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THE NEUROENDOCRINE SYSTEM OF THE GUT

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Abstract

The digestive tract is the richest source of regulatory peptides outside the brain. Such peptides occur all along the gut in the neuroendocrine system which is composed of endocrine/paracrine cells disseminated in the epithelium and of intrinsic neurons that form continuous ganglionic chains in the submucosa and in the muscle layer. Some endocrine/paracrine cells, particularly in the stomach, still have not been associated with an identified regulatory peptide implying that our present knowledge is far from complete. The intracellular processing of regulatory peptide precursors involves multi-step proteolytic cleavage generating several fragments. In many instances more than one biologically active peptide is generated from one and the same precursor. In addition, certain endocrine/paracrine cells and neurons have been found to produce more than one peptide precursor and some are known to harbour 'classical' neurotransmitters, such as 5-hydroxytryptamine, histamine and GABA as well as regulatory peptides. Key questions for the future are the functional significance of the coexistence of multiple messengers within the same cells and the details of how the endocrine/paracrine cells and the neurons in the gut interact.

Key words: Gut hormones, endocrine cells, regulatory peptides, enteric neurons.

The gut is probably the richest source of biologically active peptides outside the brain. The rapid increase in the number of identified gut peptides over the last decades (Fig. 1) is mainly the result of methodological advances within the fields of biochemistry and molecular biology.

Up till 1980 most regulatory peptides were isolated and identified by virtue of their biological actions, which were used to monitor their purification. The fact that many of these peptides had amidated C-terminal residues led Mutt and coworkers to screen gut and brain extracts for peptides with this property (1–3). So far, this screening method has resulted in the discovery of peptide histidine isoleucine (PHI), peptide YY (PYY), neuropeptide Y (NPY) and galanin, all of which are present in the gut. Each peptide is produced from a large precursor molecule. During the last years molecular biologists have identified the precursors of a great number of gut peptides. Some newly reported precursors are illustrated in Fig. 2. Each

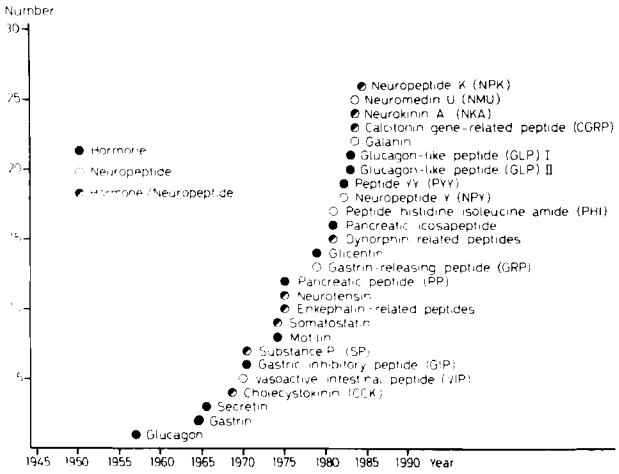

Fig. 1. Diagram listing identified regulatory peptides in the gut according to the year in which their amino acid sequence was published. Despite the fact that many of the gut hormones were discovered early in the century they were not obtained in pure form (to be sequenced) until decades later. The reasons for the difficulty in purifying them is their low tissue concentration and the presence of proteolytic enzymes in the tissues. With the development of highly efficient separation techniques and greatly improved methods for amino acid sequencing the interval between discovery and chemical characterization of a regulatory peptide has become quite short. As shown in the diagram a great number of the peptides listed has a dual occurrence in endocrine cells as well as in neuronal elements.

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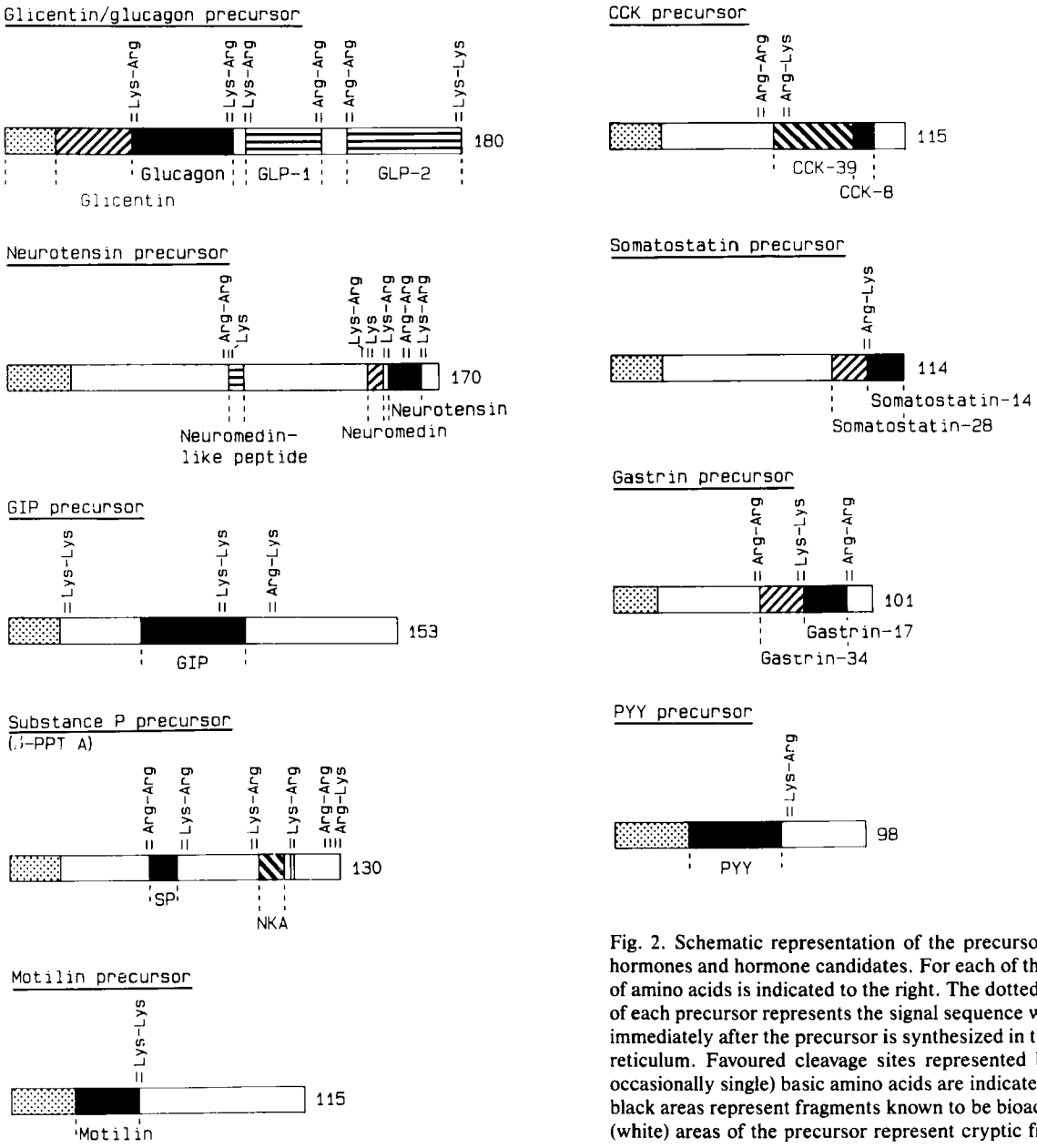

Fig. 2. Schematic representation of the precursors of some gut hormones and hormone candidates. For each of them the number of amino acids is indicated to the right. The dotted area at the left of each precursor represents the signal sequence which is split off immediately after the precursor is synthesized in the endoplasmic reticulum. Favoured cleavage sites represented by pairs of (or occasionally single) basic amino acids are indicated. Hatched and black areas represent fragments known to be bioactive. The other (white) areas of the precursor represent cryptic fragments.

precursor is processed within the cell by a series of proteolytic cleavages, preferentially at pairs of basic amino acids, to yield multiple fragments. Presently, several proteolytic enzymes thought to be involved in the precursor processing have been described (cf. 4). As a rule, processing is complete in the sense that very little intact precursor is secreted. The precursor processing, which includes secondary modifications such as glycosylation, is initiated during transport of the precursor from the endoplasmic reticulum via the Golgi apparatus to the secretory granule. Proteolytic cleavage and α-amidation are thought to occur within the secretory granules while in transit to the cell surface. Thus, by the time the granule is ready to release its content at the site of exocytosis the precursor has been replaced by a large number of proteolytic fragments (cf. 5–7).

It is not uncommon to find two or more chemically related peptide sequences in a precursor molecule. Thus, PHI and vasoactive intestinal peptide (VIP) both derive from the same precursor (8) and are structurally very similar. This is also the case with substance P and neurokinin A (9), and with glucagon and the glucagon-like peptides (GLP) I and II (10). The functional significance of the coexistence of several chemically related peptides is unknown. Apart from the predictable coexistence of e.g. VIP and PHI, SP and NKA, or glucagon and GLP I and II it has become evident during recent years that also peptides arising from different precursors may coexist (cf. 7,

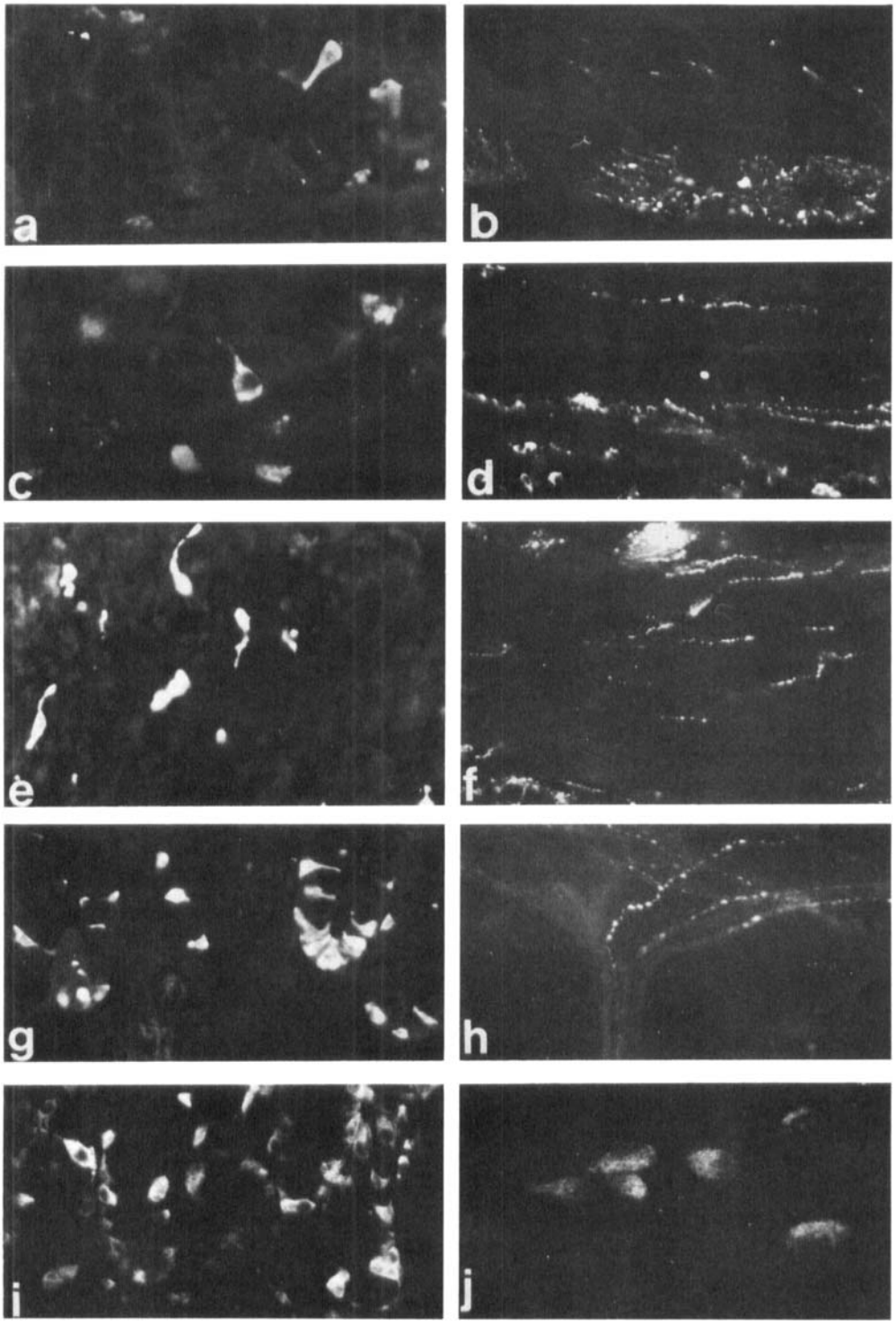

Fig. 3. Examples of the fact that the same messenger compound (peptide or amine) may occur in both endocrine cells and neurons in the gut. a) and b) enkephalin in endocrine cells and myenteric neurons in the porcine duodenum. c) and d) substance P in endocrine cells and myenteric neurons in the human ileum. e) and f) somatostatin in endocrine/paracrine cells in the oxyntic mucosa of the rat stomach and in myenteric neurons in the porcine duodenum. g) and h) 5HT in enterochromaffin cells and in myenteric neurons in the guinea pig ileum. i) and j) histamine in enterochromaffin-like (ECL) cells in the oxyntic mucosa of the rat stomach and in myenteric neurons in the rat small intestine.

Table

Organization of intercellular communication in gut and pancreas

The signal systems involved in communication			
Endocrine cells	Paracrine cells	Neurons	
The lines of communication			
Neuron ↓ Neuron	Neuron ↓ Endocrine/Paracrine cell	Endocrine/Paracrine cell ↓ Endocrine/Paracrine cell	Endocrine cell ↓ Neuron
Functional interactions			
Excitatory neuropeptide Substance P → GRP →	*Enhanced neurotransmission* Enteric cholinergic pathways Enteric cholinergic pathways	*Excitatory neuropeptide* GRP → VIP →	*Hormone released* Gastrin, CCK, Insulin Insulin
Inhibitory neuropeptide Enkephalin → Galanin →	*Suppressed neurotransmission* Enteric cholinergic pathways Enteric cholinergic pathways	*Inhibitory neuropeptide* Galanin →	*Hormone suppressed* Insulin
Excitatory hormone CCK → Gastrin → GIP →	*Hormone released* Calcitonin, Insulin Gastrocalcin Insulin	*Excitatory hormone* Motilin →	*Enhanced neurotransmission* 'Enteric' pathways
Inhibitory hormone Somatostatin →	*Hormone suppressed* Gastrin, Insulin, Glucagon	*Inhibitory hormone* PP → Neurotensin →	*Suppressed neurotransmission* 'Vagal' cholinegic pathways 'Enteric' pathways

11, 12). Thus, a major population of enteric neurons of the gut harbours both VIP and NPY (13) and some of these neurons contain in addition galanin (14, 15). Glicentin and PYY coexist in a major population of endocrine cells in the distal intestine (16, 17). It may also be mentioned in this context that certain large molecular weight proteins, such as chromogranins, have been found to be universally distributed in granules of neuroendocrine cells including those of the gut (18–21).

In addition, biologically active peptides may coexist with 'classical' neuromessengers both in endocrine cells and in neurons (cf. 22–24). This is the case in many adrenergic neurons where noradrenaline coexists with NPY (cf. 25) and in gut endocrine cells where 5-hydroxytryptamine may coexist with enkephalin (26) or substance P (27).

Cells designated for chemical communication within the gut include endocrine cells, paracrine cells and enteric neurons (cf. 22). Not only are they closely integrated in terms of function but they also seem to share a common genetic programming illustrated by the fact that they all seem capable of producing chromagranins, biologically active peptides (Fig. 1) and amines (Fig. 3).

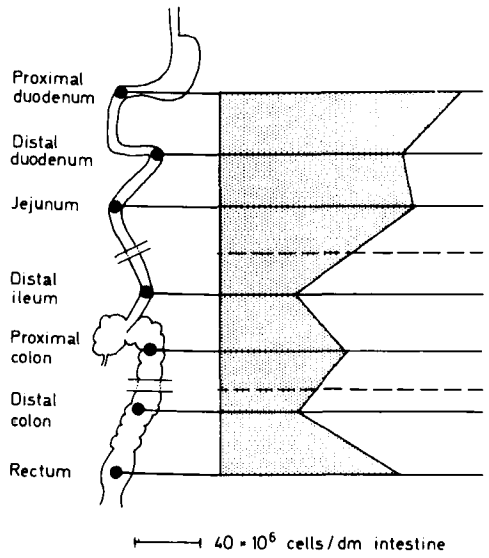

Fig. 4. The regional distribution and frequency of endocrine cells in the human intestines. The cell number is expressed per dm intestine. The data are from samples collected at positions indicated by black dots. Attempts to estimate the total number of endocrine cells in the human intestine have given a figure of about 3 billion (28).

Endocrine/paracrine cells

Endocrine/paracrine cells occur scattered in the epithelium throughout the gut (cf. 28, 29). The overall density of such cells along the human intestine is illustrated in Fig. 4. In the intestine and in the antral portion of the stomach the cells endocrine/paracrine are open, i.e. they reach the lumen via an apical process carrying numerous microvilli (Fig. 5). This probably enables the cells to respond to specific stimuli in the gut lumen, such as changes in pH and in the concentration of various nutrients. Most secretory granules are found basally in the cells. In the oxyntic mucosa of the stomach, endocrine/paracrine cells generally fail to reach the glandular lumen and it is possible that these cells react to non-luminal types of stimuli, such as

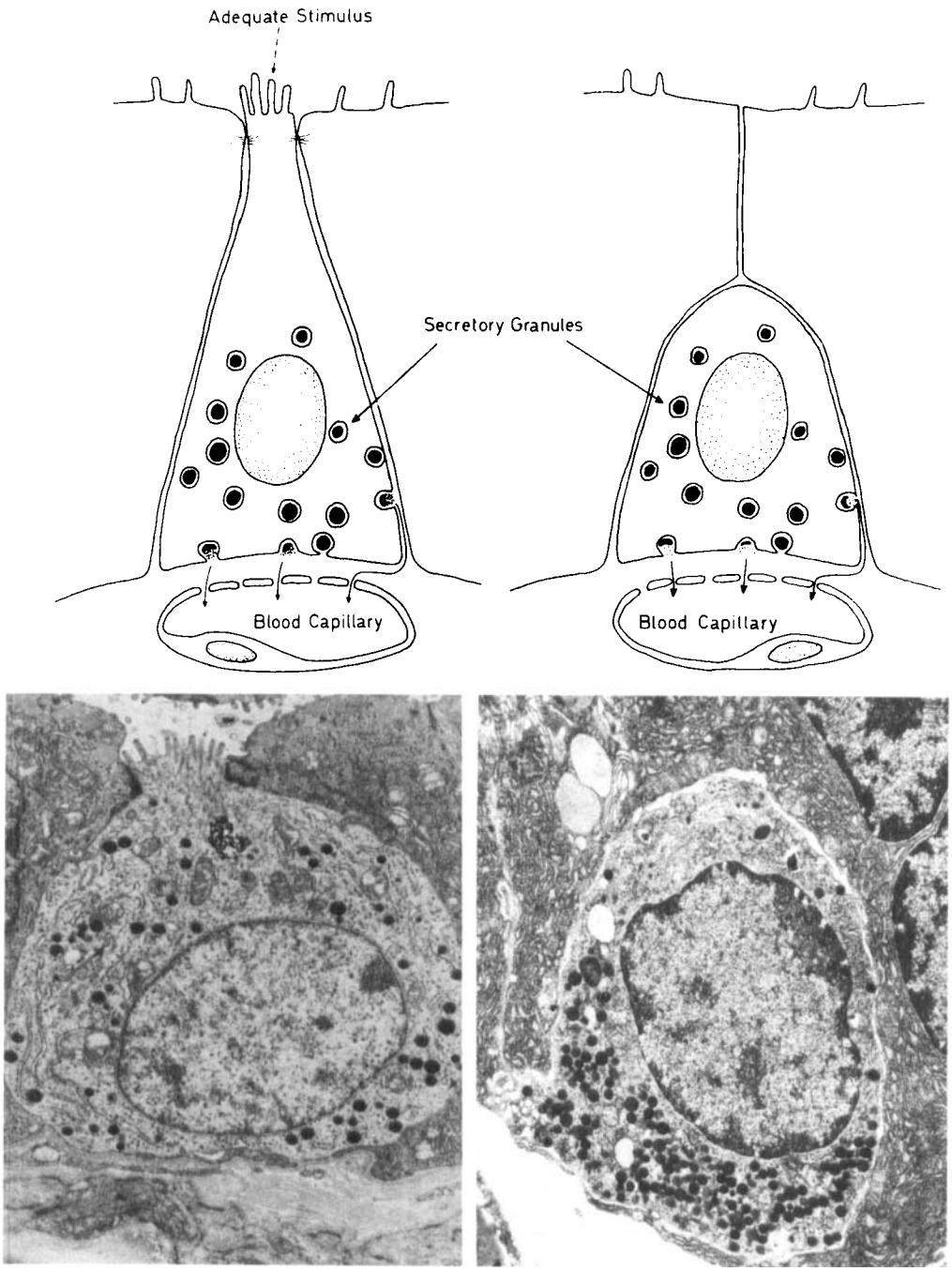

Fig. 5. Schematic drawings and electron micrographs illustrating endocrine cells of open type (left) and closed type (right). The electron micrographs show a gastrin cell in the chicken antrum (left) and an A-like cell in the oxyntic mucosa of the rat stomach (right). Open type cells reach the lumen via an apical process furnished with microvilli.

distension and temperature changes, or are under neuronal and hormonal control.

Among the first endocrine cell types to be described in the gut was the 5-HT-containing enterochromaffin cell. During the last 20 years a great number of cell types have been identified by virtue of the peptide hormones they contain. Each cell type has a characteristic distribution pattern (Fig. 6) (cf. 28, 30). A few cells e.g. the 5-HT-containing enterochromaffin cells and the somatostatin cells, occur throughout the gut. It should be noted, however, that the enterochromaffin cells probably comprise several subpopulations based on the differing ultrastructure of their secretory granules (cf. 29–31) (Fig. 7), and on their diverse chemical coding in terms of peptide content (cf. 29). However, the major populations of enterochromaffin cells have not been defined with respect to their peptide hormones and there are several other endocrine cell types (identified as such by their histochemical and

ultrastructural properties) that have had no peptide hormone ascribed to them yet. Such cells include the two predominating endocrine cell types in the oxyntic mucosa, the so-called ECL cells, and the A-like (or X) cells (cf. 32) (Fig. 8). The ECL cells contain histamine (Fig. 9) and respond to gastrin (33, 34). This response is reflected in the release of histamine and the activation of the histamine-forming enzyme, histidine decarboxylase, upon acute gastrin challenge, and in the marked hypertrophy and hyperplasia that follow a period of sustained hypergastrinemia (33, 35, 36) (Fig. 9). In contrast to endocrine cells, which deliver their messengers to the blood, paracrine cells are thought to influence their neighbouring cells by the release of locally acting messenger from cytoplasmic processes (Figs 10 and 11). These processes, which usually run along the base of the epithelium, may be of considerable length. They often end with a club-like swelling, filled with secretory granules. The somatostatin cells in the gastric mucosa are typical paracrine cells (37, 38). Admittedly the distinction between endocrine and paracrine cells is far from clear and probably cannot be based solely upon the mere presence or absence of basal cytoplasmic processes. Thus, cells which are now considered endocrine may well turn out to be paracrine and vice versa and it cannot be ruled out, in fact, that they may serve both endocrine and paracrine functions.

On the whole, gut hormones and paracrine messengers seem to be involved in the control of digestive processes, such as acid secretion, bicarbonate secretion, enzyme secretion from the pancreas and gut, gallbladder motility and local blood flow. In addition, they may have indirect effects on these processes by activating enteric neurons and/or other endocrine cells. Interestingly, there is evidence for a gastrin-dependent hormonal role of the stomach in calcium homeostasis and extracts of the oxyntic mucosa of the stomach have been found to contain peptide components that enhance calcium uptake into bone (39–41). This peptide, tentatively named gastrocalcin, may turn out to be an ECL cell hormone, keeping in mind the remarkable sensitivity of these cells to gastrin (32).

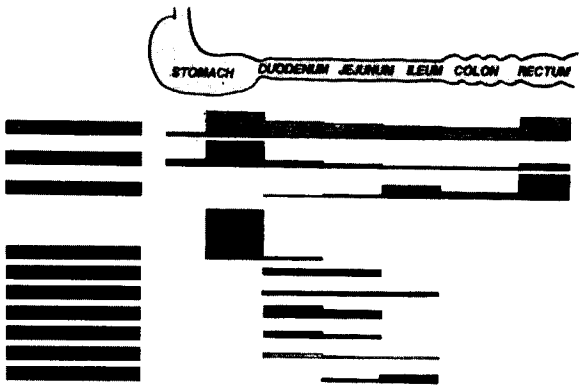

Fig. 6. Schematic diagram illustrating the distribution of the many individual populations of endocrine/paracrine cells in the antrum and intestines of man based on immunocytochemical observations. The width of the horizontal bars reflect the relative cell density. A small population of the enterochromaffin cells (5-HT containing endocrine cells) contains substance P. Besides gastrin and CCK cells a third cell population occurs in the small intestine that is recognized by antibodies against the common C-terminal part of gastrin and CCK but not by gastrin- or CCK-specific antibodies.

Enteric neurons

The nervous control of the gut is exerted primarily by an intrinsic nervous system originating in the two ganglionated plexuses, the submucosal (Meissner's) and the myenteric (Auerbach's) plexuses, that occur throughout the gastrintestinal tract (cf. 11). The enteric neurons are as numerous as those in the spinal cord and the enteric nervous system has been referred to as 'little brain'. Although characterized by a high degree of autonomy the enteric neurons are controlled by vago-sacral parasympathetic nerves, sympathetic nerves emanating in the prevertebral ganglia, and sensory nerves originating in the jugular-nodose ganglionic complex (projecting mainly to the stomach) or in the dorsal root ganglia (projecting mainly to the intestines). Sympathetic fibers can readily be demonstrated all along the gut. Fibers which are sensitive to the neurotoxic agent capsaicin and therefore regarded as sensory fibers have been demonstrated in the gut. Thus, a prominent population of CGRP- and SP-containing capsaicin-sensitive fibers occurs in the gastric mucosa (42, 43). In the intestine such fibers are found mainly around blood vessels and occasionally in the intramural ganglia (cf. 11). The distribution of parasympathetic neurons within the gut wall has been more difficult to define. Observations based on the use of methods for neuronal tracing indicate that vagal fibers terminate mainly in the myenteric ganglia (44, 45).

It seems that virtually all enteric neurons contain peptides. A schematic outline of the distribution of different peptide-containing fibers in the rat small intestine is given in Fig. 12. In order to understand the neuronal circuitry regulating such events as peristalsis, we need to know the pathways of the different neuronal systems. In order to be able to convey relevant information, enteric neurons engaged in the motor control of the gut have to issue projections that are both descending (ahead of the peristaltic wave to mediate relaxation of the circular muscle) and ascending (to mediate the contractile wave). Using microsurgical techniques for local denervation of the gut wall first used by Furness & Costa in their elegant studies of projections of guinea-pig enteric neurons (cf. 11) we have studied the rat small and large intestine. In these studies we also examined some newly discovered enteric neuronal systems, such as those storing gastrin releasing peptide (GRP), galanin and calcitonin gene-related peptide (CGRP) (46–49). Briefly, the results indicate that nerves

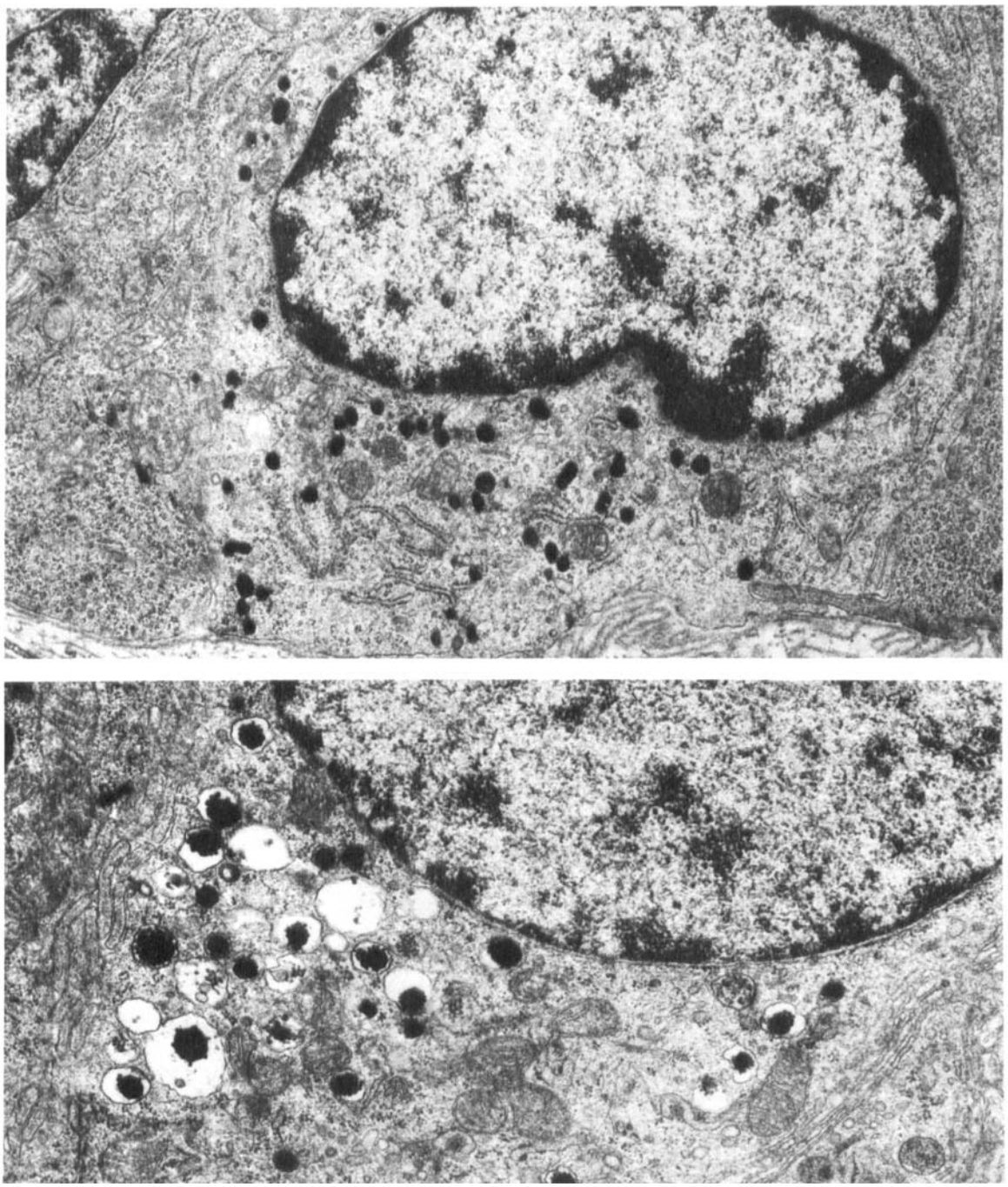

Fig. 7. Electron micrographs showing portions of two different types of enterochromaffin cells in the rat antrum. Although both cells contain highly electron-dense pleomorphic granules typical of enterochromaffin cells, the size of the granules and the width of the zone separating the dense core and the limiting membrane differ markedly. ×12000.

emanating from the myenteric ganglia project mainly to other myenteric ganglia and to the smooth muscle (see also 11). Usually the different subpopulations of myenteric nerves issue descending projections of various length. Such descending neurons include those storing NPY/VIP, somatostatin, galanin, substance P/NKA (small intestine) and GRP. The projections of GRP neurons and of galanin neurons are the longest, reaching 15–20 mm in length (see also 14, 50). CGRP-containing neurons in the small intestine issue both ascending and descending projections while enkephalin neurons in both small and large intestines and SP/NKA neurons in the large intestine were found to issue ascending projections only. Neurons in the submucous ganliga supply the mucosa and project to other submucous ganglia. Most of the different neuronal populations issue both ascending and descending projec-

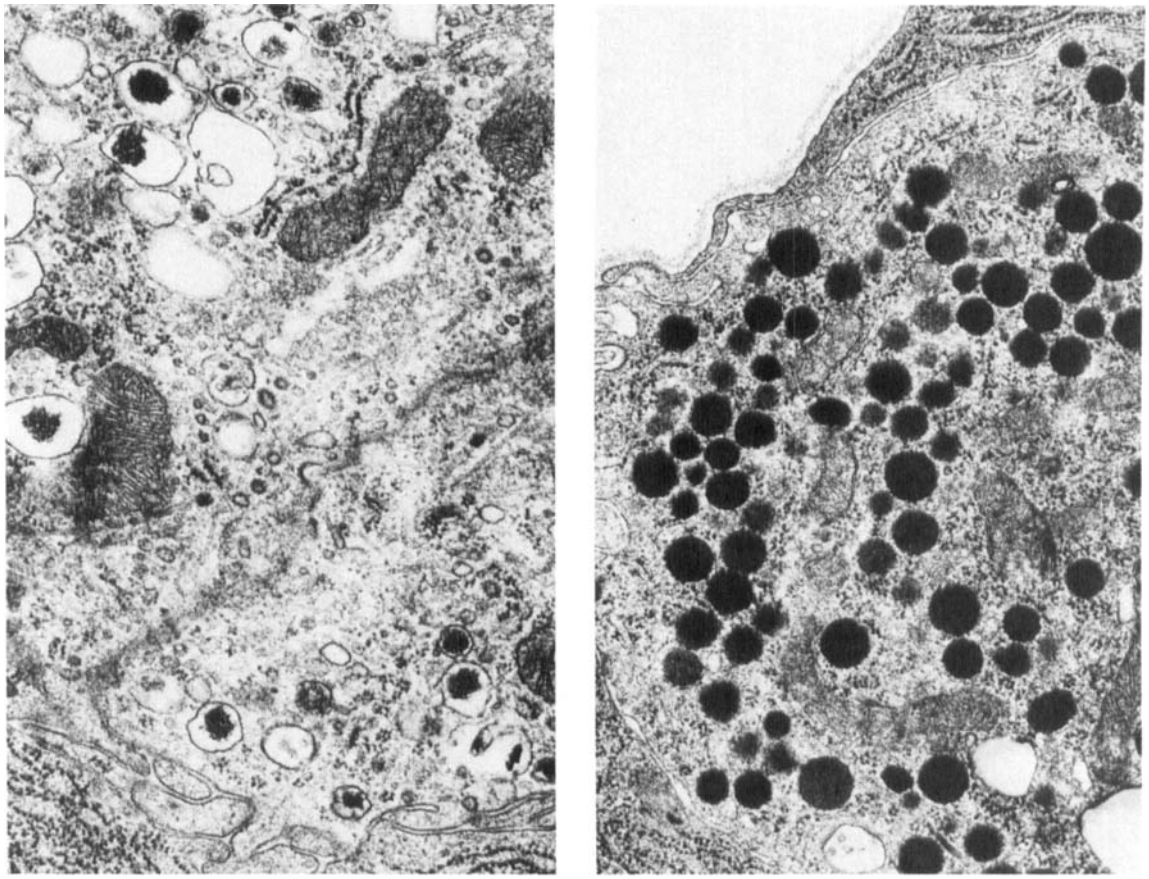

Fig. 8. Electron micrographs of portions of ECL cell (left) and A-like cell (right) in the oxyntic mucosa of the rat stomach. These cell types represent the two predominating endocrine cell types in this area. Most of the ECL cell granules have a characteristical vesicular-type appearance without dense core or with a wide zone separating a fine-dotted dense core from the limiting membrane. The A-like cell granules have a solid electron-dense core with a tightly applied limiting membrane. (×20000).

tions. The projections of submucous neurons are on the whole shorter than those of myenteric neurons. The results are summarized in Fig. 13. The functional significance of many of these neuronal projections has yet to be defined. It should also be mentioned in this context that certain enteric (mainly myenteric) neurons project also outside the gut (50–52, cf. 11). Thus, VIP neurons and GRP neurons project to the prevertebral ganglia indicating reflex loops involving the sympathetic nervous system. Extra-intestinal reflex loops involving the sensory nervous system are also known to exist and are readily explained by the prominent sensory input to the gut, the stomach in particular. Short sensory reflexes within the gut in which sensory impulses are propagated anti-dromically from one branch (e.g. situated at the mucosal surface) to another branch of the same neuron (e.g. situated in the wall of a blood vessel) (axonal reflexes) are thought to at least partly explain the increase in local blood flow upon irritation evoked by noxious stimuli at the mucosal surface (cf. 53).

Little information is available with respect to the capacity of regeneration of enteric neurons. We have studied how myenteric GRP-containing neurons disappear and reappear after local denervation (54). In summary, the results indicate a very slow process of reinnervation. However, after 20–40 weeks there is actually a hyperinnervation of the circular muscle. Thus, there is evidence that enteric neurons display plasticity and are capable of reinnervating the gut after local denervation.

Neuroendocrine integration

There are several possibilities for interaction between the different components of the gut neuroendocrine system. Neurons may interact with other neurons, neurons may interact with endocrine cells, endocrine cells may interact with other endocrine cells and, finally, endocrine cells may influence neuronal activity. Examples of such interactions are given in the Table. Our present knowledge of such interactions is only at its infancy. Nevertheless, the functional integration of the neuronal and endocrine/paracrine systems and their common genetic pro-

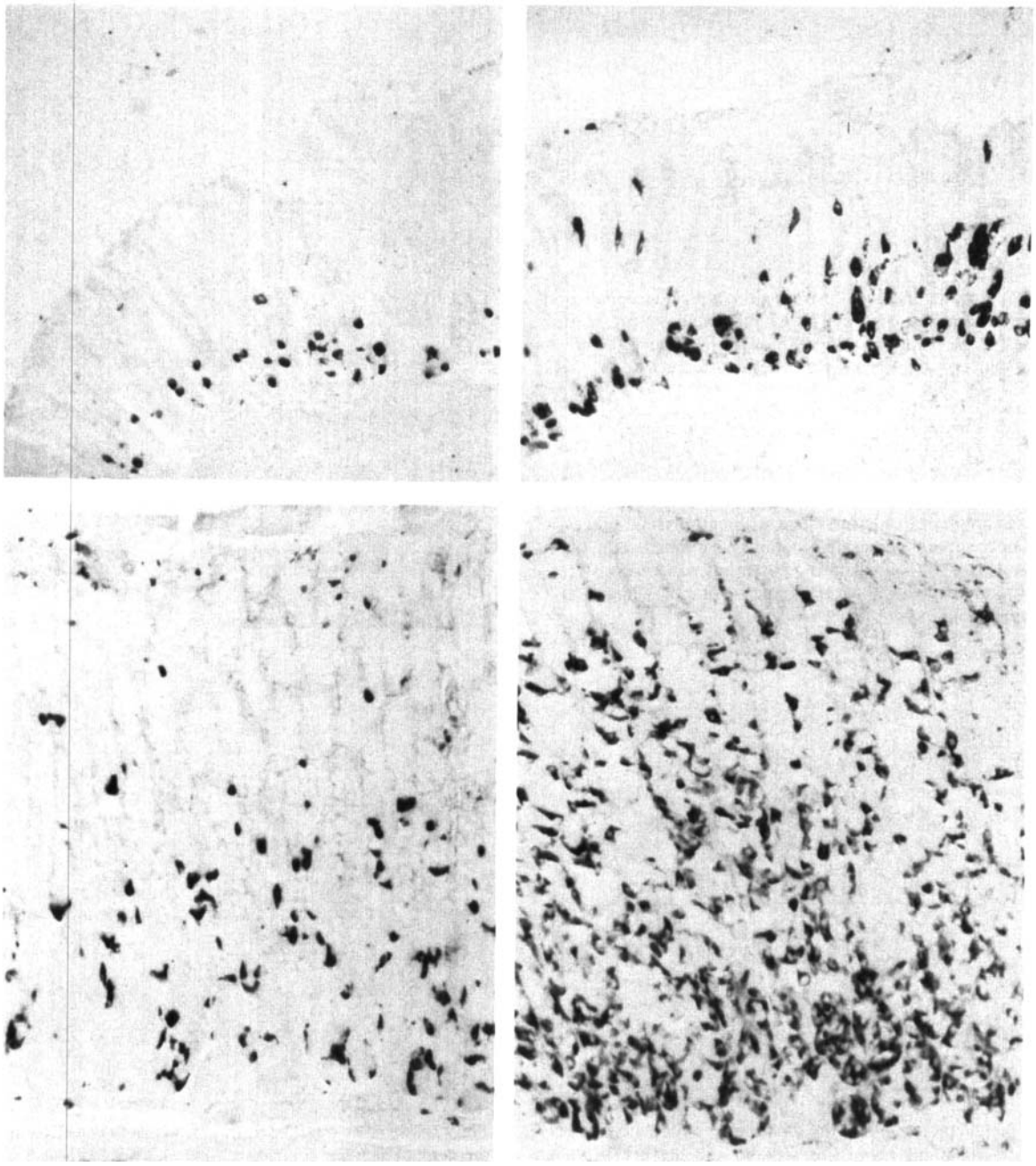

Fig. 9. Photomicrographs showing gastrin-immunoreactive cells in the antrum (top row) and histamine immunoreactive (ECL) cells in the oxyntic mucosa or bottom row of a control rat (left) and of a rat treated with large doses of the antisecretagogue omeprazole for 10 weeks (right). Note the hyperplasia of both gastrin cells and ECL cells.

gramming are good enough reasons to use the term 'neuroendocrine system of the gut'.

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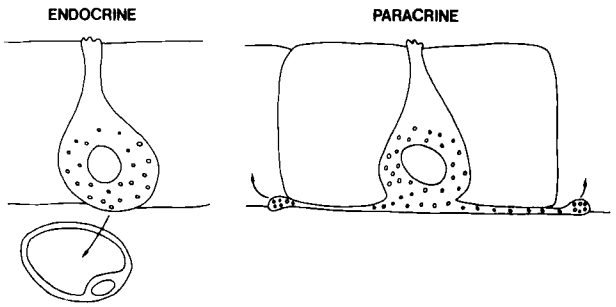

Fig. 10. Schematic drawing illustrating the typical morphology of an endocrine and paracrine cell in the gut. The basal cytoplasmic processes of the paracrine cell are thought to provide the means for local delivery of messenger to neighbouring cells.

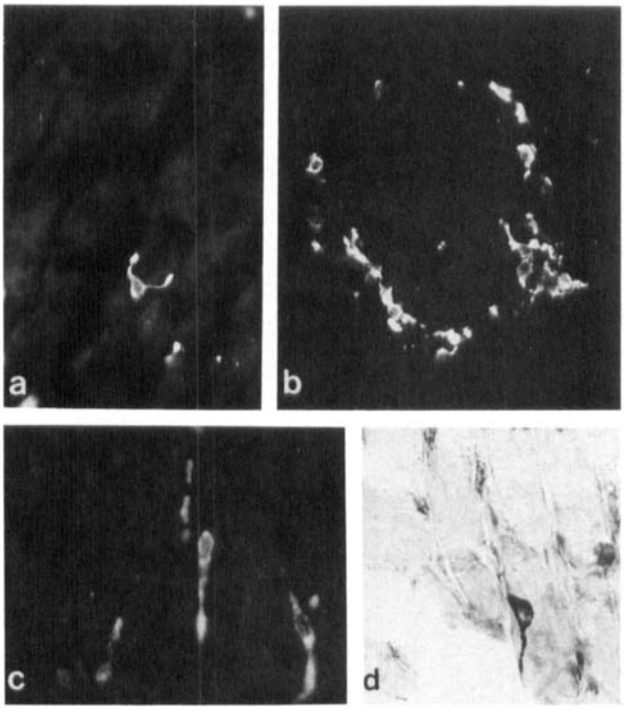

Fig. 11. Examples of cells furnished with cytoplasmic processes and possibly serving paracrine functions. a) somatostatin cell in the rat stomach. b) somatostatin cells in rat pancreatic islet. c) enterochromaffin cells in the rectal mucosa of mouse and d) ECL cell in guinea-pig oxyntic mucosa.

Fig. 11

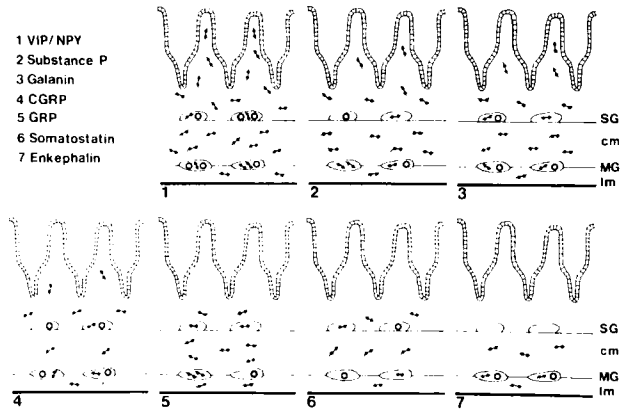

Fig. 12. Schematic outline of the topography and relative density of peptide-containing nerve fibres (●—●) and nerve cell bodies (○) in the rat small intestine. SG, submucous ganglia; cm, circular muscle; MG, myenteric ganglia; lm, longitudinal muscle.

	Myenteric neurons		Submucous neurons	
	SI	LI	SI	LI
CGRP	← → (2mm)	→ (5mm)	← (8mm)	← → (6mm)
Enkephalin	← (7mm)	← (5mm)	0	0
Galanin	→ (15mm)	→ (6mm)	← → (2mm)	↑
GRP	→ (20mm)	→ (10mm)	← → (2mm)	← → (3mm)
Som	→ (6mm)	→ (6mm)	← → (5mm)	0
Som/CGRP	0	0	0	← → (5mm)
SP	→ (7mm)	← (5mm)	← → (4mm)	ne
VIP	0	→ (3mm)	0	← (2mm)
VIP/NPY	→ (2mm)	→ (4mm)	← (4mm)	↑

Fig. 13. Polarities (marked with arrows) and projection distances (in mm) of peptide-containing neuronal populations in the rat small (SI) and large (LI) intestine. → indicates descending projections, ← indicates ascending projections and ↑ indicates that no oro-anal projections can be demonstrated, 0 means absence of nerve fibers, ne stands for not examined.

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