Evaluation of the trophic effect of longterm treatment with the histamine H_2 receptor antagonist loxtidine on rat oxyntic mucosa by differential counting of dispersed cells

E Brenna, H G P Swarts, C H W Klaassen, J J H H M de Pont, H L Waldum

Abstract

To evaluate whether the general trophic effect of gastrin on the oxyntic mucosa is an indirect effect mediated by histamine H_2 receptors, sustained 24 hour hypergastrinaemia was induced in Sprague-Dawley rats by treatment with the long acting and potent histamine H_2 antagonist loxtidine for five months. The trophic effect was assessed by weight, enumeration of total mucosal cells, parietal cells, and enterochromaffin like cells in smears stained for the actual cells after enzymatic dispersion of the mucosa, and by biochemical analysis of oxyntic mucosal homogenates. The weight of the whole stomach and the oxyntic mucosa increased by 12·7% (p=0·016) and 27·5% (p=0·006), respectively. Total oxyntic mucosal protein content increased by 28·7% (p=0·058). Total numbers of mucosal cells and parietal cells increased by 11·9% (NS) and 24·1% (NS), respectively. The amount of the parietal cell specific enzyme H^+,K^+-ATPase was unchanged. On the other hand, the number of enterochromaffin like cells and related parameters, histidine decarboxylase activity and histamine content of the oxyntic mucosa, showed a pronounced and significant increase. It is concluded that the general trophic effect of gastrin on the oxyntic mucosa is not mediated by the histamine H_2 receptor. The tropic effect of gastrin on the parietal cell seems, in contrast with that on the enterochromaffin like cell, not to be specific but only reflecting the general trophic effect on the oxyntic mucosa.

(*Gut* 1994; 35: 1547–1550)

Department of Medicine, University Hospital, Trondheim, Norway
E Brenna
H L Waldum

Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands
H G P Swarts
C H W Klaassen
J J H H M de Pont

Correspondence to:
Professor H L Waldum, Department of Medicine, University Hospital, N-7006 Trondheim, Norway.

Accepted for publication 23 March 1994

Gastrin stimulates the function[1] and growth of the oxyntic mucosa of the rat[2] as well as human.[3] Initially, the positive trophic effect of gastrin was thought to be particularly noticeable on the acid producing parietal cell.[2] It has been realised, however, that gastrin has a specific trophic effect on the enterochromaffin like cell,[4] whereas the effect on the parietal cell seems to be parallel to the general trophic effect of gastrin on the oxyntic mucosa.[5] The enterochromaffin like cell produces histamine in all vertebrates.[6] Gastrin stimulates release from[7] and synthesis[8] of histamine in this cell in quantities that, at least in the rat, fully explain the acid stimulatory effect of gastrin.[9] Hence, it was reasonable to question whether not only the functional, but also the general trophic effect of gastrin could be mediated by stimulation of histamine released from the enterochromaffin like cell.[10] There is evidence supporting this hypothesis: histamine producing enterochromaffin like cell carcinoids in mastomys are surrounded by a zone of parietal cell hyperplasia,[11] and the long acting potent histamine H_2 antagonist tiotidine has been reported to induce parietal cell atrophy.[12]

Quantification of the parietal cell mass has previously been done either functionally by assessing the maximal acid secretory capacity[13–15] or histomorphometrically by determining the parietal cell density and the oxyntic mucosal area.[2 5 13 14 16] The histomorphometric method is tedious, however, and also inaccurate, as the parietal cell density is not uniform and the estimation of the oxyntic area is uncertain. Moreover, the assessment of the parietal cell mass by means of maximal secretory capacity requires that a direct stimulus of the parietal cell is used in a dose giving maximal acid secretion.[10] At least in the rat, gastrin stimulates acid secretion mainly by the stimulation of histamine release,[17] and thus maximal gastrin stimulated acid secretion depends not only on the parietal, but also on the enterochromaffin like cell mass.[10 18 19] There is, therefore, obviously a need for an improved method to assess the parietal cell mass.

In this study, the parietal cell mass was assessed by differential counting of dispersed mucosal cells, and indirectly by quantification of the parietal cell specific enzyme H^+,K^+-ATPase.

Methods

The study was approved by the animal welfare committee of the University Hospital of Trondheim. Sprague-Dawley female rats (weighing about 225 g) were used because of their well known tendency to respond with hyperplasia of the enterochromaffin like cells secondary to hypergastrinaemia.[20] Fifteen rats received the potent and long acting histamine H_2 antagonist loxtidine[21] (a gift from Glaxo, UK) and 16 rats received the vehicle (water) by gavage once daily for five months. The

loxtidine dose was 100 mg/kg body weight, a dose similar to that previously shown to induce a sustained 24 hour hypergastrinaemia.[19] Otherwise, the two groups were housed and fed similarly. After three months, blood samples were taken from a superficial vein for determination of plasma gastrin concentration 24 hours after the last dosing. At the end of the study period, the rats were anaesthetised with 0·2 ml/100 g body weight of a combination of (per ml) 2·5 mg fluanisone, 0·05 mg phentanyl, and 1·25 mg midazolam. The animals were allowed to eat freely until anaesthetised. The last dose of loxtidine was given two hours before the rats were killed. The rats were killed by exsanguination by heart puncture. The blood was collected in tubes containing EDTA and centrifuged. Plasma gastrin concentration was determined by radioimmunoassay.[22]

CELL DISPERSION AND COUNTING

The stomach was removed from seven rats in each group and transformed to an everted sack. The mucosal cells were dispersed by means of proteolytic enzymes (Pronase-E; Merck, Darmstadt, Germany) installed into the everted stomach and incubated at 37°C for 105 minutes, essentially as described by Lewin *et al.*[23] All cells from each stomach were collected and counted. Smears were made for differential counting of parietal cells after staining with haematoxylin and eosin, and of enterochromaffin like cells after immunocytochemical staining, using histamine antibodies raised in rabbit (no 8431 Milab, Malmö, Sweden) as primary antibodies, goat antirabbit immunoglobulins as secondary antibodies, and finally visualised using the peroxidase-antiperoxidase technique. The completeness of the dispersion of the gastric mucosa was ascertained by histological examination of the denuded mucosa. All these methods have been described in detail previously.[24]

ANALYSIS OF OXYNTIC MUCOSAL HOMOGENATES

From the remaining rats (eight rats in the loxtidine group and nine rats in the control group), the stomach was removed, opened along the greater curvature, rinsed in ice cold 0·9% saline, and weighed. After removal of the antrum, the oxyntic mucosa was scraped off the remaining body of the stomach, diluted in distilled water to 100 mg/ml and homogenised. The homogenates were stored at −70°C until analysis.

The histamine content of the oxyntic mucosa was determined by radioimmunoassay, as described previously (Immunotech, Marseilles, France).[25 26]

Enzymatic activity of mucosal histidine decarboxylase was determined by the procedure described by Beaven *et al*[27] with some modifications.[28] We incubated an 80 µl aliquot of the oxyntic mucosal homogenate with [1-^{14}C]L-histidine (24 nCi, 0·48 nmol; New England Nuclear, Boston, MA, USA), 0·5 mM L-histidine, and 10 mM pyridoxal-5-phosphate in a total reaction volume of 160 µl at 37°C for 60 minutes. The reaction was stopped by adding 80 µl 2 M perchloric acid followed by an incubation at 37°C for 30 minutes. The expelled $^{14}CO_2$ was trapped in 50 µl Protosol (New England Nuclear) and counted in scintillation fluid. Results are expressed as nmol $^{14}CO_2$ produced per hour.

The amount of H^+,K^+-ATPase in the homogenates was determined by an inhibition enzyme linked immunosorbent assay. This consisted of plate A: on a 96-well microtitre plate (Greiner, Alphen a/d Rijn, The Netherlands) 0·5 µg of purified pig gastric H^+,K^+-ATPase[29] (50 µl in phosphate buffered saline (PBS)) was immobilised in each well by overnight incubation at 4°C. Blocking was done by incubating with 200 µl 1% gelatin in PBS for two hours at room temperature and then the plates were washed with PBS containing 0·05% (wt/vol) TWEEN 20. Plate B: this plate was precoated with PBS-gelatin. Twenty µl of oxyntic mucosal homogenates and control samples containing purified pig H^+,K^+-ATPase preparation (containing 150 µg and 15 µg H^+,K^+-ATPase per ml) were incubated for 30 minutes with 5 µl 5% (wt/vol) TWEEN 20 at room temperature. After dilution to 200 µl with PBS, 40 µl was used to determine the protein concentration according to the Bio-Rad procedure (using bovine serum albumin as a standard) and either 60 µl sample or 60 µl of twofold serial dilutions were incubated with 60 µl of the monoclonal antibody 5B6.[30] After 60 minutes 100 µl of this mixture was transferred to plate A and the unbound monoclonal antibody was allowed to react for 60 minutes with H^+,K^+-ATPase. After washing with PBS-TWEEN 20 (five times), the antibody-antigen complex was incubated with peroxidase conjugated rabbit antimouse immunoglobulins (Dakopatts, Denmark), which was diluted 800 times in PBS with 1% gelatin. After 60 minutes and washing for five times with PBS-TWEEN 20, a substrate solution containing 0·4 mg/ml o-phenylenediamine (Sigma Chemicals, St Louis, MO, USA) in 24 mM citric acid and 51 mM sodium hydrogenphosphate buffer and 0·012% (wt/vol) hydrogen peroxide was added. After 10 minutes the reaction was stopped with 2 M sulphuric acid and the absorption was measured at 492 nm on a Titertek Multiskan (Bio-Rad).

After correction for unspecific binding (no H^+,K^+-ATPase), the dilution that gave 50% reduction of the absorption was determined and compared with that of pig gastric H^+,K^+-ATPase preparations. The values are expressed as the amount of H^+,K^+-ATPase per mg protein. The calculated total amount of H^+,K^+-ATPase in the oxyntic mucosa is also given. The significance of the differences was evaluated by the Student's *t* test. All values are given as mean (SD).

Effect on the rat oxyntic mucosa of loxtidine treatment (100 mg/kg body weight) for five months

	Loxtidine	*Control*	*p Value*
Rat weight (g)	298 (19·2)	294 (15·2)	NS
Plasma gastrin (pM) 2 hours after dosing	149·8 (80·6)	80·7 (43·7)	0·006
Plasma gastrin (pM) 24 hours after dosing	104·0 (70·5)	41·8 (13·3)	0·002
Plasma histamine (nM) 2 hours after dosing	554·3 (305·1)	316·3 (178·3)	0·012
Whole stomach weight (g)	1·960 (0·208)	1·739 (0·120)	0·016
Weight of oxyntic mucosa (g)	0·403 (0·065)	0·316 (0·047)	0·006
Mucosal protein content (mg)	51·28 (11·28)	39·83 (11·64)	0·058
Mucosal cells$\times 10^7$	22·5 (5·7)	20·1 (4·2)	NS
Parietal cells$\times 10^6$	57·7 (12·5)	46·5 (13·6)	NS
(% of total)	25·9 (2·2)	22·9 (2·4)	p=0·033
Mucosal H^+,K^+-ATPase concentration (μg/mg protein)	24·5 (4·7)	27·9 (5·3)	NS
Total amount of mucosal H^+,K^+-ATPase (μg)	1184·5 (273·5)	1139·8 (378·4)	NS
Enterochromaffin like cells$\times 10^6$	15·3 (4·2)	5·9 (2·5)	0·001
(% of total)	6·9 (1·0)	2·8 (0·8)	0·001
Mucosal histamine content (nmol)	512·1 (242·9)	157·2 (47·1)	0·001
Histidine decarboxylase activity (nmol CO_2/h)	153·2 (100·0)	19·6 (9·0)	0·001

All values are given as mean (SD).

Results

The Table summarises the results.

Loxtidine induced a significant increase in plasma gastrin concentration two hours and 24 hours after the last dose. Loxtidine did not affect the weight of the rats. The weights of the whole stomach and oxyntic mucosa were significantly increased in the rats treated with loxtidine. The total amount of protein also increased, although of borderline statistical significance. On the other hand, the number of gastric mucosal cells did not increase significantly. The increase in parietal cell mass induced by loxtidine was more noticeable, but did not reach significance. The mucosal amount of the parietal cell specific enzyme H^+,K^+-ATPase was unaffected by the loxtidine treatment.

The enterochromaffin like cell mass was three times as high in the loxtidine treated rats with an even more noticeable increase in the activity of the enterochromaffin like cell specific enzyme histidine decarboxylase, and mucosal histamine content.

Discussion

In this study we have assessed the total masses of the parietal and enterochromaffin like cells by enzymatic dispersion and counting of the mucosal cells followed by differential counting on smears stained for the actual cells. This method is rapid, and permits determination of the total cell masses, not only the density of the different cell types. Furthermore, a new enzyme linked immunosorbent assay for the determination of H^+,K^+-ATPase in mucosal homogenates is described, and quantification of this specific enzyme is also used for assessment of the parietal cell mass. An immunoassay of pig and human gastric H^+,K^+-ATPase has been described previously, but that method could not be used on homogenates.[31]

The findings confirm the specific trophic effect of profound acid inhibition on the enterochromaffin like cells,[4 28] an effect mediated by hypergastrinaemia.[4 32] The magnitude of the enterochromaffin like cell hyperplasia recorded in this study, is comparable with what has been found previously after prolonged hypergastrinaemia, assessed by histomorphomtery.[4 18 19 28] The histidine decarboxylase activity increased more than the enterochromaffin like cell mass, showing that loxtidine treatment increased the enzyme activity in the individual enterochromaffin like cells. Gastrin seems to regulate the histidine decarboxylase gene expression,[33] and the increase in enzyme activity in the individual cells therefore probably reflects the hypergastrinaemia in the loxtidine treated rats.

The whole stomach weight increased in the loxtidine treated group, and this increase was mainly caused by an increased weight of the oxyntic mucosa. The oxyntic mucosal cell number and the total amount of mucosal protein increased only slightly and non-significantly. Taken together, these findings suggest that the increase in the oxyntic mucosal weight may partly be caused by an increase in the intercellular matrix. The parietal cell mass showed a stronger tendency to increase than the total mucosal cell mass, and in percentage terms the increase in parietal cells reached significance. Accordingly, the general trophic effect of gastrin on the oxyntic mucosa seems not to be mediated by the histamine H_2 receptor. This is in agreement with a recent study by Andersson *et al*[34] showing that hypergastrinaemia induced a general trophic effect on the oxyntic mucosa also when the enterochromaffin like cells were totally depleted of histamine.

Initially, gastrin was thought to have a specific trophic effect on the parietal cell,[2] but this has been difficult to confirm.[5 35 36] In this study, the number of parietal cells increased by 24·1%, whereas the total amount of the parietal cell specific enzyme H^+,K^+-ATPase was unaffected by the loxtidine induced hypergastrinaemia, suggesting a reduction in the concentration of the enzyme per parietal cell. This may show that longterm potent histamine H_2 blockade inhibits the production of the H^+,K^+-ATPase. In contrast with omeprazole induced hypergastrinaemia, hypergastrinaemia secondary to histamine H_2 blockade does not activate the H^+,K^+-ATPase gene in short term experiments.[37] On the other hand, the H^+,K^+-ATPase gene expression was unchanged during seven days of treatment with omeprazole.[38] To our knowledge, however, the effect of more longterm hypergastrinaemia, either induced by histamine H_2 antagonists or H^+,K^+-ATPase inhibitors, on the H^+,K^+-ATPase gene expression has not been examined.

In conclusion, we describe a new and simplified method for the determination of different mucosal cell masses, and confirm that hypergastrinaemia secondary to longterm acid inhibition induced by a histamine H_2 blocker evokes a specific trophic effect of the enterochromaffin like cell and a less pronounced general trophic effect of the oxyntic mucosa. Accordingly, it is highly unlikely that the general trophic effect of gastrin on the oxyntic mucosa is mediated by a histamine H_2 receptor. The enterochromaffin like cell, however, having ultrastructural

features of a peptide producing cell,[39] may also secrete peptides with trophic effect on the gastric progenitor cells.

1 Makhlouf GM, McManus JPA, Card WI. The action of the pentapeptide (ICI 50,123) on gastric secretion in man. *Gastroenterology* 1966; **51:** 455–65.
2 Crean GP, Marshall MW, Rumsey RDE. Parietal cell hyperplasia induced by the administration of pentagastrin (ICI 50,123) to rats. *Gastroenterology* 1969; **57:** 147–56.
3 Ellison EH, Wilson SD. Further observations on factors influencing the symptomatology manifest by patients with Zollinger-Ellison syndrome. In: Shnitka TK, Gilbert JAL, Harrison RC, eds. *Gastric secretion.* New York: Pergamon, 1967: 363–9.
4 Creutzfeldt W, Stöckmann F, Conlon JM, Fölsch UR, Bonatz G, Wülfrath M. Effect of short- and long-term feeding of omeprazole on rat gastric endocrine cells. *Digestion* 1986; **35** (suppl 1): 84–97.
5 Håkanson R, Blom H, Carlsson E, Larsson H, Ryberg B, Sundler F. Hypergastrinaemia produces trophic effects in stomach but not in pancreas and intestines. *Regul Pept* 1986; **13:** 225–33.
6 Håkanson R, Böttcher G, Ekblad E, Panula P, Simonsson M, Dohlsten M, *et al.* Histamine in endocrine cells in the stomach. A survey of several species using a panel of histamine antibodies. *Histochemistry* 1986; **86:** 5–17.
7 Sandvik AK, Waldum HL, Kleveland PM, Schulze Søgnen B. Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. *Scand J Gastroenterol* 1987; **22:** 803-8.
8 Kahlson G, Rosengren E, Svahn D, Thunberg R. Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J Physiol* 1964; **174:** 400–16.
9 Waldum HL, Sandvik AK, Brenna E, Schulze Søgnen B. Radioimmunoassay of histamine. *Scand J Gastroenterol* 1991; **26** (suppl 180): 32–9.
10 Waldum HL, Brenna E, Sandvik AK, Petersen H. Trophic effect of histamine on the stomach. *Scand J Gastroenterol* 1991; **26** (suppl 180): 137–42.
11 Modlin IM, Zucker KA, Zdon MJ, Sussman J, Adrian TE. Characteristics of the spontaneous gastric endocrine tumor of mastomys. *J Surg Res* 1988; **44:** 205–15.
12 Streett CS, Cimprich RE, Robertson JL. Pathologic findings in the stomachs of rats treated with the H_2-receptor antagonist tiotidine. *Scand J Gastroenterol* 1984; **19** (suppl 101): 109–17.
13 Marks IN, Komarov SA, Shay H. Acid secretory responses to histamine and the parietal cell mass in the dog. *Am J Physiol* 1958; **195:** 528–34.
14 Card WI, Marks IN. The relationship between the acid output of the stomach following 'maximal' histamine stimulation and the parietal cell mass. *Clin Sci* 1960; **19:** 147–63.
15 Carlsson E, Larsson H, Mattsson H, Ryberg B, Sundell G. Pharmacology and toxicology of omeprazole – with special reference to the effect on the gastric mucosa. *Scand J Gastroenterol* 1986; **21** (suppl 118): 31–8.
16 Hogben CAM, Kent TH, Woodward PA, Sill AJ. Quantitative histology of the gastric mucosa: man, dog, cat, guinea pig, and frog. *Gastroenterology* 1974; **67:** 1143–54.
17 Waldum HL, Sandvik AK, Brenna E, Petersen H. Gastrin-histamine sequence in the regulation of gastric acid secretion. *Gut* 1991; **32:** 698–701.
18 Waldum HL, Lehy T, Brenna E, Sandvik AK, Petersen H, Schulze Søgnen B, *et al.* Effect of the histamine-1 antagonist astemizole alone or with omeprazole on rat gastric mucosa. *Scand J Gastroenterol* 1991; **26:** 23-35.
19 Brenna E, Waldum HL, Sandvik AK, Schulze Søgnen B, Kristensen A. Effects on the rat oxyntic mucosa of the histamine-2 antagonist loxtidine and the H^+,K^+-ATPase inhibitor omeprazole. *Aliment Pharmacol Ther* 1992; **6:** 335–49.
20 Ekman L, Hansson E, Havu N, Carlsson E, Lundberg C. Toxicological studies on omeprazole. *Scand J Gastroenterol* 1985; **20** (suppl 108): 53–69.
21 Brittain RT, Jack D, Reeves JJ, Stables R. Pharmacological basis for the induction of gastric carcinoid tumours in the rat by loxtidine, an unsurmountable histamine H_2-receptor blocking drug. *Br J Pharmacol* 1985; **85:** 843–7.
22 Kleveland PM, Haugen SE, Waldum HL. The preparation of bioactive ^{125}I-gastrin, using iodo-gen as oxidizing agent, and the use of this tracer in receptor studies. *Scand J Gastroenterol* 1985; **20:** 569–76.
23 Lewin M, Cheret AM, Soumarmon A, Girodet J. Métode pour l'isolement et le tri des cellules de la muqueuse fundique de rat. *Biol Gastroenterol (Paris)* 1974; **7:** 139–44.
24 Brenna E, Waldum HL. Studies of isolated parietal and enterochromaffin-like cells from the rat. *Scand J Gastroenterol* 1991; **26:** 1295–306.
25 Morel AM, Delaage MA. Immunoanalysis of histamine through a novel chemical derivatization. *J Allergy Clin Immunol* 1988; **82:** 646–54.
26 Waldum HL, Brenna E, Sandvik AK, Kleveland PM, Petersen H, Søgnen B. Histamine and gastrin in plasma of patients with upper gastrointestinal diseases. *Digestion* 1989; **42:** 121–7.
27 Beaven MA, Wilcox G, Terpstra GK. A microprocedure for the measurement of $^{14}CO_2$ release from [^{14}C] carboxyl-labelled amino acids. *Anal Biochem* 1978; **84:** 638–41.
28 Larsson H, Carlsson E, Mattsson H, Lundell L, Sundler F, Sundell G, *et al.* Plasma gastrin and gastric enterochromaffin-like cell activation and proliferation. Studies with omeprazole and ranitidine in intact and antrectomized rats. *Gastroenterology* 1986; **90:** 391–9.
29 Swarts HGP, Van Uem TJF, Hoving S, Fransen JAM, De Pont JJHHM. Effects of free fatty acids and detergents on H,K-ATPase. The steady-state ATP phosphorylation level and the orientation of the enzyme in membrane preparations. *Biochim Biophys Acta* 1991; **1070:** 283–92.
30 Van Uem TJF, Peters WHM, De Pont JJHHM. A monoclonal antibody against pig gastric H^+/K^+-ATPase, which binds to the cytosolic $E_1{\cdot}K^+$ form. *Biochim Biophys Acta* 1990; **1023:** 56–62.
31 Smolka A, Weinstein WM. Immunoassay of pig and human gastric proton pump. *Gastroenterology* 1986; **90:** 532–9.
32 Håkanson R, Sundler F. Proposed mechanism of induction of gastric carcinoids: the gastrin hypothesis. *Eur J Clin Invest* 1990; **20** (suppl 1): 65–71.
33 Dimaline R, Sandvik AK. Histidine decarboxylase gene expression in rat fundus is regulated by gastrin. *FEBS Lett* 1991; **281:** 20–2.
34 Andersson K, Håkanson R, Mattsson H, Ryberg B, Sundler F. Hyperplasia of histamine-depleted enterochromaffine-like cells in rat stomach using omeprazole and α-fluoromethylhistidine. *Gastroenterology* 1992; **103:** 897–904.
35 Larsson H, Carlsson E, Ryberg B, Fryklund J, Wallmark B. Rat parietal cell function after prolonged inhibition of gastric acid secretion. *Am J Physiol* 1988; **254:** G33–9.
36 Helander HF, Rutgersson K, Helander KG, Pisegna JP, Gardner JD, Jensen RT, *et al.* Stereologic investigations of human gastric mucosa. II. Oxyntic mucosa from patients with Zollinger-Ellison syndrome. *Scand J Gastroenterol* 1992; **27:** 875–83.
37 Tari A, Yamamoto G, Sumii K, Sumii M, Takehara Y, Haruma K, *et al.* Role of histamine$_2$ receptor in increased expression of rat H^+-K^+-ATPase alpha-subunit induced by omeprazole. *Am J Physiol* 1993; **265:** G752–8.
38 Swarovsky B, Eissele R, Gross M, Körtner G, Koop H, Arnold R, *et al.* Expression of the gastric H^+/K^+-ATPase and histidine decarboxylase during omeprazole treatment. *Digestion* 1994; **55:** 97–102.
39 Capella C, Finzi G, Cornaggia M, Usellini L, Luinetti O, Buffa R, *et al.* Ultrastructural typing of gastric endocrine cells. In: Håkanson R, Sundler F, eds. *The stomach as an endocrine organ.* Fernström Foundation Series. Vol 15. Amsterdam: Elsevier, 1991: 27–51.