1902 by the British physiologists William Maddox Bayliss and Ernest Henry Starling (12, 13). Following up on the observation of Pavlov and co-workers, that acidification of the upper intestine resulted in secretion of pancreatic juice (for review, see Ref. 178), Bayliss and Starling extracted from the duodenal mucosa a substance, which

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injected into blood stimulated pancreatic bicarbonate secretion irrespective of whether the pancreas was innervated or not. They called the substance secretin, a somewhat unspecific designation. However, in 1905, Starling in his Croonian lecture proposed the word *hormone* as a general designation for blood-borne chemical messengers (227). That same year, John Sidney Edkins, also from University College in London, discovered another hormonal substance in extracts of the antral mucosa (67, 68). This substance stimulated gastric acid secretion. In view of its origin, Edkins called it "gastric secretin," but abbreviated it to gastrin. Hence, the two hormones first discovered in history were both gastrointestinal hormones. In the following decades, however, endocrinology as such blossomed by the discovery and isolation of steroid hormones from the adrenals, ovaries, and testes; larger protein hormones from the anterior lobe of the pituitary and oxytocin and vasopressin from the posterior pituitary lobe; and insulin from the pancreatic islets. In light of the immediate and often life-saving implications of these breakthroughs, the interest for secretin and gastrin faded to a degree that figuratively returned them to the darkness of the bowel. Subsequently, only a small priesthood of physiologists maintained an interest in the hormonal control of digestion. One of them was Andrew Ivy in Chicago, who in 1928 assisted by Eric Oldberg found evidence of a gallbladder-emptying hormone in extracts of the small intestine (115). He called the substance cholecystokinin (CCK). A stimulator of pancreatic enzyme secretion, termed pancreozymin, was subsequently discovered in the 1940s in small intestinal extracts by Harper and Raper in Newcastle (99). However, as shown in the 1960s by Erik Jorpes and Viktor Mutt in Stockholm, CCK and pancreozymin are one and the same substance (124, 165), for which now only the acronym CCK is used.

Secretin, gastrin, and CCK are the classical gut hormones. The troika was not only discovered first but structurally identified first (91, 93, 165–167). It was also by many believed that the endocrine regulation of digestion might be excreted only by these three hormones. A leading gastrointestinal physiologist in the 1960s and 1970s, Morton Grossman, even suggested that they acted via the same receptor (95). The trinity doctrine soon, however, turned out to be incorrect. There are many more gut hormones, and each has its own or even more receptors, although, vice versa, there are also examples showing that different gastrointestinal peptides may act on the same receptor. This complexity is also part of the new biology. However, so as not to lose sight in the avalanche of new information, the general and fundamental characteristics of the new biology are primarily exemplified with data for the classical gastrointestinal hormones. Moreover, only the molecular and cellular biology of the gastrointestinal hormones as such is reviewed. Neither transport mechanisms, receptors, nor signal transduction in target cells are discussed here. In other words, this review attempts to present general principles governing structure and expression of gastrointestinal hormones. Readers interested in details about individual hormones and their effects should consult comprehensive multi-author volumes comprising the entire range of gastrointestinal endocrinology (211, 251) or the many volumes about individual gut hormones published in the 1990s. Before the characteristics and principles of the new biology of gastrointestinal hormones are presented, a summary of the old concept may be pertinent.

II. THE CLASSIC CONCEPT

The old concept about gastrointestinal hormones, which prevailed unaffected through the century until the late 1970s, still dominates most general textbooks in physiology, biochemistry, and endocrinology. According to the classic biology, a gut hormone is a substance produced by one type of endocrine cell dispersed in a relatively well-defined region of the proximal gastrointestinal tract. From here it is released to blood by a specific stimulus to reach its target organ that subsequently elicits an acute response (secretion or muscle contraction).

In the 1960s, it was shown that gastrointestinal hormones could be peptides of some 20–30 amino acid residues. Hence, secretin, a 27-amino acid peptide, is released by gastric acid from S cells in the duodenum to stimulate bicarbonate secretion from exocrine pancreatic cells. Gastrin, a 17-amino acid peptide, is by protein-rich food in the stomach released from antral G cells to stimulate acid secretion from parietal cells in the fundic mucosa. And CCK, a 33-amino acid peptide, is by fat, protein, and acid released from small intestinal I cells to stimulate pancreatic enzyme secretion and gallbladder contraction. On the basis of simple physiological studies mainly in dogs, a number of additional gastrointestinal hormonal mechanisms were proposed in the first half of this century and as candidate hormones named according to function and origin (incretin, enterogastrone, duocrinin, antral chalone, villikinin, and others). However, when the classic troika of hormones became available as pure peptides in the late 1960s and early 1970s, proper experimentation showed that several of the observed hormonal mechanisms could be explained either by interaction and/or additional effects of secretin, gastrin, and CCK or by newly identified gut hormones.

The new biology of gastrointestinal hormones maintains in accordance with the classic conceptions that the hormones are peptides, which are released to blood from cells in the gastrointestinal tract upon appropriate stimulation. However, the new biology contains a wealth of additional features revealed by modern molecular and cell biology. These features have been collected under five headings in this review.

For the sake of completeness, it should be added here that a number of monoamines (including histamine and dopamine) and various eicosanoids with hormonelike activities also are produced in the gastrointestinal tract. This review is, however, restricted to proper gut hormones, which are peptides. It should also be emphasized that the designations gastrointestinal hormones and gut hormones are used synonymously in this review.

III. THE NEW CONCEPTS

A. Many Hormones and Their Families

Gastrointestinal endocrinology has since 1970, when it was confined to only three identified peptides (91, 166, 167), virtually exploded in number of regulatory gut peptides, i.e., hormones, peptide transmitters, and growth factors (Fig. 1). Not only have new peptides with all features of a hormone been found in gut extracts [gastric inhibitory polypeptide (GIP), Refs. 32, 34, 36; motilin, Refs. 31, 33, 35, 210; peptide tyrosine tyrosine (PYY), Refs. 236, 237; galanin, Refs. 72, 238; glucagon-like peptides (GLPs), Refs. 16, 109, 176, 177, 234, 239, 245], but also neuropeptides isolated from the central nervous system and hormones identified first in other endocrine organs have been found to be present in endocrine cells and/or neurons in the gastrointestinal tract [substance P, Refs. 43, 46, 71; the enkephalins, Refs. 73, 113; dynorphin, Ref. 224; neurotensin, Refs. 42, 98, 130; neuropeptide Y (NPY), Refs. 157, 232; the neurokinins, Ref. 55; pituitary adenylate cyclase-activating peptide, Refs. 158, 231; somatostatin, Refs. 8, 29, 47; pancreatic polypeptide (PP), Refs. 45, 129; and calcitonin gene-related peptide (CGRP), Refs. 2, 3, 164, 205, 241]. Moreover, potent regulatory peptides originally believed to be classical hormones but later shown to be widespread neurotransmitters have been isolated from gut extracts [vasoactive intestinal polypeptide (VIP), Refs. 137, 168, 207; peptide histidine isoleucine, Refs. 20, 114, 148, 237; and gastrin-releasing peptide (GRP), Refs. 153, 154, 156, 160]. Finally, a number of growth factors with hormonal effects have now been shown to be present in the gut: epidermal growth factor (EGF), originally isolated as a gut hormone; urogastrone, from urine independently of Cohen's EGF identification (49, 50), the isolation being monitored by its effect on gastric acid secretion by Harold Gregory (90); insulin-like growth factor I and II (30, 56, 209, 229); transforming growth factor (TGF)-α and -β (14, 44, 134, 250); and amphiregulin (182).

The complexity is further increased by the fact that individual genes for gut regulatory peptides encode different peptides, which in a tissue- and cell-specific manner release a number of different bioactive peptides. 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 (3). Also, the secretin gene is expressed in different molecular forms in the gut because of alternative splicing (84, 131).

Additional features contributing to the plurality are physiological studies which have indicated that there still are gut hormonal activities that are not easily explained by known peptides and, therefore, require identification of new hormones. Hence, as shown in Figure 1, 10 hormonal factors are still awaiting structural determination of both peptides and genes. Perhaps some of them can be partly explained by identified peptides. Hence, the incretin effect (135, 155) is probably exerted by a combination GIP (66) and GLP-I (54, 109) stimulations, whereas the entero-, vago-, and bulbogastrone effects may be explained by various combinations of somatostatin, GIP, EGF, and TGF-α. However, villikinin, duo- and enterocrinins, and the recently suggested gastrocalcin (181) still await a structural identification of new substances, most probably peptides.

It may be difficult to overlook the multiplicity of the gut peptide systems. The structural identifications, however, have simplified the matter by showing striking homologies between groups of peptides. Consequently, one-half of the hormones can be classified in families based on homology. Table 1 shows the major gastro-entero-pancreatic hormone families. The expression of several peptide genes both in the gut and the pancreas reflects that the pancreas is of intestinal origin, both in onto- and phylogenetic terms.

The nature of the homology varies from family to family. It may be an overall similarity in the primary structure as illustrated by the PP-fold family, which comprises PP, PYY, and NPY (Table 1). The family members display similarities varying between 45 and 70% (Fig. 2). The extensive similarity of the primary structures is coupled to an almost identical and stable tertiary structure because the homology explicitly comprise residues, which are important for stabilization of the three-dimensional PP-fold structure (88). The PP-fold motif consists of a polyproline-like helix (residues 1–8) and an amphiphilic α-helix (residues 15–30). The two helixes are joined by a type I β-turn (residues 9–12) and held in the folded configuration by hydrophobic interdigitations between side chains of the α-helix residues and the NH_2-terminal proline residues (Fig. 2). Not only are the bioactive 36-amino acid peptides in the family highly homologous, the cDNA-deduced prepropeptides also display remarkable similarities in their organization (21, 147, 157).

Another type of homology is that of the gastrin family (Table 1 and Fig. 3). The family comprises, in addition to the mammalian hormones, gastrin and CCK, also the protochordean neuropeptide cionin (120), and the frog skin peptide cerulein (4). The decisive homology of this family is concentrated in and around the precisely defined active site, the common COOH-terminal tetrapeptide am-

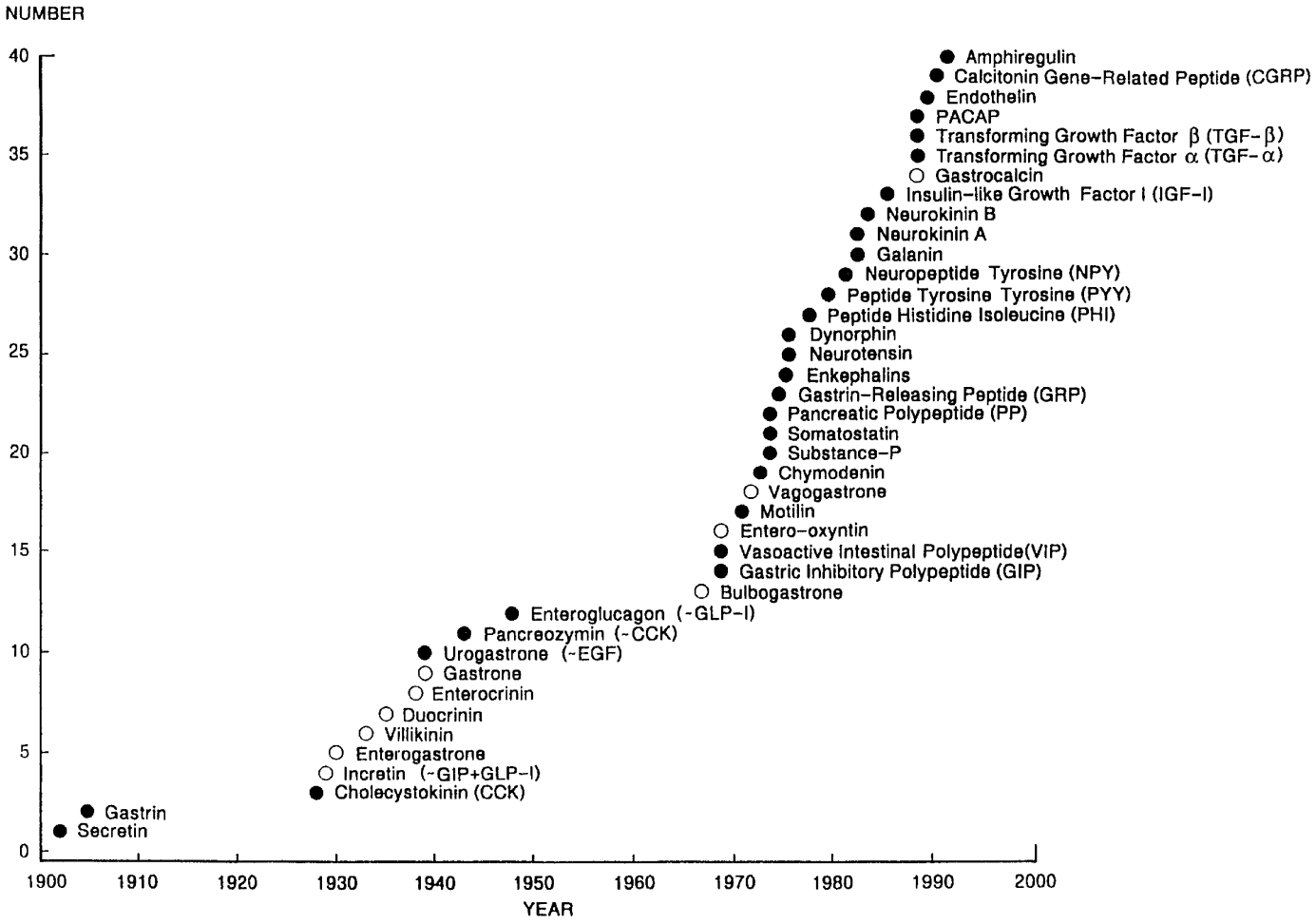


FIG. 1. Discovery and identification of regulatory peptides in gastrointestinal tract. Peptides may act as hormones, neurotransmitters, and growth factors. Sometimes 1 peptide acts in 2 or all of the 3 roles. Discovery is indicated by year of first report. Solid circles indicate structural identification, and open circles indicate hormonal activities, which still require identification of responsible hormone(s). Some of structurally unidentified hormonal activities can be partly explained by activity of later identified hormones; for instance, incretin activity is partly due to gastric inhibitory polypeptide (GIP) and glucagon-like peptide I (GLP-I) activities. Commonly used acronyms are indicated in brackets after full name, except for PACAP, which is an acronym for pituitary adenylate cyclase-activating peptide.

ide -Trp-Met-Asp-Phe-NH_2 (the box in Fig. 3). Any modification of this site grossly reduces or abolishes the receptor binding and consequently the biological effects of the hormones (162). Comparison of propeptide and gene structures reveals a complex pattern with an overall similarity in the organization of progastrin, proCCK, and procionin as well as their genes (22, 57, 58, 161, 203, 256, 257, 259), but with only little similarity in the amino acid residues and DNA sequences outside the sequence corresponding to the common active site and its COOH-terminal flanking peptide. Hence, this family is in contrast to the PP-fold family and the secretin family primarily defined by the conserved active site sequence and by the neighboring *O*-sulfated tyrosyl residues (Fig. 3).

The frequent occurrence of homology among gastrointestinal hormones, peptide neurotransmitters in the gut, and intestinal growth factors is not a feature specific for regulatory peptides in the gut. On the contrary, it is common among all kinds of regulatory peptides, enzymes, and other proteins in the organism (for reviews, see Refs. 1, 64, 65, 118, 260). Each family is assumed to reflect the phylogenetic evolution by duplication and subsequent mutations of an ancestral gene. It is, however, possible that the homology not only reflects divergent evolution. The occurrence of homologous peptides in submammalian species may also demonstrate the existence of several related genes, of which some do not evolve into genes of mammalian species or of other vertebrates.

The question of phylogenetic origin has recently been examined in detail for the gastrin family by Johnsen (118). His study tested an immunochemically based hypothesis suggesting that CCK and gastrin originate from a single common ancestral gene, which during evolution duplicated into separate CCK and gastrin genes

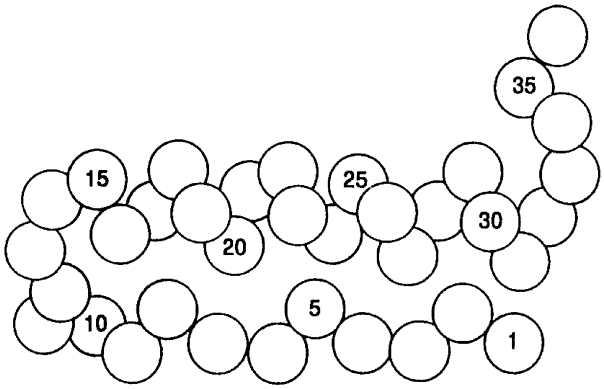
TABLE 1. *Gastroenteropancreatic peptide families*

Secretin family	*Gastrin family*
Secretin	Gastrin
* Glucagon and glucagon-like peptides	Cholecystokinin
Gastric inhibitory polypeptide	Cerulein†
* Vasoactive intestinal polypeptide and Peptide histidine isoleucine	Cionin†
Growth hormone releasing hormone	*PP-fold family*
Pituitary adenylyl cyclase-activating peptide	Pancreatic polypeptide
	Peptide YY
Insulin family	Neuropeptide Y
Insulin	*Tachykinin family*
Insulin-like growth factor I	Substance P
Insulin-like growth factor II	Neurokinin A
Relaxin	Neurokinin B
EGF family	*Somatostatin family*
Epidermal growth factor	Somatostatin
Transforming growth factor-α	Corticostatin
Amphiregulin	

* Peptides encoded by one gene. † Not present in mammals.

at the level of reptiles (140). Identification of peptides from brain and gut tissues of species representing the entire animal kingdom (including invertebrates) showed, however, the following: so far it has not been possible in invertebrates to identify true CCK-/gastrin-like peptides having an intact COOH-terminal tetrapeptide amide (Fig. 3), although invertebrate neurons may express a family of less related -Asp-Phe-NH_2 peptides (122). Possibly, however, the gastrin family may represent a subclass of a larger DFamide family. The earliest occurrence of true gastrin/CCK peptides in evolution is apparently cionin as expressed in protochordean neurons (120; Fig. 3). Procionin, the cionin gene, and its expression pattern resemble mammalian CCK rather than gastrin (161). So far, therefore, the gastrin family appears to originate in protochordates with expression of the CCK-like cionin gene at the evolutionary level, where vertebrates branch off from invertebrates ~500 million years ago or earlier. In cartilaginous fish or elasmobranchs (the earliest animals to secrete gastric acid), the CCK-like gene has duplicated to express two very similar peptides of which one is likely to regulate gastric acid secretion (119). Two such CCK-like peptides are also expressed in the gut of bony fish, amphibians, reptiles, and birds (18, 121). Only mammals express gastrins with a structure that differs grossly from CCK outside the active site sequence (22, 118, 259).

The phylogenetic story of the gastrin family shows that gastrointestinal hormones indeed are very old. So far, the data also support the idea that each gut peptide family has evolved from a single ancestor. An associated trait is that gastrointestinal hormones, at least in the gastrin family, to a large degree have preserved their tissue-specific



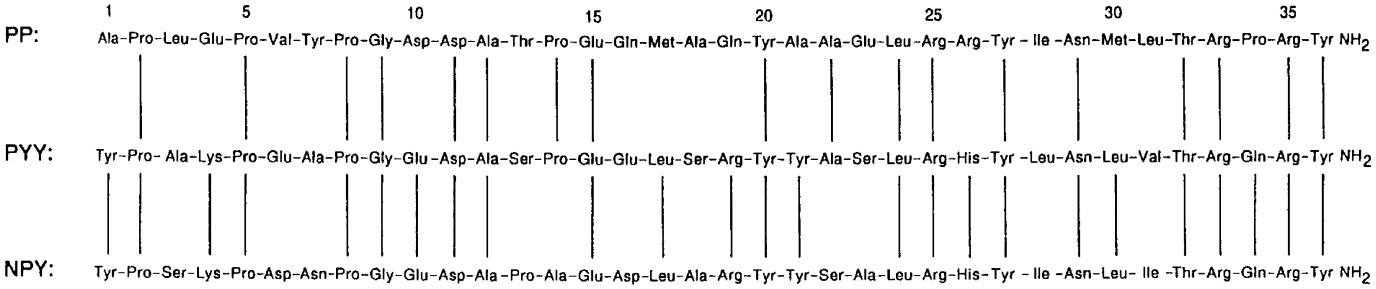


FIG. 2. Structural homology of members of pancreatic polypeptide (PP)-fold family: PP, peptide tyrosine tyrosine (PYY), and neuropeptide tyrosine (NPY). *Top*: PP-fold configuration (tertiary structure) of the three 36-amino acid peptides. *Bottom*: amino acid sequences (primary structure) of the three porcine peptides.

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