YALE JOURNAL OF BIOLOGY AND MEDICINE 71 (1998), pp. 163-171.

Physiology of the ECL Cells

R. Håkanson[a], D. Chen, E. Lindström, P. Norlén, M. Björkqvist and D. Lehto-Axtelius

Department of Pharmacology, University of Lund, Lund, Sweden

The enterochromaffin-like (ECL) cells of the oxyntic mucosa (fundus) of the stomach produce, store and secrete histamine, chromogranin A-derived peptides such as pancreastatin, and an unanticipated but as yet unidentified peptide hormone. The cells are stimulated by gastrin and pituitary adenylate cyclase activating peptide and suppressed by somatostatin and galanin. Choline esters and histamine seem to be without effect on ECL cell secretion. The existence of a gastrin-ECL cell axis not only explains how gastrin stimulates acid secretion but also may help to explore the functional significance of the ECL cells with respect to the nature and bioactivity of its peptide hormone. From the results of studies of gastrectomized/fundectomized and gastrin-treated rats, it has been speculated that the anticipated ECL-cell peptide hormone acts on bone metabolism.

INTRODUCTION

This review attempts to summarize what is known of the mechanisms that control the activity of the enterochromaffin-like (ECL)[b] cells in the rat stomach and describes also current speculations about their functional significance. Morphological aspects of the ECL cells are discussed in another chapter in this volume [1]. The ECL cells are known to produce, store and secrete histamine and chromogranin A (CGA)-derived peptides, such as pancreastatin; in addition, they are suspected to manufacture an as yet unidentified peptide hormone [2, 3]. The secretory products are stored in secretory vesicles, which have a very characteristic ultrastructural appearance [1, 4-7]. The products appear to be released in parallel in response to stimulation [7, 8]. The functional significance of the ECL cells can be expected to reflect the nature and bioactivity of the secreted products.

ECL CELLS: OPERATIONAL CONTROL

In vivo studies

Much information on the mechanisms that control the activity of the ECL cells is already available in the literature from decades of *in vivo* studies [2, 3]. It is generally accepted that gastrin is a major stimulus for the ECL cells, causing secretory activation as well as hypertrophy/hyperplasia. Secretory activation is manifested in the prompt mobilization of histamine and pancreastatin and followed by activation of the histamine-forming enzyme histidine decarboxylase (HDC). If the gastrin stimulus is sustained for a long period of time the ECL cells will respond with hypertrophy and general, diffuse hyperplasia, and ultimately with dysplasia (focal hyperplasia and micronodules) and neoplasia (frank tumors) [9].

Although it seems to be generally accepted that the ECL cells operate under vagal control the evidence for this view is not overwhelming. Gastrin and the vagus are thought

[a]*To whom all correspondence should be addressed:* R. Håkanson, Department. of Pharmacology, University of Lund, Lund, Sweden.
[b]*Abbreviations:* ECL, enterochromaffin-like; CGA, chromogranin A; HDC, histidine decarboxylase; PACAP, pituitary adenylate cyclase activating peptide; VIP, vasoactive intestinal peptide.

to interact in an intricate manner in regulating various activities in the oxyntic mucosa (e.g., ECL cell activation, parietal cell activation), and it is difficult to define the precise contribution of each individual stimulus on each individual target. From published reports it appears that vagally denervated ECL cells respond poorly to the trophic action of gastrin [10-12]. It appears to be generally accepted that the vagus controls the ECL cells and that acetylcholine is the neurotransmitter involved. In fact, however, the vagal control of the ECL cells (and of all other epithelial cells in the oxyntic mucosa) is mediated by enteric neurons (probably those in the myenteric plexus) [13], and the nature and identity of the relevant transmitters are unknown.

The activity of the ECL cells can be suppressed by exogenously administered somatostatin [14] and histamine [15, 16]. Somatostatin occurs in paracrine cells in the oxyntic mucosa [14, 17] and is thought to modulate the activity of the ECL cells by way of direct contact or per diffusionem. In the past, exogenous histamine has been found to be quite effective in suppressing the HDC activity of the ECL cells [15]. In fact, histamine was proposed to suppress the activity of the ECL cell by way of auto feedback inhibition; the receptor involved was tentatively identified as a histamine H_2-receptor [16]. However, the design of these experiments was such that indirect effects of histamine could not be excluded.

Table 1. Screening of various regulatory peptides and neurotransmitters to demonstrate stimulated secretion and suppression of gastrin-induced secretion from isolated ECL cells.

Peptides	Stimulation	Suppression	Peptides	Stimulation	Suppression
Gastrin	++++	-	NMU	+	-
Gastrin-Gly	-	-	Substance P	-	-
CCK-8s	++++	-	Somatostatin	-	++++
PACAP-27	++++	-	Met-enkephalin	-	-
PACAP-38	++++	-	Leu-enkephalin	-	-
VIP	+++	-	β-Endorphin	-	-
Helodermin	+	-	Dynorphin	-	-
Helospectin II	+	-	Pancreastatin	-	-
Glucagon	-	-			
GLP-1	-	-	*Amino acids*		
GLP-2	-	-	Aspartate	-	-
Secretin	+	-	GABA	-	-
GIP	-	-	Glutamate	-	-
PTH	-	-			
Calcitonin	-	-	*Amines, choline esters*		
CGRP	-	-	Acetylcholine	-	-
GRP	-	-	Bethanechol	-	-
Galanin	-	+++	Carbachol	-	-
NPY	-	-	Histamine	-	-
PYY	-	-	Serotonin	+	-
PP	-	-	Dopamine	+	-
Bradykinin	-	-	Noradrenaline	++	-
Motilin	-	-	Adrenaline	++	-
Neurotensin	+	-	Isoprenaline	++	-
Insulin	-	-			

Each agent was tested on at least four separate isolated cell preparations, except in the case of CGRP, NPY, PYY, PP, PACAP, choline esters and histamine. The latter agents were screened at least 10 times. Peptides were tested at a concentration 10^{-7} M. All other agents were screened at 10^{-4} M concentration. Suppression was studied with cells stimulated with EC_{80} gastrin. ++++, potent and powerful; +++, strong; ++, moderate; +, weak; -, no effect.

Table 2. Stimulatory effect of gastrin, PACAP and VIP on secretion of histamine and pancreastatin from ECL cells.

Peptide	EC_{50} histamine secretion	Maximum secretion (% increase)	EC_{50} pancreastatin secretion	Maximum secretion (% increase)
Gastrin	6×10^{-10} M	320	2×10^{-10} M	410
CCK-8	4×10^{-10} M	310	2×10^{-10} M	390
PACAP-27	10×10^{-11} M	310	6×10^{-11} M	360
PACAP-38	9×10^{-11} M	300	3×10^{-11} M	380
VIP	10×10^{-10} M	220	4×10^{-10} M	280

Mean values of 6-10 concentration response curves.
EC_{50}, concentration of peptide that elicits 50% of maximum effect.

In vitro studies

The fairly recent development of a method for the preparation of isolated, near-pure ECL cells by Prinz et al. [18, 19] will make it possible to demonstrate receptors and binding sites on the ECL cells directly and to examine in detail the signal transduction mechanisms that are activated by the different stimuli. We have accumulated data from studies of more than 200 preparations of isolated ECL cells with a reproducible yield of $2\text{-}3 \times 10^6$ ECL cells from five rat stomachs; the ECL cells constitute 80-90 percent of the cells in the final preparation [8]. A number of regulatory peptides and neurotransmitters were tested for their ability to affect secretion from the ECL cells. A fairly limited number of the agents tested were found to be active (Table 1). Briefly, gastrin causes a parallel release of histamine and pancreastatin, and also the neuropeptides pituitary adenylate cyclase activating peptide (PACAP) and vasoactive intestinal peptide (VIP) were able to stimulate secretion. PACAP was more potent than gastrin, while VIP seemed to be less potent (Table 2). Of the regulatory peptides tested not only somatostatin but also galanin were able to suppress gastrin-evoked (and PACAP-evoked) secretion (Table 2). Choline esters and the amino acid transmitters tested were inactive, while catecholamines and 5-hydroxytryptamine were moderately active. Our results differ from previously published reports in several instances. Thus, we failed to observe an inhibiting effect of histamine. We also failed to find a stimulating effect of choline esters. Since acetylcholine was inactive, it is unlikely that the vagal control of the ECL cells is cholinergic. Instead, the nervous control may be exercised by enteric neurons that use PACAP (or possibly VIP) as the excitatory transmitter and galanin as the inhibitory transmitter. Interestingly, the oxyntic mucosa is richly supplied with nerve fibers that contain PACAP and/or VIP [13, 20-22]. Also galanin occurs in nerve fibers in the oxyntic mucosa [23]. We conclude from our data that not only gastrin but also PACAP is a powerful excitant of the ECL cells, that not only somatostatin but also galanin can suppress secretion, that muscarinic receptor agonists fail to evoke secretion, and that histamine (and pancreastatin) does not evoke auto feedback inhibition [8].

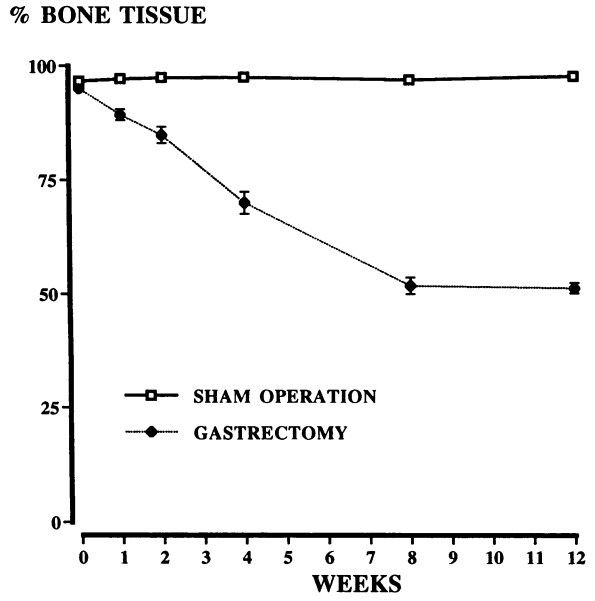

Figure 1. The percentage of bone in rat calvaria at different times after gastrectomy or sham operation. The analysis was performed by computer-assisted image analysis on decalcified sections. The bone surface area is expressed as percentage of the total biopsy surface. Mean values ± SEM, 33-56 rats in each group. The number of rats examined at each point varied between 5 and 15. Data from Ref. [37].

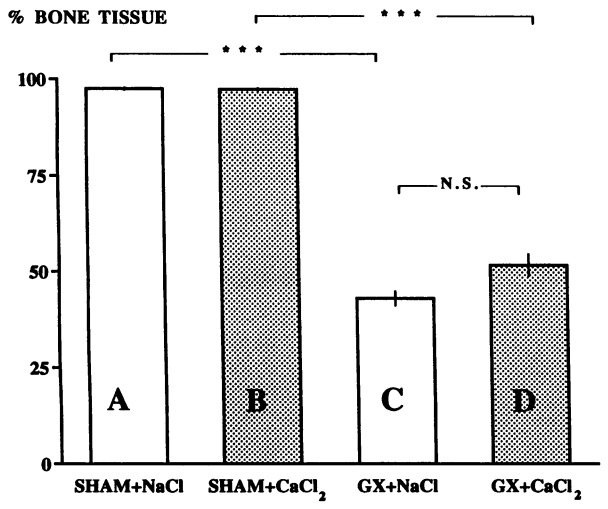

Figure 2. Morphometric analysis of sections of calvaria from rats subjected to: sham operation, SHAM and treatment with NaCl, (A); SHAM and treatment with $CaCl_2$, (B); gastrectomy, GX and treatment with NaCl, (C); and GX and treatment with $CaCl_2$, (D). Each rat received 100 mg calcium chloride dihydrate or equivalent amount of sodium chloride by the oral route daily for 7 weeks. The analysis was performed by computer-assisted image analysis of decalcified sections. *** $p < .01$; ANOVA, multiple comparisons, two-way analysis. Mean values ± SEM; 8-10 rats in each group. NS, not significant. Data from Ref. [37].

THE GASTRIN-ECL-CELL AXIS

The ECL cells operate under the control of circulating gastrin [2, 3]. The relationship between gastrin and the ECL cells is so close that we have proposed the term gastrin-ECL cell axis. The biological significance of the ECL cells is linked with the actions of locally released histamine and the systemic actions of the anticipated ECL-cell peptide hormone. ECL-cell histamine is mobilized by gastrin to stimulate the parietal cells [24, 25] and it appears to be generally accepted by now that the acid-stimulating effect of gastrin is mediated by ECL-cell histamine. The evidence for this view is summarized in another chapter of this book [26]. It may be pointed out here that ECL-cell histamine does not seem to mediate vagus-stimulated acid secretion [25].

It is not unusual to see the ECL cells dismissed as representing merely the local source of gastric histamine necessary for the activation of the parietal cells. In fact, however, there is compelling evidence that the ECL cells produce not only histamine but also a peptide hormone. The concomitant formation of an amine and a regulatory peptide is a feature shared with a great many different peptide hormone-producing cell populations [27]. Admittedly, the arguments in favor of the view that the ECL cells are the source of a peptide hormone are indirect. First, the ECL cells share the properties of all other peptide hormone-producing endocrine cells in the body (Table 3). Recently, they were found to be supplied with the full range of exocytotic proteins associated with neurons and endocrine cells [28]. In addition, observations documented in the literature make it possible to speculate that the ECL cells are engaged in specific functions not related to histamine (see below).

FUNCTIONAL SIGNIFICANCE OF THE ECL CELLS

Circulating pancreastatin—a marker of ECL cell activity and number

The ECL cells constitute one of the largest endocrine cell populations in the body. They are rich in CGA-derived peptides, such as pancreastatin [7, 29, 30]. The ECL cells contain about 25 percent of the body's total content of pancreastatin and account for 75 percent of the circulating pancreastatin [31]. Furthermore, at least 90 percent of circulating pancreastatin in hypergastrinemic rats (omeprazole treatment) derive from the ECL cells [32]. From these data it can be surmised that the ECL cell population represents a quantitatively prominent endocrine organ. CGA and the CGA-derived peptides are generally thought to play a role in the packaging, processing and storage of the actual secretory products rather than being messenger compounds in their own right [33]. It is possible,

Table 3. Characteristic features of ECL cells in oxyntic mucosa of rat stomach.

Properties in common with known peptide hormone-producing cells	Unique properties
Positive argyrophil staining	Production, storage and secretion of histamine
Cytoplasmic (secretory) granules	Responsiveness to gastrin
Exocytotic proteins	
Chromogranins in the granules	
Proteolytic "processing" enzymes in the granules	
APUD properties*	
Co-storage of amines and peptides in granules*	

*For a discussion of these features see Sundler et al. (1980).

even likely, that the formation, packaging, processing and secretion of the anticipated ECL-cell hormone occurs in parallel with CGA and that what we learn about the manufacture and handling of CGA in the ECL cells will help us understand the mechanisms that control the manufacture and handling of the ECL-cell peptide hormone.

Anticipated ECL-cell peptide hormone

We have speculated in the past that the key role of the ECL cells is associated with the anticipated peptide hormone rather than with histamine. In endocrinology, a classical approach to detect unknown but anticipated hormonal principles is to examine the consequences of resection of the organ under suspicion. Resection of the stomach is known to be associated with osteopathy. This was first demonstrated in young dogs and monkeys by Ivy and co-workers [34, 35]. It was generally assumed at the time that the osteopathy reflected nutritional deficiency. Since then numerous observations both in the clinical setting and in experimental animals have confirmed the importance of the stomach for skeletal health.

As expected, gastrectomy causes the development of osteopathy in the rat [36-40]. This was manifested in all bones examined, most dramatically in the calvaria [37]. Also, trabecules were lost to a great extent [36]. The effect was near-maximal within 8-12 weeks (Figure 1). There are several possible explanations for the gastrectomy-evoked osteopathy. First, general nutritional deficiency may be responsible. However, the food intake appeared normal and the curve illustrating the body weight development was not much affected by the surgery. Hence, it is unlikely that a general nutritional deficiency accounts for the gastrectomy-evoked bone loss. Also, calcium supplementation by the peroral or systemic route failed to prevent the gastrectomy-evoked osteopathy [36, 37] (Figure 2). At present, we have no evidence that vitamin D deficiency contributes to the gastrectomy-evoked osteopathy [41]. Circulating concentrations of Ca^{+2}, PTH and calcitonin were unaffected by gastrectomy (Håkanson et al., to be published). Hence it is unlikely that hyperparathyroidism is responsible for the gastrectomy-evoked bone loss. Lack of gastric acid, as after treatment with the proton pump inhibitor omeprazole, did not induce osteopathy [36]. It appears therefore that the gastrectomy-evoked bone loss is a consequence of the loss of the stomach per se and that the stomach plays an as yet unidentified role in controlling bone status. In another series of experiments we tried to establish whether defined parts of the stomach were as important to bone metabolism as was the whole stomach [39]. Removal of the acid-producing part of the stomach (fundectomy) resulted in the same degree of osteopathy as did gastrectomy, whereas antrectomy had only little effect. We conclude that while fundectomy completely reproduces the effects of gastrectomy, antrectomy has little effect. We speculate therefore that the bone loss observed after gastrectomy reflects the loss of the oxyntic mucosa and of a circulating hormone originating in one of the endocrine cell populations in this location.

Schulak and Kaplan [42, 43] and Kaplan et al. [44] reported that gastrin evokes transient hypocalcemia in the rat. This effect was unaffected by thyroparathyroidectomy but abolished by gastrectomy or fundectomy. We were able to confirm these observations with respect not only to exogenous but also to endogenous gastrin [45-47]. In addition, we demonstrated that gastrin-free extracts of the oxyntic mucosa mimicked the effect of gastrin on blood calcium. The gastrin-induced hypocalcemia reflected the stimulated uptake of $^{45}Ca^{2+}$ into bone. We suggest that gastrin causes hypocalcemia by releasing a blood calcium-lowering hormone from the acid-producing part of the stomach and speculated that the oxyntic mucosa contains a hormone (gastrocalcin), which is released in response to gastrin (which in turn is released in response to food intake) [48]. The ECL cells are likely candidates for such a hormone because they operate under the control of gastrin.

If the ECL cells secrete a calciotropic hormone it is to be expected that they should respond to perturbations in the concentration of blood Ca^{2+}. While changes in the blood

Ca^{2+} concentration induced the expected changes in the circulating concentrations of calcitonin and PTH, the ECL cells failed to respond [49]. We conclude that the ECL cells do not secrete a calciotrophic hormone. It cannot be excluded, however, that they produce a hormone with a primary action on bone rather than on Ca^{2+}

REFERENCES

1. Chen, D., Zhao, C.-M., Andersson, K., Meister, B., Panula, P., and Håkanson, R. ECL cell morphology. Yale J. Biol. Med. 71:217-231 1998.
2. Håkanson, R., Chen, D., Andersson, K., Monstein, H.-J., Zhao, C.-M., Ryberg, B., Sundler, F. and Mattsson, H. The biology and physiology of the ECL cell. Yale J. Biol. Med. 67:123-134, 1994.
3. Håkanson, R., Chen, D. and Sundler, F. The ECL cells. In: Johnson, L.R., ed. *Physiology of the Gastrointestinal Tract*, Vol. 2, 3rd Ed. New York: Raven Press; 1994, pp 1171-1184.
4. Capella, C., Vassallo, G., and Solcia, E. Light and electron microscopic identification of the histamine-storing argyrophil (ECL) cell in mufine stomach and of its equivalent in other mammals. Z. Zellforsch. 188:68-84, 1971.
5. Håkanson, R., Owman, Ch., Sporrong, B., and Sundler, F. Electron microscopic identification of the histamine-storing argyrophil enterochromaffin-like cells in the rat stomach. Z. Zellforsch. 122:460-466, 1971.
6. Böttcher, G., Håkanson, R., Nilsson, G., Seensalu, R., and Sundler, F. Effects of long-term hypergastrinaemia on the ultrastructure of enterochromaffin-like cells in the stomach of the rat, hamster and guinea pig. Cell Tissue Res. 256:247-257, 1989.
7. Chen, D., Monstein, H.-J., Nylander, A.-G., Zhao, C,M., Sundler, F., and Håkanson, R. Acute responses of rat stomach enterochromaffinlike cells to gastrin: Secretory activation and adaptation. Gastroenterology 107:18-27, 1994.
8. Lindström, E., Björkqvist, M., Boketoft, A., Chen, D., Zhao, C.-M., Kimura, K., and Håkanson, R. Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. Regul. Pept. 71:73-86, 1997.
9. Håkanson, R. and Sundler, F. Proposed mechanism of induction of gastric carcinoids: The gastrin hypothesis. J. Clin. Invest. 20(suppl. 1):S65-S71, 1990.
10. Håkanson, R., Vallgren, S., Ekelund, M., Rehfeld, J.F., and Sundler, F. The vagus exerts trophic control of the stomach in the rat. Gastroenterology 86:28-32, 1984.
11. Håkanson, R., Axelson, J., Tielemans, Y., Johansson, A.-G., Willems, G., and Sundler, F. Unilateral vagal denervation suppresses omeprazole-induced trophic effects on the denervated side of the rat stomach. Scand. J. Gastroenterol. 27:65-70, 1992.
12. Axelson, J., Ekelund, M., Håkanson, R., and Sundler, F. Gastrin and the vagus interact in the trophic control of the rat oxyntic mucosa. Regul. Pept. 22:237-243, 1988.
13. Ekblad, E., Håkanson, R., and Sundler, F. Microanatomy and chemical coding of peptide containing neurons in the digestive tract. In: Daniel, E.E., ed. *Neuropeptide Function in the Gastrointestinal Tract.* Boca Raton, FL:CRC Press; 1990, pp. 131-179.
14. Alumets, J., Ekelund, M., El Munshid, H.A., Håkanson, R., Loren, I., and Sundler, F. Topography of somatostatin cells in the stomach of the rat: possible functional significance. Cell Tissue Res. 202:177-188, 1979.
15. Håkanson, R., Larsson., L.-I., Liedberg, G., Rehfeld, J.F., and Sundler, F. Suppression of rat stomach histidine decarboxylase activity by histamine: H_2-receptor-mediated feed back. J. Physiol. 269:643-667, 1977.
16. Håkanson, R., Larsson, L.-I., Liedberg, G., and Sundler, F. Evidence for H_2-receptor-mediated feed-back regulation of histamine release from endocrine cells in the rat stomach. J. Physiol. 276:151-157, 1978.
17. Alumets, J., Sundler, F., and Håkanson, R. Distribution, ontogeny and ultrastructure of somatostatin immunoreactive cells in the pancreas and gut. Cell Tissue Res. 185:465-479, 1977.
18. Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H.F., and Sachs, T. Histamine secretion from rat enterochromaffinlike cells. Gastroenterology 105:449-461, 1993.
19. Prinz, C., Scott, D.R., Hurwitz, D., Helander, H.F., and Sachs, G. Gastrin effects on isolated rat enterochromaffin-like cells in primary culture. Am. J. Physiol. 267:G663-G675, 1994.
20. Ekblad, E., Ekelund, M., Graffner, H., Håkanson, R. and Sundler, F. Peptide-containing nerve-fibers in the stomach wall of rat and mouse. Gastroenterology 89:73-85, 1985.

21. Ekblad, E., Håkanson, R., and Sundler, F. Innervation of the stomach of rat and man with special reference to the endocrine cells. In: Håkanson, R. and Sundler, F., eds. *The Stomach as an Endocrine Organ.*, Fernstrom Symposium, No. 15. Amsterdam: Elsevier; 1991, pp. 79-95.
22. Sundler, F., Ekblad, E., Absood, A., Håkanson, R., Koves, K., and Arimura, A. Pituitary adenylate cyclase activating peptide: A novel vasoactive intestinal peptide-like neuropeptide in the gut. Neuroscience 46:439-454, 1992.
23. Ekblad, E., Rökaeus, A., Håkanson, R., and Sundler, F. Galanin nerve fibers in the rat gut: distribution, origin and projections. Neuroscience 16:355-363, 1985.
24. Waldum, H., Sandvik, A.K., Brenna, E., and Petersen, H. The gastfin-histarnine sequence in the regulation of gastric acid secretion. Gut 32:698-700, 1991.
25. Andersson, K., Cabero, J.L., Mattsson, H., and Håkanson, R. Gastric acid secretion after depletion of enterochromaffin-like cell histamine. A study with α-fluoromethylhistidine in rats. Scand. J. Gastroenterol. 31:24-30, 1996.
26. Andersson, K., Chen, D., Mattsson, H., Sundler, F., and Håkanson, R. Physiological significance of ECL-cell histamine. Yale J. Biol. Med. 71:185-195, 1998.
27. Sundler, F., Håkanson, R., Loren, I,. and Lundquist, I. Amine storage and function in peptide hormone producing cells. Invest. Cell. Pathol. 3:87-103, 1980.
28. Zhao, C.-M., Jacobsson, G., Chen, D., Håkanson, R. and Meister, B. Exocytotic proteins in enterochromaffin-like (ECL) cells of the rat stomach. Cell Tissue Res. 290:539-551, 1997.
29. Håkanson, R., Böttcher, G., Ekblad, E., Panula, P., Simonsson, M., Dohlsten, M., Hallberg, T., and Sundler, F. Histamine in endocrine cells in the stomach: a survey of several species using a panel of histamine antibodies. Histochemistry 86:5-17, 1986.
30. Håkanson, R., Ding, X., Norlen, P., and Chen, D. Circulating pancreastatin is a marker for the enterochromaffin-like cells of the rat stomach. Gastroenterology 108:1445-1452, 1995.
31. Norlén, P., Curry, W.J., Chen, D., Zhao., C.-M., Johnston, C.F., and Håkanson, R. Expression of the chromogranin A-derived peptides pancreastatin and WE_{14} in rat stomach ECL cells. Regul. Pept. 70:121-133, 1997.
32. Kimura, K., Chen, D., Lindström, E., Zhao, C.-M. and Håkanson, R. Evidence that rat stomach ECL cells represent the main source of circulating pancreastatin. Regul. Pept. 68:177-180, 1997.
33. Helle, K.B. Chromogranins: Universal proteins in secretary organelles from paramecium to man. Neurochem. Int. 17:165-175, 1990.
34. Bussaberger, R.A., Freeman, S., and Ivy, A.C. The experimental production of severe homogeneous osteoporosis by gastrectomy in puppies. Am. J. Physiol. 121:137-148, 1938.
35. Freeman, S., Hough, V.H., Wigodsky, H., and Ivy A.C. The effect of gastrectomy upon growing monkeys. Gastroenterology, 1:199-210, 1943.
36. Persson, P., Gagnemo-Persson, R., Chen, D., Axelson, J., Nylander, A.-G., Johnell, O., and Håkanson, R. Gastrectomy causes bone loss in the rat. Is lack of gastric acid responsible? Scand. J. Gastroenterol. 28:301-306, 1993.
37. Klinge, B., Axtelius, D., Akerman, M., and Håkanson, R. Structure of calvaria following gastrectomy: an experimental study in the rat. Scand. J. Gastroenterol. 30:952-957, 1995.
38. Stenstrom, M., Olander, B., Carlsson, C.A., Alm-Carlsson, G., and Håkanson, R. Computerised microtomography for monitoring the development of osteoporosis in gastrectomized rats: methodological aspects of *in vivo* measurements. Acad. Radiol. 2:785-791, 1995.
39. Lehto-Axtelius, D., Stenström, M., and Johnell, O. Osteopenia after gastrectomy, fundectomy or antrectomy: an experimental study in the rat. Regul. Pept. 78:41-50, 1998.
40. Mühlbauer, R.C., Schenk, R.K., Chen, D., Lehto-Axtelius, D., and Håkanson, R. Morphometric analysis of gastrectomy-evoked osteopenia. Calcif. Tiss. Int. 62:323-326, 1998.
41. Axelson, J., Persson, P., and Håkanson, R. Importance of the stomach in maintaining calcium homeostasis in the rat. Gut 32:1298-1302, 1991.
42. Schulak, J.A. and Kaplan, E.L. Gastrin-induced hypocalcemia in thyroparathyoidectomized rats. Metabolism 23:1103-1106, 1974.
43. Schulak, J.A. and Kaplan, E.L. The importance of the stomach in gastrin-induced hypocalcemia in the rat. Endocrinology 96:1217-1220, 1975.
44. Kaplan, E.L., North, P.T., Norberg, H.T., Schulak, J.A., and Hill, B.J. Evidence for the role of the stomach in serum calcium regulation. J. Surg. Res. 22:237-141, 1977.
45. Persson, P., Håkanson, R., Axelson, J., and Sundler, F. Gastrin releases a blood calcium-lowering peptide from the acid-producing part of the stomach. Proc. Natl. Acad. Sci. USA 86:2834-2838, 1989.
46. Håkanson, R., Persson, P., Axelson, J., Johnell, O., and Sundler, F. Evidence that gastrin enhances ^{45}Ca uptake into bone through release of a gastric hormone. Regul. Pept. 28:107-118, 1990.

47. Håkanson, R., Persson, P., and Axelson, J. Elevated serum gastrin after food intake or acid blockade evokes hypocalcemia. Regul. Pept. 28:131-136, 1990.
48. Persson, P. and Håkanson R. The gastrin-gastrocalcin hypothesis. In: Håkanson, R. and Sundler, F., eds. The Stomach as an Endocrine Organ. Fernström Symposium, Vol. 15. Amsterdam: Elsevier; 1991, pp. 341-350.
49. Håkanson, R., Gagnemo-Persson, R., and Surve, V. Perturbations in blood Ca^{2+} do not affect the activity of rat stomach ECL cells. Scand. J. Gastroenterol. 31:217-221, 1996.