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ECL Cell Morphology

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Using immunohistochemistry at the conventional light, confocal and electron microscopic levels, we have demonstrated that rat stomach ECL cells store histamine and pancreastatin in granules and secretory vesicles, while histidine decarboxylase occurs in the cytosol. Furthermore the ECL cells display immunoreactivity for vesicular monoamine transporter type 2 (VMAT-2), synaptophysin, synaptotagmin III, vesicle-associated membrane protein-2, cysteine string protein, synaptosomal-associated protein of 25 kDa, syntaxin and Munc-18. Using electron microscopy in combination with stereological methods, we have evidence to suggest the existence of both an exocytotic and a crinophagic pathway in the ECL cells. The process of exocytosis in the ECL cells seems to involve a class of proteins that promote or participate in the fusion between the granule/vesicle membrane and the plasma membrane. The granules take up histamine by VMAT-2 from the cytosol during transport from the Golgi zone to the more peripheral parts of the cells. As a result, they turn into secretory vesicles. As a consequence of stimulation (e.g., by gastrin), the secretory vesicles fuse with the cell membrane to release their contents by exocytosis. The crinophagic pathway was studied in hypergastrinemic rats. In the ECL cells of such animals, the secretory vesicles were found to fuse not only with the cell membrane but also with each other to form vacuoles. Subsequent lysosomal degradation of the vacuoles and their contents resulted in the development of lipofuscin bodies.

INTRODUCTION

The mucosa of the digestive tract is rich in endocrine cells, which have been classified into enterochromaffin and enterochromaffin-like cells. They can be collectively demonstrated by so-called argyrophil staining techniques. The enterochromaffin cells contain serotonin or catecholamines, which accounts for the fact that they can be stained with chromaffin or argentaffin staining techniques. The enterochromaffin-like cells do not contain serotonin or catecholamines and cannot be stained with chromaffin or argentaffin staining techniques. Like the enterochromaffin cells, they have the capacity to convert DOPA or 5-HTP to dopamine and serotonin, respectively, and to store the amine in the secretory granules for some time. The term enterochromaffin-like derives from the fact that after these cells have been induced to store monoamines they are structurally and histochemically indistinguishable from the enterochromaffin cells [1]. The oxyntic mucosa of the rat stomach is rich in enterochromaffin-like cells [1]. They comprise several distinct cell types: ECL cells, A-like cells, D cells and D_1/P cells. This classification is based on the ultrastructural char-

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[f]*Abbreviations:* HDC, histidine decarboxylase; VMAT, vesicular monoamine transporter; CSP, cysteine string protein; VAMP-2, vesicle-associated membrane protein-2; CSP, cysteine string protein; SNAP-25, synaptosomal-associated protein of 25 kDa; α-FMH, alpha-fluoromethylhistidine.

acteristics of the different cell types [2, 3]. The ECL cells have an irregular, slender shape, sometimes with quite long extensions, and the cytoplasm contains numerous, quite large electron-lucent vesicles and a few, small electron-dense granules [4, 5].

Due to their content of histamine and of the histamine-forming enzyme histidine decarboxylase (HDC)[f], the ECL cells can be demonstrated by immunocytochemistry using antibodies to histamine [6] or to the HDC [7]. In addition, the ECL cells are known to display immunoreactivity for chromogranin A-derived peptides such as pancreastatin [6, 8-11]. Of the various endocrine cells (enterochromaffin and enterochromaffin-like cells) in the oxyntic mucosa, only the ECL cells produce histamine [6, 7, 12, 13] and respond to gastrin by the release of histamine [11, 14-16], which mediates the gastrin-induced stimulation of acid secretion from the parietal cells [17, 18]. The ECL cells respond to gastrin according to a precise time- table [19, 20]. Among acute effects (manifested within minutes) are the release of histamine and pancreastatin [11]. Among less immediate effects (within hours) are those manifested first in the activation of HDC and subsequently in the increased expression of HDC mRNA [11]. Long-term effects of hypergastrinemia include hypertrophy (after several days of hypergastrinemia), hyperplasia (after weeks of hypergastrinemia), and dysplasia/neoplasia (after one to two years) [21, 22].

HISTAMINE, PANCREASTATIN AND EXOCYTOTIC PROTEINS IN ECL CELLS

Light microscopy

The ECL cells are numerous in the oxyntic mucosa of rat, mouse and hamster, while mast cells are comparatively few. In these species, histamine immunostaining provides an excellent method for the demonstration of ECL cells. In mammals, the ECL cells are restricted to the oxyntic mucosa and occur in the basal half of the mucosa (Figure 1). Double immunostaining with antibodies against HDC and vesicular monoamine transporter type 2 (VMAT-2) revealed the presence of VMAT-2 immunoreactivity in the ECL cells (Figure 2). The protein-protein interactions behind the intracellular transport of cytoplasmic organelles and behind the process of exocytosis has been extensively studied in neurons. The ECL cells were found to display immunoreactivity for synaptophysin (Figure 3), synaptotagmin III, vesicle-associated membrane protein-2 (VAMP-2), cysteine string protein (CSP), synaptosomal-associated protein of 25 kDa (SNAP-25), syntaxin and Munc-18,

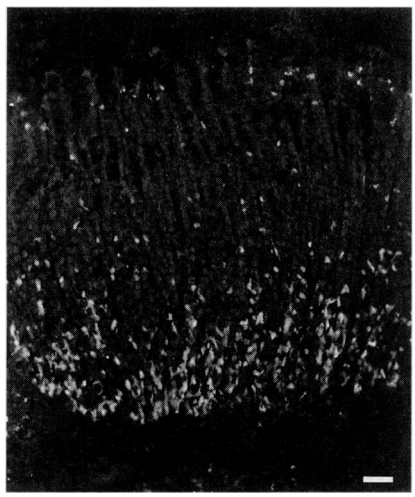

Figure 1. Immunofluorescence photomicrograph of rat oxyntic mucosa after staining with antiserum to histamine. Numerous immunoreactive ECL cells in the basal part of the glands, scattered immunoreative mast cells at the mucosal surface and in the submucosa. Bar = 50 μm.

but not for synaptotagmin I/II and VAMP-1 [23]. These findings suggest that the proteins that promote or participate in the docking and fusion of granules/vesicles to the plasma membrane are similar in neurons and ECL cells.

Confocal microscopy

The precise subcellular localization of ECL cell histamine remains unknown. The failure to demonstrate histamine at the subcellular level, e.g., by immunoelectron microscopy, may reflect the diffusion of histamine from its storage site because of inadequate fixation or because of the destruction of the antibody-recognizing epitopes by the fixation. By confocal microscopy of immunostained section, we were able to show that histamine is present

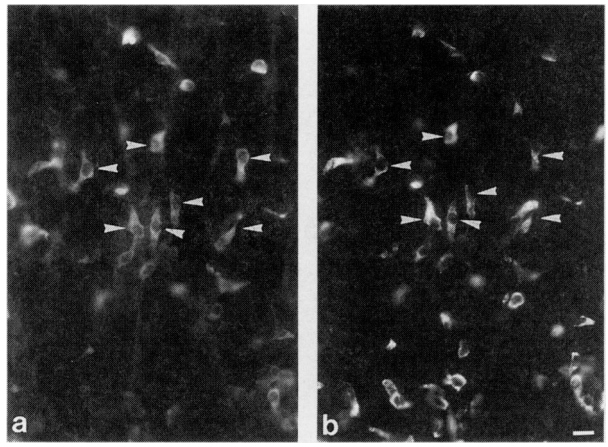

Figure 2. Immunofluorescence photomicrographs of rat oxyntic mucosa after double-labeling with antisera to VMAT-2 (a) and histidine decarboxylase (HDC) (b). VMAT-2 immunoreactivity is present in all HDC-positive ECL cells (arrowheads) and in non-ECL cells as well. Bar = 25 μm.

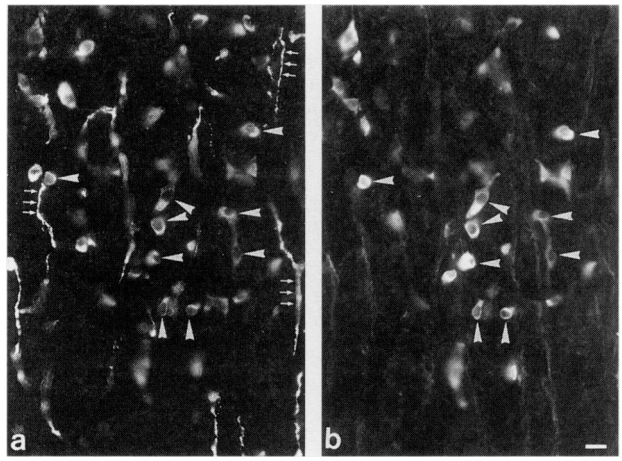

Figure 3. Immunofluorescence photomicrographs of rat oxyntic mucosa after double-labeling with antisera to synaptophysin (a) and histidine decarboxylase (HDC) (b). Synaptophysin immunoreactivity is present in all HDC-positive ECL cells (arrowheads). Arrows indicate synaptophysin-positive nerve fibers. Bar = 25 μm.

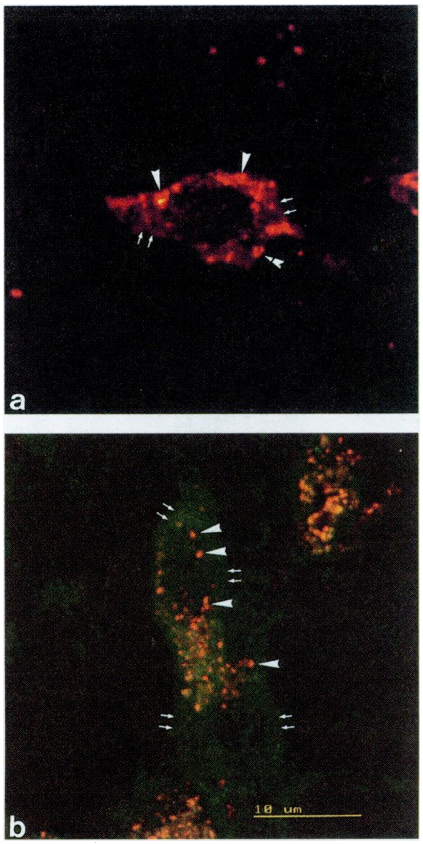

Figure 4. Confocal immunofluorescence photomicrographs of rat oxyntic mucosa after staining with antiserum to histamine (a) and after double-labeling with antisera to pancreastatin and histidine decarboxylase (HDC) (b). Histamine immunoreactivity (red fluorescence) has punctate localization to granules/vesicles (arrowheads) and in the cytosol (arrows). Pancreastatin immunoreactivity (red fluorescence) has a punctate localization to granules/vesicles (arrowheads) while HDC immunoreactivity (green fluorescence) is in the cytosol (arrows). Bar = 10 μm.

both in the cytosol and in the secretory vesicles but usually at a much higher concentration in the latter site. HDC immunoreactivity was found to occur in the cytosol only (Figure 4).

Electron microscopy

ECL cells have been identified as the only endocrine cell type in rat oxyntic mucosa that contains histamine by electron microscopic immunocytochemistry for histamine [12]. The ECL cells contain not only histamine but also pancreastatin. In fact, the ECL cells are the main source of circulating pancreastatin in the rat [24-26]. Pancreastatin is derived from chromogranin A. Chromogranin/pancreastatin is probably synthesized, packaged, stored, processed and secreted in parallel with the anticipated ECL cell-peptide hormone. The gastrin-induced release of pancreastatin parallels that of histamine, while pancreastatin can be released independently of histamine, for instance, after blocking histamine synthesis [27]. At the electron microscopic level, we could show that ECL cell-pancreastatin occurs in the dense cores of the granules/vesicles (Figure 5). We suggest that the electron dense parts of the organelles are the storage site for pancreastatin and other secretory peptides and proteins while the electron lucent parts of the organelles represent the storage site for histamine.

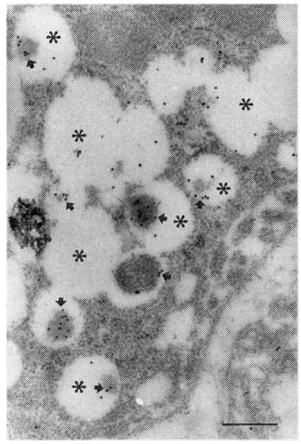

Figure 5. Immuno-electron micrograph showing the enterochromaffin-like cell ultrastructure after staining with antiserum to pancreastatin. Pancreastatin immunoreactivity, revealed by immunogold staining, is present in the electron-dense cores (arrows) of the secretory vesicles (asterisks). Bar = 200 nm.

DYNAMICS OF ECL CELL GRANULES AND VESICLES

Classification of organelles

The cytoplasm of the ECL cells is rich in fairly large, electron-lucent vesicles, which have a small and eccentrically located dense core (Figure 6). In addition, a few granules with prominent electron-dense core and a few clear microvesicles can be observed. In the case of long-term hypergastrinemia, the ECL cells display very large vesicles and electron dense bodies in their cytoplasm. We have classified the various organelles into granules, secretory vesicles, microvesicles, vacuoles and lipofuscin bodies (Figure 7) [28, 29]. The granules are defined as cytoplasmic membrane-enclosed organelles (with a diameter of 25-200 nm), displaying an electron-dense core and a thin electron-lucent halo between the membrane and the dense core, the diameter of the dense core representing more than 50 percent of the diameter of the entire organelle. The vesicles are membrane-enclosed electron-lucent organelles without a dense core or possessing a small, often eccentrically located dense core, the diameter of the dense core being less than 50 percent of the diameter of the

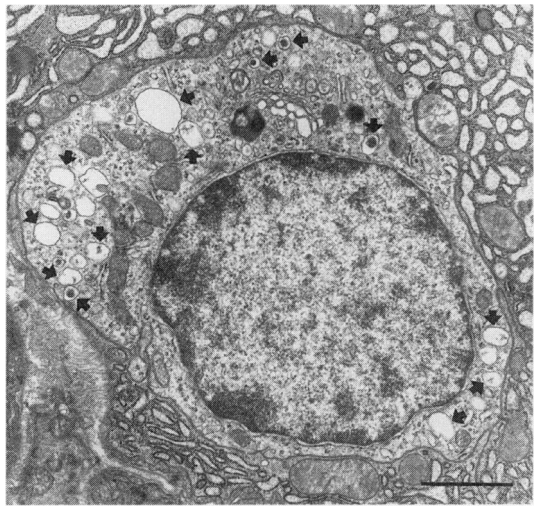

Figure 6. Electron micrograph of an ECL cell with typical secretory vesicles and a few granules (arrows). Bar = 1 μm.

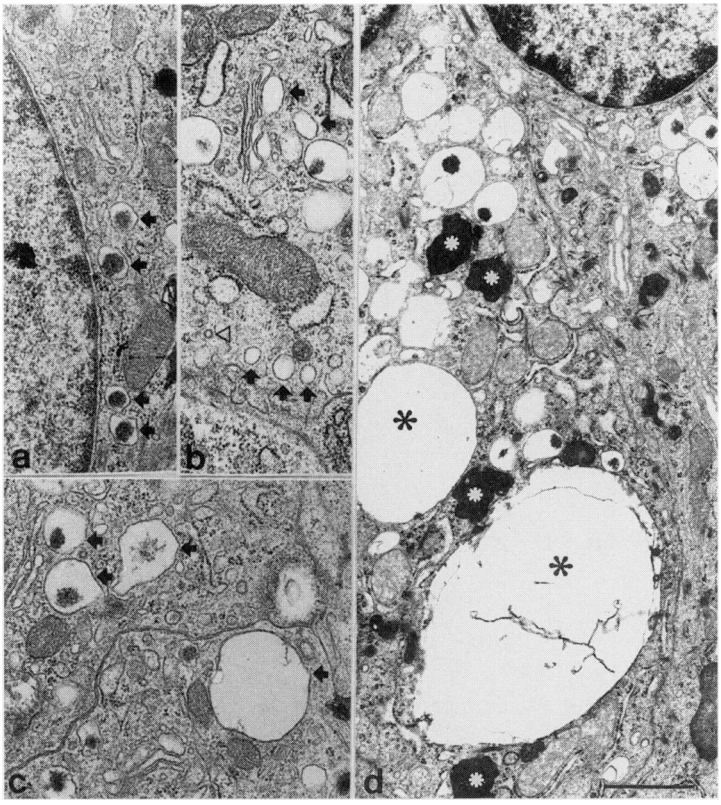

Figure 7. Electron micrographs of ECL cells showing granules (a, arrows), microvesicles (b, arrows), secretory vesicles (c, arrows), vacuoles (d, black asterisk) and lipofuscin bodies (d, white asterisks). Note, in b, a typical clathrin-coated vesicle indicated by a triangle. a-c Control rats, d Omeprazole-treated rat (10 weeks of daily administration of the drug). Bar in d = 200 nm.

organelle. Based on profile size, vesicles belong to one of three populations: 1) secretory vesicles with a diameter of 125-500 nm (with a dense core, which can be revealed by serial sectioning if not immediately apparent); 2) vacuoles with a diameter of at least 500 nm without visible dense core or possessing one (or more than one) dense core; and 3) electron-lucent microvesicles with a diameter of 25-125 nm. Lipofuscin bodies are identified by their irregular shape and high electron density (osmiophilia).

We have proposed a model for the development and maturation of secretory organelles and for the mechanism behind the fusion of membranes in these cells [11, 30]. The granules with their content of chromogranin A and peptide hormone precursor bud off from the Golgi apparatus and start to take up histamine during their transport to the more peripheral parts of the cells. As a result, they turn into secretory vesicles, possibly through osmotic forces, generated by the progressive accumulation of histamine and other small molecules. Among small molecules that will accumulate in the secretory vesicles are cleavage products of chromogranin A (e.g., pancreastatin) and of the anticipated peptide hormone precursor, which are generated by proteolysis within the granules. According to this concept, we should expect the secretory vesicles to represent a readily releasable pool of histamine and other secretory products, while the granules should represent a less readily releasable pool. As a consequence of stimulation, the secretory vesicles fuse with the cell

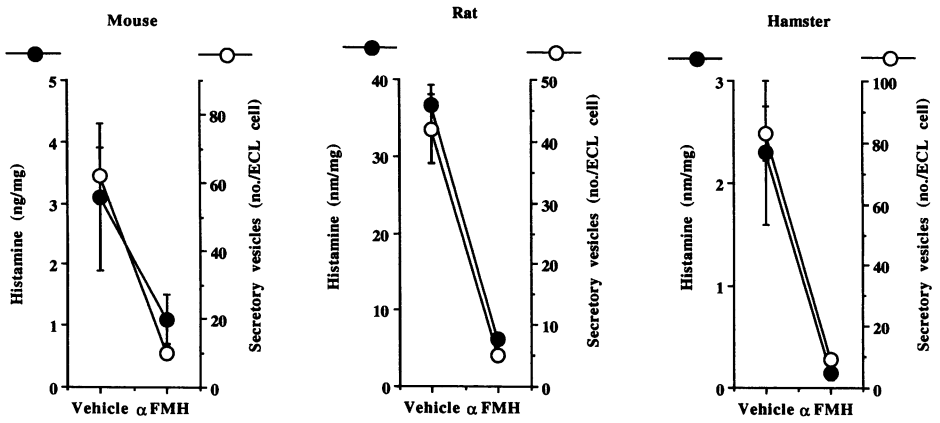

Figure 8. Histamine concentration in the oxyntic mucosa and number of secretory vesicles per ECL cell in the stomach of mice, rats and hamsters that were treated with vehicle (control) or α-FMH for 24 hr. Mean ± SEM (vertical bars, n = 7-8 animals and 36-41 cells in each group).

membrane to release their contents by exocytosis [11]. The microvesicles become numerous, perhaps as a result of stimulated recycling, which can be expected to lead to an increased number of retrieval vesicles [22]. Gastrin accelerates several of the steps that control the formation and subsequent maturation of the storage/secretory organelles, namely the formation of granules, their transformation into secretory vesicles, the excytosis and endocytosis (resulting in microvesicles), and the fusion of secretory vesicles with each other (resulting in vacuoles). Vacuoles and lysosomes probably play a role in the degradation of superfluous products (granule disposal or crinophagia). The highly electron dense material that accumulates within the lumen of lysosomes as a result of autophagic degradation is referred to as ceroid or lipofuscin (age pigment) [31-33]. We interpret the vacuoles and lipofuscin bodies that appear in ECL cells in response to long-term hypergastrinemia as crinophagic (autophagic) organelles.

Development of granules into secretory vesicles

α-Fluoromethylhistidine (α-FMH) is an irreversible and selective inhibitor of HDC [34]. Treatment with α-FMH results in a loss of 65 to 90 percent of the histamine in the oxyntic mucosa of mouse, rat and hamster, and the number of secretory vesicles in the ECL cells is reduced in parallel (Figure 8) [see also 13, 28, 35]. VMAT-2 has been found to exist in ECL cell granules/vesicles. Histamine is thought to accumulate in granules/secretory vesicles through action of VMAT-2. This transporter can be blocked by reserpine. Treatment with reserpine reduced greatly the number of large secretory vesicles while increasing the number of granules (Figure 9). The combination of gastrin and reserpine still reduced the number of large secretory vesicles but increased greatly the number of granules (Figure 10). Together, these findings support our hypotheses 1) that pre-formed histamine in the cytosol is taken up by granules, probably via VMAT-2 and 2) that the accumulation of histamine is necessary for transforming granules into secretory vesicles.

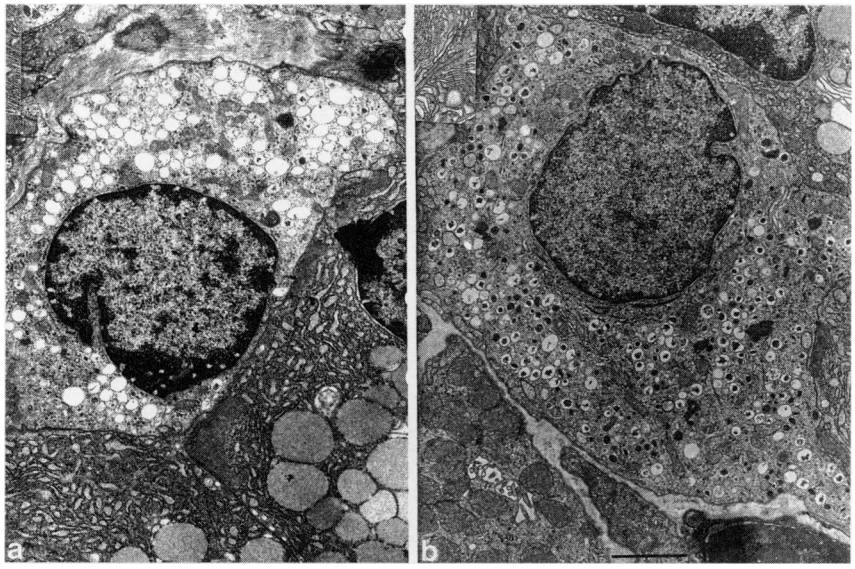

Figure 9. Electron micrographs showing ECL cells from a control rat (a) and from a rat treated with reserpine for two days (25 mg/kg i.p. once per day) (b). Numerous granules and few secretory vesicles in b. Bar in b = 1000 nm.

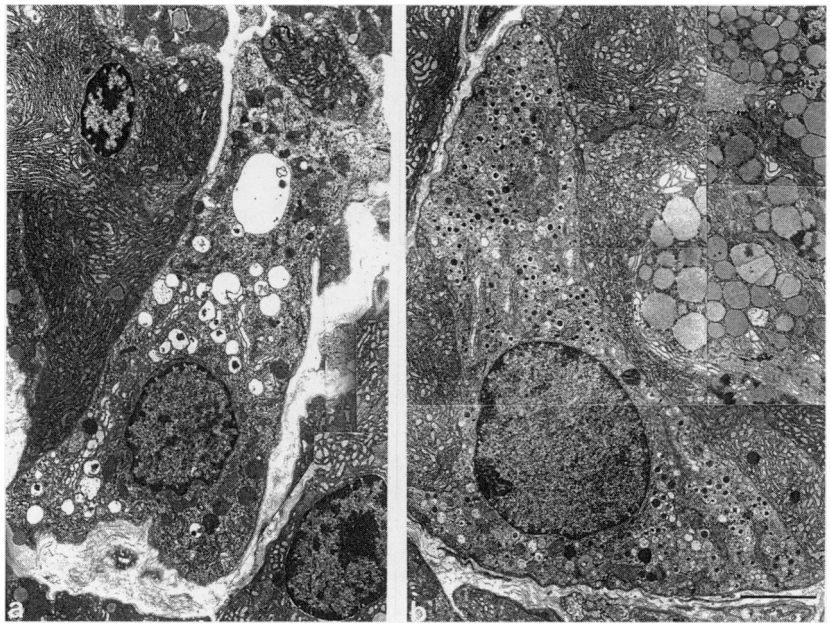

Figure 10. Electron micrographs showing ECL cells of rats treated with gastrin for 4 days (a) or gastrin for 4 days plus reserpine for the last 2 days (b). Note that after reserpine, large secretory vesicles are missing and vacuoles fail to develop in response to gastrin (b). Bar in b = 700 nm.

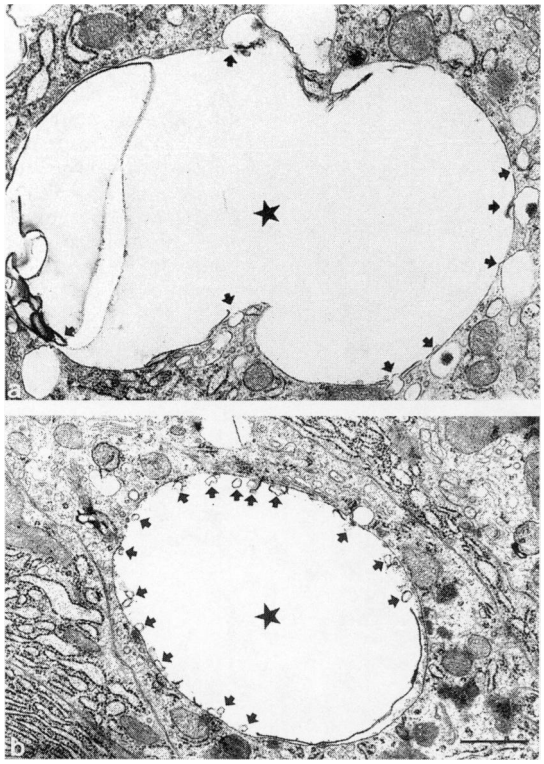

Figure 11. Electron micrograph showing (a) a vacuole (asterisk) and numerous secretory vesicles (arrows), and (b) a vacuole (asterisk) with membrane residues (arrows). The two vacuoles gives the impression of being engaged in fusion (a) and having been engaged in fusion (b). Bar in b = 200 nm.

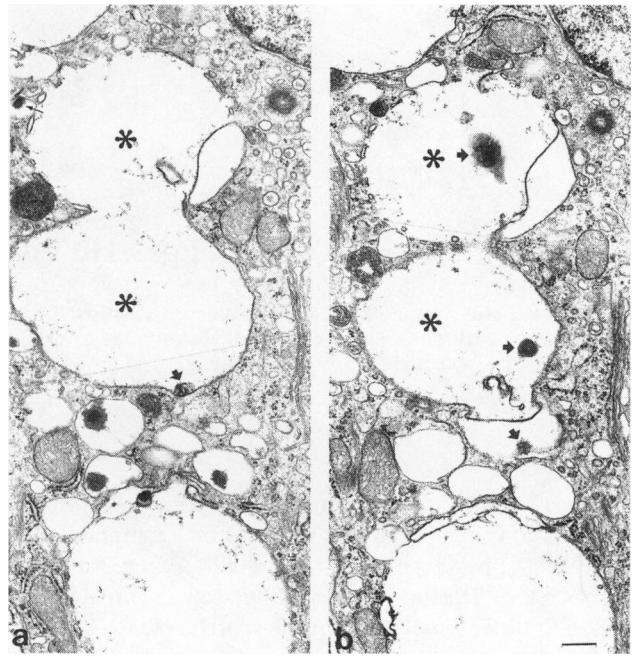

Figure 12. Two consecutive sections of a serially sectioned ECL cell from an omeprazole-treated rat (6 weeks of treatment) revealing two electron-dense cores (arrows) (b) in a vacuole that appeared to be empty when examined in the previous section (a). The vacuole in (a) appears as two vacuoles in (b) (asterisks). Bar in b = 300 nm.

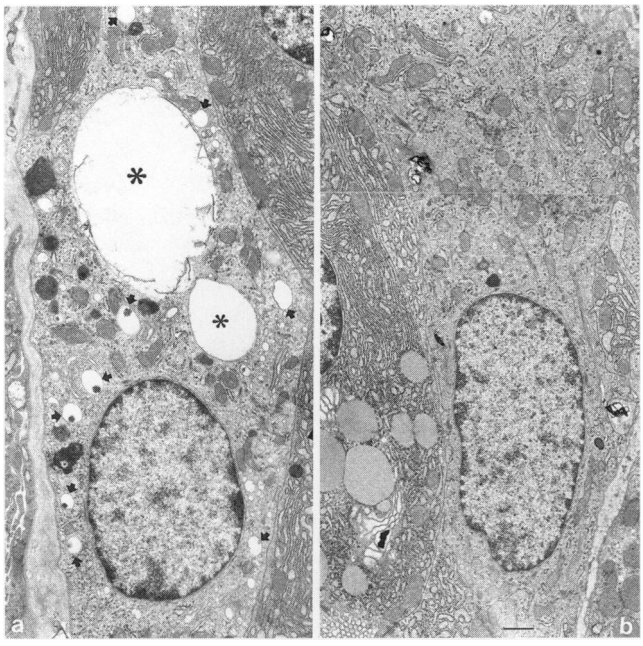

Figure 13. Electron micrographs showing ECL cells from an omeprazole-treated rat (a) and from an *a*-FMH and omeprazole- treated rat (b). Following omeprazole treatment (six weeks of treatment), granules are scarce, secretory vesicles (arrows) are fairly numrous and vacuoles are prominent (asterisks). α-FMH treatment eliminates the secretory vesicles and vacuoles. Bar in b = 200 nm.

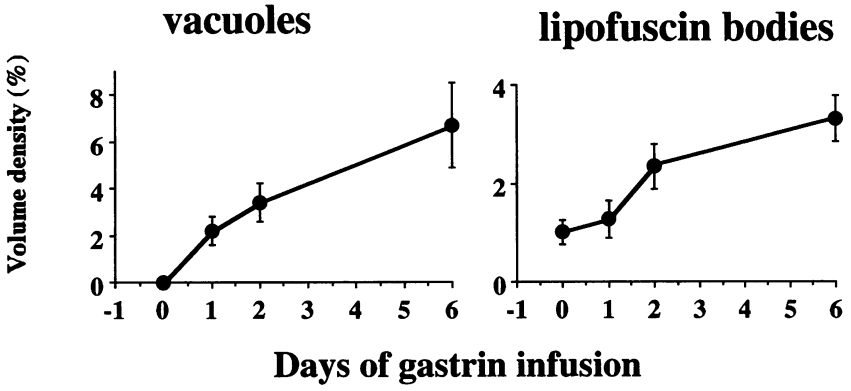

Figure 14. Time course of change in volume densities of vacuoles and of lipofuscin bodies in response to continuous, subcutaneous infusion of gastrin over a time period of six days. Mean ± SEM (n = 6-7 rats and 30-35 cells in each group).

Development of secretory vesicles into vacuoles

Vacuoles occur in response to long-term sustained hypergastrinemia induced by gastrin infusion or by treatment with gastric acid inhibitors such as omeprazole [22, 28, 36-38]. Their appearance is associated with a decrease in the number and volume density of the secretory vesicles. Conceivably, vacuoles arise as a result of the gastrin-evoked activation of the enterochromaffin-like cells, perhaps because the stimuli that induce intracellular membrane-membrane interactions result not only in exocytosis but also in vesicle fusion. The secretory vesicles in many of these cells line up in long rows or form aggregates, and the vesicles are often in such intimate contact that they give the impression of

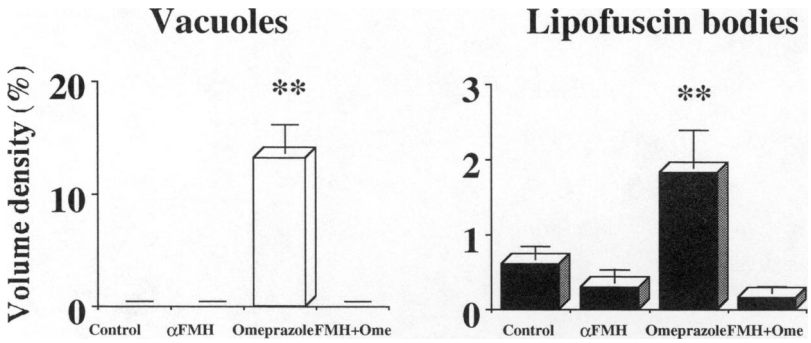

Figure 15. Volume densities of vacuoles and lipofuscin bodies in rats treated with vehicle, α-FMH (FMH), omeprazole (OME), or OME plus FMH for six weeks. Omeprazole treatment causes vacuoles and lipofuscin bodies to develop. α-FMH prevents this. Mean ± SEM (n = 8-9 rats and 30-35 cells). ** for $p < .01$; – in A and C = zero.

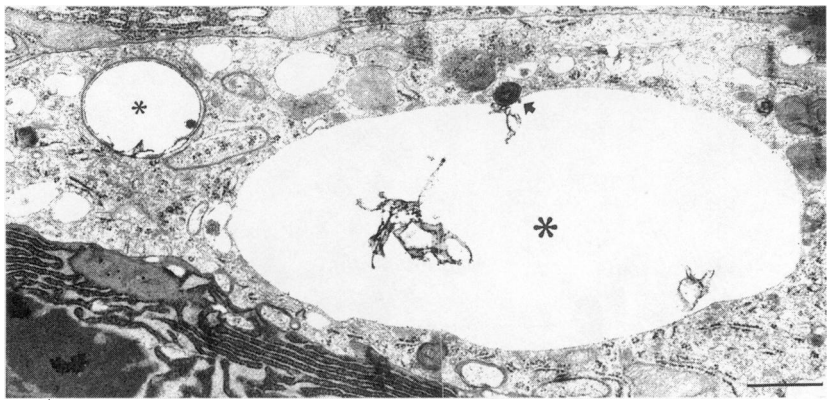

Figure 16. Electron micrograph suggesting that vacuoles (asterisks) and lipofuscin-containing secondary lysosomes may fuse (arrow). Omeprazole-treated rat (10 weeks of treatment). Bar = 300 nm.

being engaged in fusion (Figure 11). The following additional findings support the view that they result from the fusion of several secretory vesicles, namely 1) that the vacuoles at times contain more than one dense core (Figure 12) and 2) that α-FMH or reserpine treatment prevented the formation of vacuoles, conceivably by preventing or impairing the formation of secretory vesicles (Figure 13).

Development of vacuoles into lipofuscin

The increased number and volume density of vacuoles was associated with an increased number and volume density of lipofuscin bodies in the ECL cell cytoplasm in response to sustained gastrin stimulation (Figure 14). There was a good correlation between the number of vacuoles and of lipofuscin bodies ($r = .965$, $p < .001$) [29]. α-FMH-evoked ECL cell histamine depletion prevented the formation of vacuoles and also the development of lipofuscin (Figure 15). These observations indicate that vacuoles contribute to the formation of

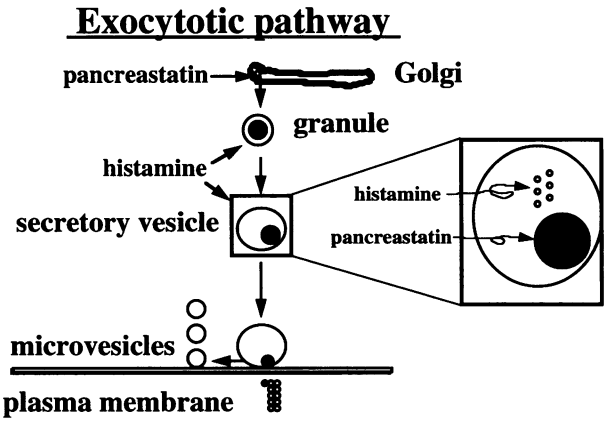

Figure 17. Schematic drawing of the proposed exocytotic pathway of the ECL cells (for details, see text). Rectangle indicates that histamine is stored in the electron lucent part, while pancreastatin is in the electron dense part.

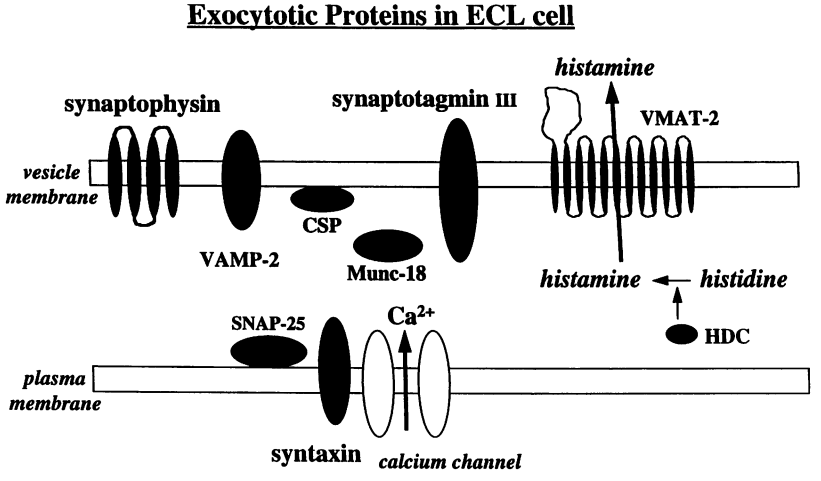

Figure 18. Schematic drawing of the suggested localization of different exocytotic proteins. Synaptophysin, VAMP and synaptotagmin III are all proteins that have transmembrane domains, which anchors them to the secretory vesicle membrane. CSP has so far only been shown to be associated with granules/secretory vesicles. SNAP-25 and syntaxin are associated with the plasma membrane, where syntaxin has a close relationship with calcium-channels. Munc-18 is located in the cytoplasm and has been shown to interact with syntaxin. Histamine is synthesized in the cytoplasm from histidine via the enzyme histidine decarboxylase (HDC), and is thereafter taken up via VMAT-2, located at the membrane of the granules/vesicles.

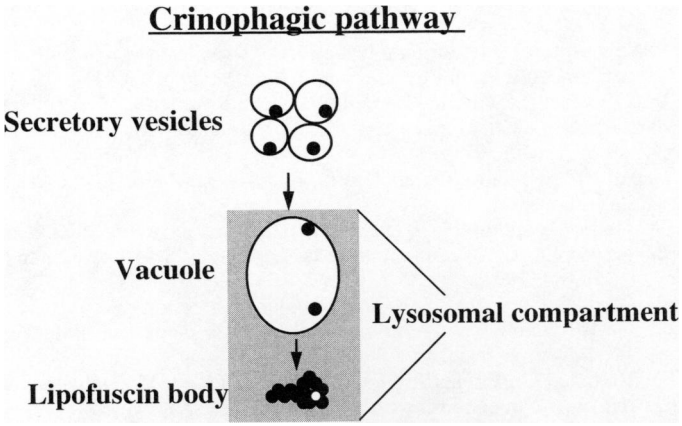

Figure 19. Schematic drawing of the proposed crinophagic pathway in the enterochromaffin-like cells (for details, see text).

lipofuscin. In ECL cells of hypergastrinemic rats, membrane-membrane fusion was seen not only between the secretory vesicles (resulting in vacuoles) but also between vacuoles and lipofuscin-containing secondary lysosomes (Figure 16). The findings are in line with the view that vacuoles form part of the lysosomal compartment. In fact, it has been suggested previously that material within lysosomal membrane-bound vacuoles (secondary lysosomes) can aggregate or disperse through fusion and fission [32, 39].

SUMMARY

1. Exocytotic pathway. The granules bud off from the Golgi apparatus. The intragranular proteins condense in the process, forming an electron-dense core. The newly formed granules take up histamine from the cytosol by VMAT-2 during their transport from the Golgi area to the more peripheral parts of the cells. As a result, the granules turn into secretory vesicles. The secretory vesicles represent a readily releasable pool of secretory products. As a consequence of stimulation, the secretory vesicles fuse with the cell membrane to release their contents by exocytosis (Figure 17). The ECL cells are rich in exocytotic proteins that promote granule/vesicle docking and fusion with the plasma membrane. Synaptophysin and VAMP-2 are vesicle proteins that participate in the docking of the organelle to the cell membrane. They bind to proteins in the cell membrane, such as SNAP-25 and syntaxin; cytosolic Munc-18 binds to syntaxin. The process of fusion of the secretory organelle with the cell membrane probably involves the interaction of synaptotagmin III, syntaxin and CSP with Ca^{2+} channels (Figure 18).

2. Crinophagic pathway. Long-lasting hypergastrinemia stimulates the development of vacuoles, which are formed by the fusion of several secretory vesicles. The development of vacuoles is associated with the development of lipofuscin bodies. The latter organelles probably result from lysosomal degradation of the vacuoles and their contents. The lipofuscin bodies contain the indigestible waste products of autophagia (Figure 19).

REFERENCES

1. Håkanson, R. New aspects of the formation and function of histamine, 5-hydroxytryptamine and dopamine in gastric mucosa. Acta Physiol. Scand. Suppl. (suppl)38-41, 1970.
2. Forsmann, W.G., Orci, L., Pictet, R., Renold, A.E., and Rouiller, C. The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. An electron microscope study. J. Cell Biol. 40:692-715, 1969.
3. Solcia, E., Capella, C., Vassallo, G., and Buffa, R. Endocrine cells of the gastric mucosa. Int. Rev. Cytol. 42:223-286, 1975.
4. Capella, C., Vassallo, G., and Solcia, E. Light and electron microscopic identification of the histamine-storing argyrophil (ECL) cell in murine stomach and of its equivalent in other mammals. Z. Zellforsch. 118:68-84, 1971.
5. Håkanson, R., Owman, C., Sporrong, B., and Sundler, F. Electron microscopic identification of histamine-storing argyrophil (enterochromaffin-like) cells in the rat stomach. Z. Zellforsch. 122:460-466, 1971.
6. Håkanson, R., Böttcher, G., Ekblad, E., Panula, P., Simonsson, H., 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.
7. Kubota, H., Taguchi, Y., Tohyama, M., Tatusura, N., Shiosaka, S., Ishihara, T., Watanabe, T., Shiotani, Y., and Wada, H. Electron microscopic identification of histidine decarboxylase-containing endocrine cells of the rat gastric mucosa: an immunohistochemical analysis. Gastroenterology 87:496-502, 1984.
8. Grube, D., Bargsten, G., Cetin, Y., and Yoshie, S. Chromogranins in mammalian GEP endocrine cells: their distribution and interrelations with co-stored amines and peptides. Arch. Histol. Cytol. 52:91-98, 1989.
9. Curry, W.J., Johnston, C.F., Shaw, C., and Buchanan, K.D. Distribution and partial characterization of immunoreactivity to the putative C-terminus of rat pancreastatin. Regul. Pept. 30:207-219, 1990.
10. Watkinson, A. and Dockray, G. Functional control of chromogranin A and B concentrations in the body of the rat stomach. Regul. Pept. 40:51-61, 1992.
11. Chen, D., Monstein, H.-J., Nylander, A.-G., Zhao, C.-M., Sundler, F., and Håkanson, R. Acute responses of rat stomach enterochromaffin-like cells to gastrin. Secretory activation and adaptation. Gastroenterology 107:17-28, 1994.
12. Nissinen, M. and Panula, P. Histamine-storing cells in the oxyntic mucosa of the rat stomach: a transmission electron microscopic study employing fixation with carbodiimide. J. Histochem. Cytochem. 41:1405-1412, 1993.
13. Andersson, K., Zhao, C.-M., Chen, D., Sundler, F., and Håkanson, R. Effect of α-fluoromethylhistidine-evoked histamine depletion on ultrastructure of endocrine cells in acid-producing mucosa of stomach in mouse, rat and hamster. Cell Tissue Res. 286:375-384, 1996.
14. Håkanson, R., Larsson, L.-I., Liedberg, G., and Sundler, F. Evidence for H_2 receptor- mediated feedback regulation of histamine release from endocrine cells in the rat stomach. J. Physiol. 276:151-157, 1978.
15. Sandvik, A.K., Waldum, H.L., Kleveland, P.M., and Schulze-Sognen, B. Gastrin produces an immmediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. Scand. J. Gastroenterol. 22:803-808, 1987.
16. Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H.F., and Sachs, G. Histamine secretion from rat enterochromaffinlike cells. Gastroenterology 105:449-461, 1993.
17. Waldum, H.L., Sandvik, A.K., Brenna, E., and Petersen, H. Gastrin-histamine sequence in the regulation of gastric acid secretion. Gut 32:698-701, 1991.
18. 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.
19. Håkanson, R., Chen, D., and Sundler, F. The ECL cells. In: Johnson, L.R., ed. Physiology of the Gastrointestinal Tract, Third Edition. New York: Raven Press; pp. 1171-1184, 1994.
20. Chen, D., Zhao, C.-M., Monstein, H.-J., Nylander, A.-G., Tielemans, Y., Sundler, F., and Håkanson, R. A time-table of rat stomach ECL cell responses to gastrin. In: Singer, M.V., Ziegler, R., and Rohr, G., eds. Gastrointestinal Tract and Endocrine System, Falk Symposium No. 77. Boston, London: Kluwer: Dordrecht.; 1994. pp. 178-185.
21. Håkanson, R., Tielemans, Y., Chen, D., Andersson, K., Mattsson, H., and Sundler, F. Time dependent changes in enterochromaffin like cell kinetics in stomach of hypergastrinemic rats. Gastroenterology 105:15-21, 1993.

22. Chen, D., Zhao, C.-M., Nylander, A-G., and Håkanson, R. Time course of hypertropic and ultrastructural response of rat stomach enterochromaffin-like cells to sustained hypergastrinemia. Cell Tissue Res. 284:55-63, 1996.
23. 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.
24. Håkanson, R., Ding, X.-Q., Norlén, P., and Chen, D. Circulating pancreastatin is a marker for the enterochromaffin-like cells of rat stomach. Gastroenterology 108:1445-1452, 1995.
25. Kimura, K., Chen, D., Linderström, 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
26. 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 WE14 in rat stomach ECL cells. Regul. Pept. 70:121-133, 1997.
27. Chen, D., Mårvik, R., Ronning, K., Andersson, K., Waldum, H.L., and Håkanson, R. Gastrin-evoked secretion of pancreastatin and histamine from ECL cells and of acid from parietal cells in isolated, vascularly perfused rat stomach. Effects of isobutyl methylxanthin and α-fluoromethylhistidine. Regul. Pept. 65:133-138, 1996.
28. Chen, D., Zhao, C.-M., Andersson, K., Sundler, F., and Håkanson, R. Ultrastructure of enterochromaffin-like cells in rat stomach: effects of α-fluoromethylhistidine-evoked histamine depletion and hypergastrinemia. Cell Tissue Res. 283:469-478, 1996.
29. Zhao, C.-M., Chen, D., Andersson, K., and Håkanson, R. Rat stomach ECL cells: development of lipofuscin in response to sustained gastrin stimulation. Cell Tissue Res. 291:315-323, 1997.
30. 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.
31. Brun, A. and Brunk, U.T. Histochemical indications for lysosomal location of heavy metals in normal rat brain and liver. J. Histochem. Cytochem. 18:820-828, 1970.
32. Brunk, U.T. Distribution and shifts of ingested marker particles in residual bodies and other lysosomes. Exp. Cell Res. 79:15-27, 1973.
33. Brunk, U.T., Jones, C.B., and Sohal, R.S. A novel hypothesis of lipofuscinogenesis and cellular aging based on interactions between oxidative stress and autophagocytosis. Mutation Res. 275:395-403, 1992.
34. Kollonitsch, J., Patchett, A.A., Marburg, S., Maycock, A.L., Perkins, L.M., Doldouras, G.A., Duggan, D.E., and Aster, S.D. Selective inhibitors of biosynthesis of aminergic neurotransmitters. Nature 274:906-908, 1978.
35. Andersson, K., Chen, D., Håkanson, R., Mattsson, H., and Sundler, F. Enterochromaffin-like cells in the rat stomach: Effect of α-fluoromethylhistidine-evoked histamine depletion. A chemical, histochemical and electron microscopic study. Cell Tissue Res. 270:7-13, 1992.
36. Böttcher, G, Håkanson, R., Nilsson, G., Seensalu, R., and Sundler, F. Effects of long-term hypergastrinemia on the ultrastructure of enterochromaffin-like cells in the stomach of the rat, hamster and guinea-pig. Cell Tissue Res. 256:247-257, 1989.
37. Chen, D., Håkanson, R., and Sundler, F. Effect of omeprazole-evoked hypergastrinemia on ultrastructure of enterochromaffin-like cells in the stomach of portacaval-shunted rats. Cell Tissue Res. 272:71-77, 1993.
38. Zhao, C.-M., Chen, D., Kimura, K., and Håkanson, R. Reversibility of omeprazole-evoked changes in rat stomach ECL cell ultrastructure. Cell Tissue Res. 291:91-95, 1998.
39. De Duve, C. The lysosome in retrospect. In: Dingle, J.T. and Fell, H.B., eds. Lysosomes in Biology and Pathology, Vol. 1. Amsterdam: North-Holland; 1969, pp. 3-40.