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ECL cells of the rat stomach: development of lipofuscin in response to sustained gastrin stimulation

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Abstract Ageing cells, especially post-mitotic cells, are known to accumulate pigments, i.e. highly electron-dense material, referred to as ceroid or lipofuscin. This material is formed as a consequence of autophagocytosis and peroxidation of the products undergoing degradation. The present study describes the development of lipofuscin in the ECL cells of the rat stomach. These cells produce and secrete histamine in response to gastrin. They are rich in secretory vesicles, which fuse to form vacuoles in hypergastrinaemic rats. Hypergastrinaemia was induced by continuous infusion of human Leu^{15}-gastrin-17 for 6 days or by daily treatment with omeprazole for 10 weeks. Either treatment caused both vacuoles and lipofuscin bodies to appear in large numbers; the vacuoles disappeared promptly after interruption of the hypergastrinaemia, whereas the lipofuscin bodies remained. Antrectomy-evoked hypogastrinaemia was associated with a reduced number and volume density of lipofuscin bodies. Treatment with α-fluoromethylhistidine, an irreversible inhibitor of the histamine-forming enzyme, resulted in depletion of ECL-cell histamine and was found to prevent the omeprazole-evoked formation of vacuoles and lipofuscin. The numbers of both vacuoles and lipofuscin bodies were well-correlated with the serum gastrin concentration, suggesting that gastrin stimulates the development not only of vacuoles but also of lipofuscin, perhaps through enhanced autophagocytosis and/or oxidative stress. Thus, lipofuscin bodies may develop from vacuoles, and both vacuoles and lipofuscin bodies may reflect the efforts of overstimulated ECL cells to cope with the excessive formation of secretory products.

Key words Stomach · ECL cells · Gastrin · Omeprazole · Vacuoles · Lipofuscin · Rat (Sprague Dawley)

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Introduction

The acid-producing mucosa of the stomach is rich in endocrine cells (Sundler and Håkanson 1991), such as ECL cells, A-like cells, D cells, D_1/P cells and, in some species, enterochromaffin cells. They can be distinguished from each other on the basis of their characteristic ultrastructure (Forssmann et al. 1969; Håkanson et al. 1971; Solcia et al. 1975; Capella et al. 1991). ECL cells produce histamine and respond to gastrin by the release of histamine (for a review, see Håkanson et al. 1994), which is thought to control the activity of the parietal cells by diffusion (Waldum et al. 1991; Andersson et al. 1996). Upon sustained gastrin stimulation, ECL-cell protein synthesis and self-replication rate are accelerated, resulting in hypertrophy and hyperplasia and with time in dysplasia and neoplasia (for a review, see Håkanson et al. 1994).

ECL cells are rich in large electron-lucent vesicles with a small, eccentrically located electron-dense core. In addition, a few small dense-cored granules and clear microvesicles can be observed (Håkanson et al. 1971; Chen et al. 1996a). α-Fluoromethylhistidine (α-FMH), an inhibitor of the histamine-forming enzyme histidine decarboxylase (HDC), can be used to deplete histamine from the ECL cells (Andersson et al. 1992a, b; Chen et al. 1996a). Depletion of ECL-cell histamine is associated with a markedly reduced number of secretory vesicles (Andersson et al. 1992a, b; Chen et al. 1996a). Furthermore, gastrin stimulation results in the transient loss of both histamine and secretory vesicles from the ECL cells; the return of histamine is associated with the return of secretory vesicles (Chen et al. 1994a). These observa-

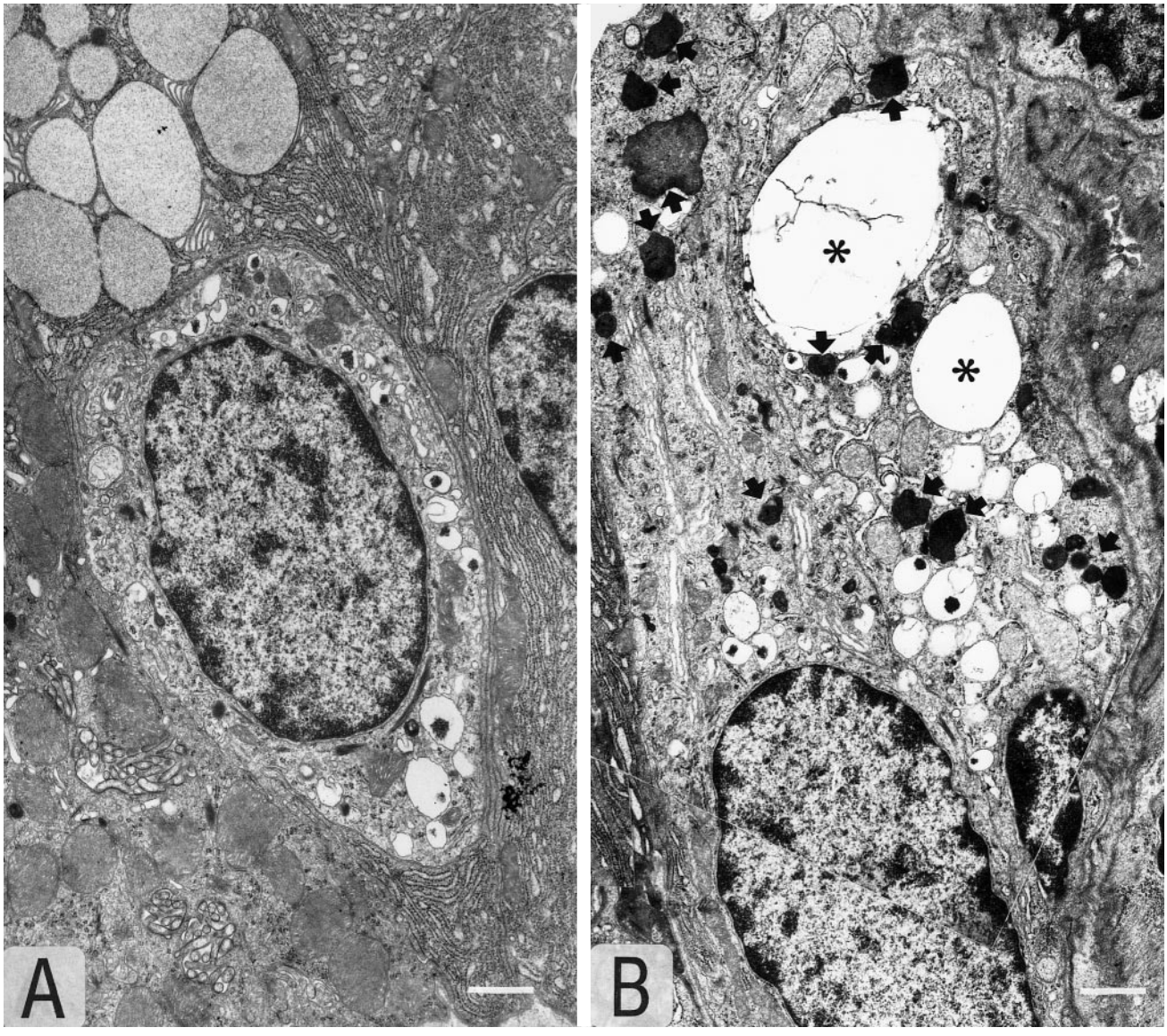


Fig. 1 Electron micrographs showing ECL cells of a control rat (**A**) and after gastrin infusion for 6 days (**B**). Note the vacuoles (*asterisks*) and lipofuscin bodies (*arrows*) in the ECL cell from the gastrin-treated rat. *Bars* 200 nm

tions suggest that the secretory vesicles represent a major storage site of ECL-cell histamine (Andersson et al. 1992a; Chen et al. 1996a, b). We have outlined a scheme for the storage and transport of secretory products (histamine, chromogranin A and peptide hormone), according to which granules develop into secretory vesicles by actively accumulating preformed histamine from the cytosol (Chen et al. 1996a, b). After sustained stimulation with gastrin, vacuoles appear in the ECL cells; they are very prominent and seem to be formed by the fusion of secretory vesicles (Böttcher et al. 1989; Chen et al. 1993, 1996a, b). We have suggested that such vacuoles interact with lysosomes in the degradation of superfluous secretory products (Chen et al. 1996b). The lysosomal compartment is the major site for the degradation of intracellular material (Essner and Novikoff 1960; Ericsson 1969). The highly electron-dense material that accumulates in the lysosomes of ageing cells and in cells subjected to sustained oxidative stress (premature ageing) is referred to as lipofuscin or ceroid, or sometimes as age pigment (Brun and Brunk 1970; Brunk 1973; Brunk and Collins 1981; Brunk and Cadenas 1988; Brunk et al. 1992).

The present paper describes the time course of the development of lipofuscin in ECL cells in response to hypergastrinaemia induced by gastrin infusion for 6 days or by omeprazole treatment for 10 weeks. Furthermore, we have examined the effect of α-FMH-induced depletion of ECL-cell histamine on the gastrin-evoked formation of lipofuscin, explored the consequences of lowering circulating gastrin by antrectomy and of normalizing the serum gastrin concentration after withdrawal of omeprazole, and evaluated the relationships between the serum gastrin concentration and the number of vacuoles or lipofuscin bodies in the ECL cells on one

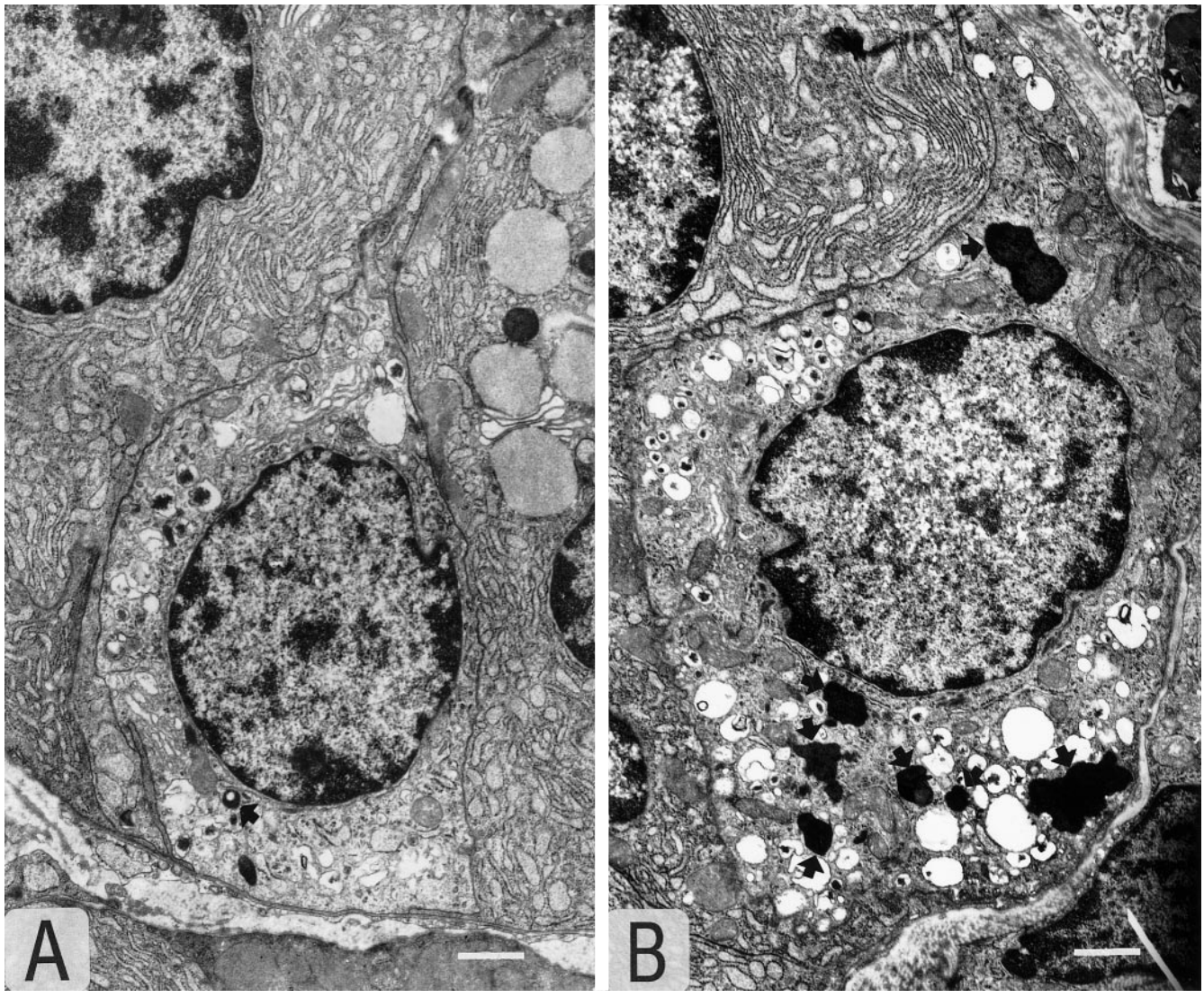


Fig. 2 Electron micrographs showing ECL cells of a control rat (**A**) and of a rat treated with omeprazole for 2 weeks followed by withdrawal of the drug for 40 days (**B**). Note the numerous lipofuscin bodies (*arrows*) but no vacuoles in a ECL cell from the omeprazole pre-treated rat. *Bars* 200 nm

hand and between vacuoles and lipofuscin bodies on the other.

Materials and methods

Drugs

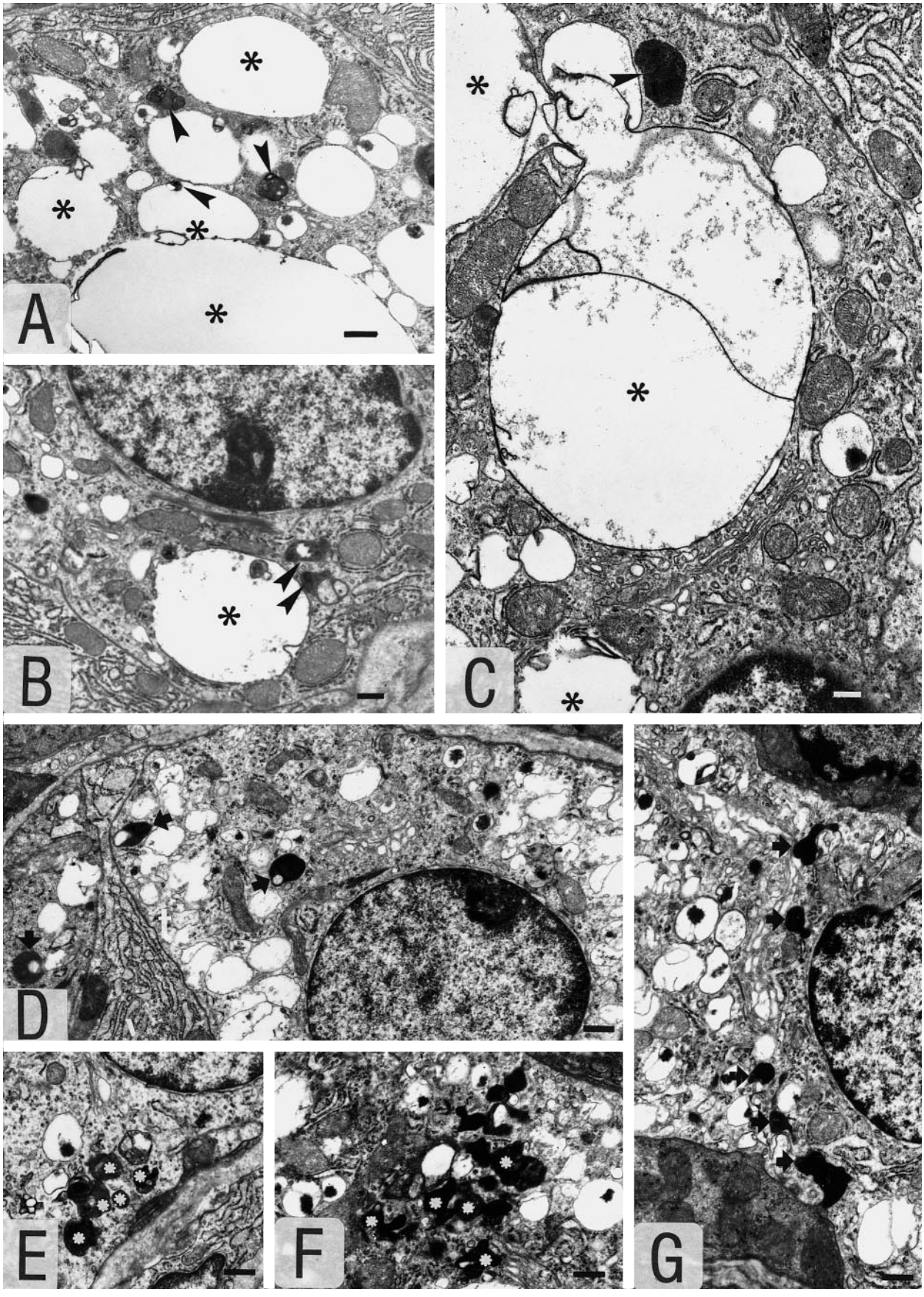
Human Leu^{15}-gastrin-17 was purchased from Research Plus, Bayonne, N.J., USA. Bovine serum albumin (BSA) was from Boehringer Mannheim, Mannheim, Germany. Gastrin was dissolved in 0.9% NaCl, containing 1% BSA, immediately before starting the infusion. Omeprazole was provided by Astra-Hässle, Mölndal, Sweden, and dissolved in 0.25% methylcellulose. α-FMH was obtained from Dr. J. Kollonitsch, Merck, Sharp and Dohme, Rahway, N.J., USA, and dissolved in 0.9% NaCl. All other chemicals were obtained commercially.

Animals

The study was approved by the local committee for animal welfare. All experiments were performed at the Department of Pharmacology at the University of Lund unless otherwise stated. Sprague-Dawley rats (131 males and 32 females, weighing 200–225 g at the start of the experiments) were kept in Macrolon cages (4–6 animals in each cage) with free access to standard rat food pellets (ALAB, Stockholm, Sweden) and tap water. Each group comprised 6–13 rats. They were killed by exsanguination via the abdominal aorta between 10:00 and 12:00 a.m. under chloral hydrate anaesthesia (300 mg/kg, i.p.). Six male rats were subjected to antrectomy (Billroth I) by resecting the distal half of the glandular stomach, including the duodenal bulb, followed by an end-to-end gastroduodenostomy under chloral hydrate anaesthesia. Six male rats underwent laparotomy and served as controls.

Gastrin infusion

Thirty-seven male rats were given continuous subcutaneous infusion of human Leu^{15}-gastrin-17 (5 nmol/kg per h) via osmotic minipumps (ALZET 2001, Alza Corporation, Palo Alto, Calif., USA), implanted in the neck under anaesthesia (see above) for 1, 2 or 6 days. This dose of gastrin is known to induce half-maximal activa-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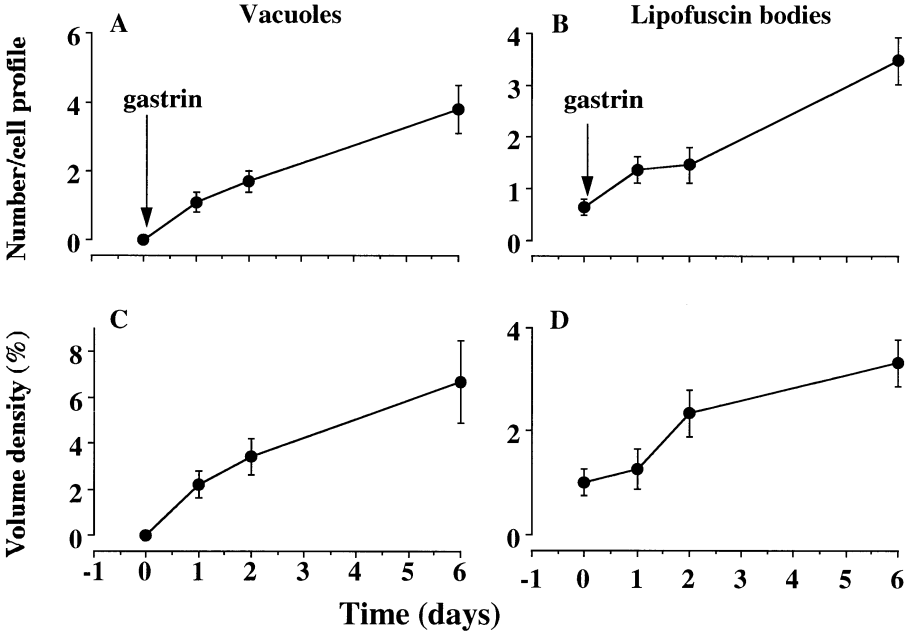

Fig. 4 Time course of the change in numbers and volume densities of vacuoles (**A**, **C**) and of lipofuscin bodies (**B**, **D**) in response to continuous subcutaneous infusion of gastrin over a time period of 6 days. Mean ±SEM (n=30–35 cells in each group)

tion of oxyntic mucosal HDC in fasted rats (Chen et al. 1994a). No antibiotics were used. Six untreated rats served as controls.

Omeprazole treatment

Eighteen male rats received omeprazole (400 μmol/kg) orally by means of a stomach tube once daily for 2, 6 or 10 weeks. This dose regime is known to induce sustained acid blockade and hypergastrinaemia (Larsson et al. 1986; Ryberg et al. 1989) and the rats were killed 2–3 h after the last dose. Controls (13 male rats) received the vehicle only. Six male rats were untreated (zero time controls).

Cessation of omeprazole treatment

Twenty-seven male rats received omeprazole (see above) for 2 weeks and were killed 1, 5, 20 or 40 days after discontinuing treatment. Twelve vehicle-treated rats served as controls and were killed 17 and 40 days after cessation of administration of the vehicle.

Treatment with omeprazole and α-FMH in combination

This experiment was carried out at Astra Hässle, Mölndal. Thirty-two female rats were divided into four groups: controls (vehicle+vehicle), vehicle+α-FMH, omeprazole+vehicle, and omeprazole+α-FMH. α-FMH was given by continuous subcutaneous infusion (3 mg/kg per h) via osmotic minipumps (Alzet 2ML1) implanted in the neck under Brietal anaesthesia (45 mg/kg i.p.; Eli Lilly, Indianapolis, Ind. USA). This dose of α-FMH is known to deplete histamine from ECL cells within 24 h (Andersson et al. 1992a). Omeprazole treatment was same as above. After 6 weeks, the rats were killed 2 h after the last dose of omeprazole or vehicle.

◀ **Fig. 3** Electron micrographs showing lipofuscin bodies (*and vacuoles*) in ECL cells from rats treated with omeprazole for 2 weeks (**A–C**) and with omeprazole for 2 weeks followed by withdrawal of the drug for 40 days (**D–G**). Note that vacuoles (*black asterisks*) are numerous in ECL cells from omeprazole-treated rats and that vacuoles and lipofuscin bodies (*arrowheads*) seem to exist in close apposition (**A–C**). Note also that, whereas lipofuscin bodies (*arrows or white asterisks*) remain, vacuoles are absent after cessation of omeprazole treatment (**D–G**). *Bars* 200 nm

Determination of serum gastrin concentration

Blood was drawn from the abdominal aorta at sacrifice (with the gastrin-containing minipump still in place or 2–3 h after the last omeprazole dose). Serum was stored at −20 °C until the determination of gastrin by radioimmunoassay. Serum gastrin concentration was determined as described in detail elsewhere (Stadil and Rehfeld 1973; Håkanson et al. 1974) and expressed as picomole equivalents of human Leu^{15}-gastrin-17 per liter of serum.

Electron-microscopic analysis of ECL cells

Small tissue specimens (1 mm^3) were collected from the oxyntic mucosa of each rat and immersed in a mixture of glutaraldehyde (1%) and formaldehyde (3%) in 0.075 M sodium phosphate buffer, pH 7.2, for 4–6 h. The specimens were postfixed for 1 h in 1% osmium tetroxide, dehydrated in graded acetone solutions, contrasted *en bloc* in a mixture of 1% phosphotungstic acid and 0.5% uranyl acetate and embedded in Epon 812. Ultrathin sections (60–80 nm) were cut on an LKB MK III Ultratome, routinely contrasted with uranyl acetate and lead citrate and examined in a Jeol 200 CX electron microscope. At least six sections from each rat were examined. The ECL cells were identified by their characteristic ultrastructure (Capella et al. 1971; Håkanson et al. 1971; Solcia et al. 1975; Rubin and Schwartz 1979; Böttcher et al. 1989; Chen et al. 1993). The granules/vesicles in the ECL cells were classified into granules, secretory vesicles, microvesicles and vacuoles (Chen et al. 1996a). The granules were defined as cytoplasmic membrane-enclosed organelles displaying an electron-dense core and a thin electron-lucent halo between the membrane and the dense core; the diameter of the dense core represented at least 50% of the diameter of the entire organelle (usually 50–250 nm in diameter). The vesicles were membrane-enclosed electron-lucent organelles with no dense core or possessing a small, often eccentrically located dense core, the diameter of the dense core being less than 50% of the diameter of the organelle. Based on their profile size, vesicles belonged to one of three populations: (1) secretory vesicles with a diameter of 150–500 nm (with a dense core, revealed by serial sectioning, if not immediately apparent), (2) vacuoles with a diameter of at least 500 nm, and (3) clear electron-lucent microves-

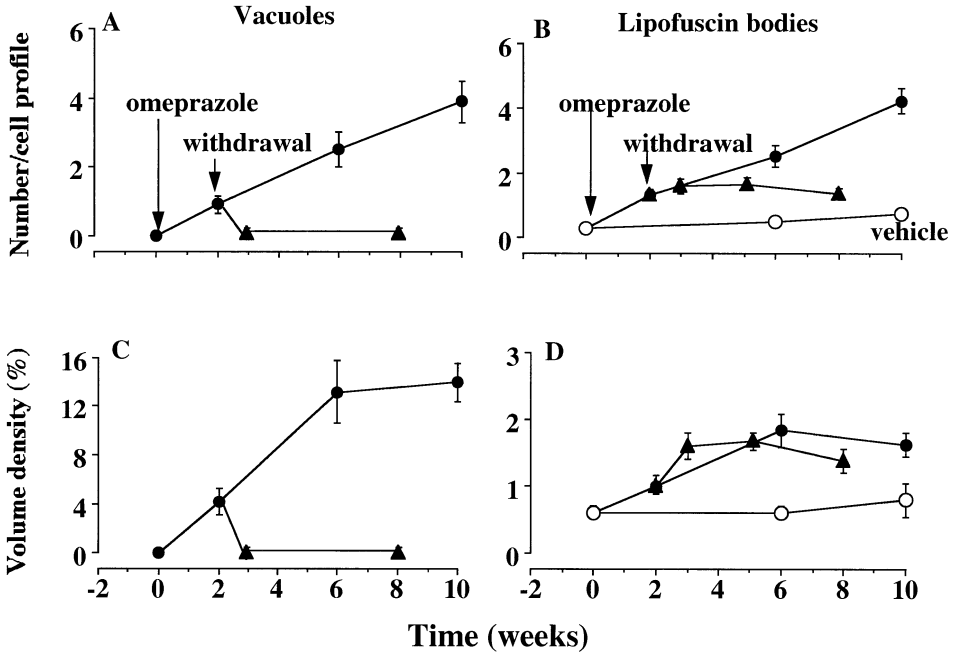

Fig. 5 Time course of the change in numbers and volume densities of vacuoles (**A**, **C**) and of lipofuscin bodies (**B**, **D**) in response to daily treatment with omeprazole (●) or vehicle (○) for 10 weeks, or to omeprazole treatment for 2 weeks followed by withdrawal of the drug for 40 days (▲). Mean±SEM (n=30–35 cells in each group)

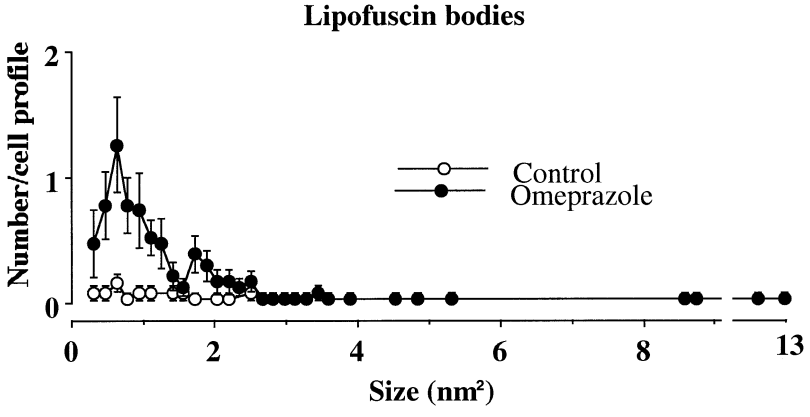

Fig. 6 Size distribution of lipofuscin bodies in ECL cells from rats treated either with vehicle (control) or omeprazole for 10 weeks. Mean±SEM (n=28 cells from 6 vehicle-treated rats, and 32 cells from 6 omeprazole-treated rats). There were 17 lipofuscin bodies in the ECL cells from control rats and 167 in those from omeprazole-treated rats

icles with a diameter of 25–200 nm (Chen et al. 1996a). Upon serial sectioning, the vacuoles could be shown to have one or more dense cores (Chen et al. 1996a). Lipofuscin bodies could be identified by their high electron density (osmiophilia). ECL cell profiles (with nuclei) were photographed and used for morphometric analysis (enlargement ×20000 or ×40000) by means of the point-counting technique described by Weibel (1969) and Weibel and Bolender (1973). The vacuoles lipofuscin bodies and per ECL cell profile were counted. The number of test points overlying the cytoplasm, vacuoles or lipofuscin bodies was determined. The volume density (% of cytoplasm) of vacuoles or lipofuscin bodies was calculated from the number of point intercepts for the various compartments. The size distribution of the lipofuscin bodies was illustrated in graphs constructed from the profile area (determined by the point-counting technique; x axis) and number (y axis) of lipofuscin bodies per cell profile (Chen et al. 1993, 1996a, b).

Statistical analysis

The results are expressed as Mean±SEM. Dunnett's test or Student's t-test for unpaired observations was used; $P < 0.05$ was considered statistically significant. Curves were drawn by a curve-fitting computer program (Cricket Graph)

Results

Development of lipofuscin in the ECL cells of hypergastrinaemic rats

Typical vacuoles and lipofuscin bodies are illustrated in electron micrographs in Figs. 1–3. The lipofuscin bodies are characterized by their high electron density and irregular shape. A small electron-lucent core can sometimes be seen in the electron-dense body. Lipofuscin bodies were few in the ECL cells of untreated, intact or sham-operated rats, and they were even fewer in antrectomized rats (sham operation *vs* antrectomy: 0.73±0.16 per cell profile *vs* 0.25±0.11; 0.60±0.09 volume density in % *vs* 0.17±0.05; $P < 0.01$ for both). The serum gastrin concentration was 65±2 pmol/l in the sham-operated rats and 5±1 pmol/l in the antrectomized rats (n=6 in each group). No vacuoles were observed in untreated intact rats or in antrectomized rats. Continuous infusion of gastrin

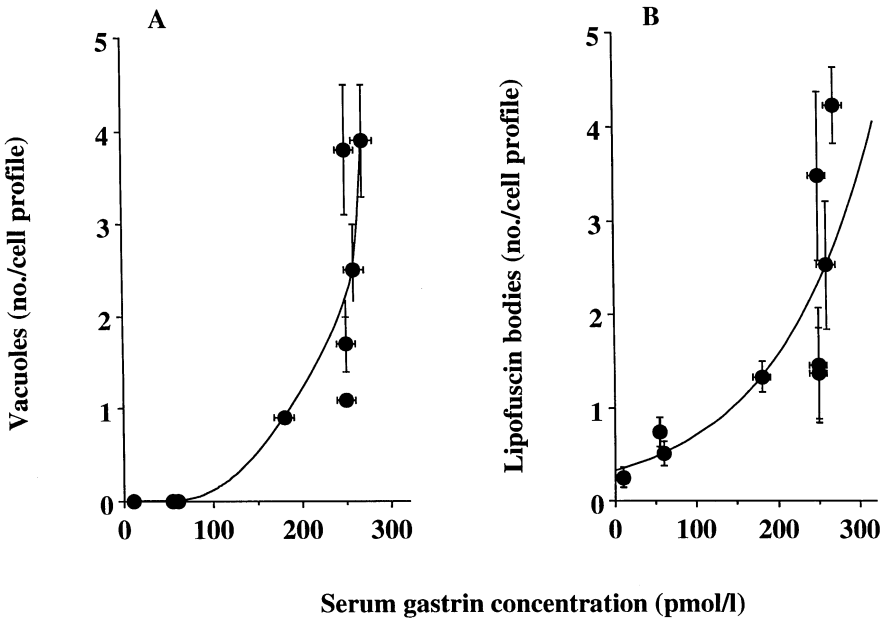

Fig. 7 Serum gastrin concentration *vs* the number of vacuoles (**A**) or lipofuscin bodies (**B**) per ECL cell profile. Data from rats treated with vehicle or gastrin infusion (for 1, 2 or 6 days) or omeprazole (for 2, 6 or 10 weeks) or subjected to sham-operation or antrectomy. r=0.741 (**A**) and 0.835 (**B**), P <0.0001 for both **A** and **B**

promptly raised the serum gastrin concentration [200±13 (n=12) *vs* 60±5 (n=6) pmol/l in untreated rats]; the elevated serum gastrin concentration remained at this level for the duration of the experiment. After six days of gastrin infusion, the numbers and volume densities of vacuoles and lipofuscin bodies were greatly increased (Figs. 1B, 4). Treatment with omeprazole raised the serum gastrin concentration. After 2 days of treatment, this concentration was 200±21 (n=6) pmol/l compared with 62±4 (n=13) pmol/l in vehicle-treated rats. It remained at this level for the duration of the study. After 10 weeks, the numbers and volume densities of vacuoles and lipofuscin bodies had increased greatly (Fig. 5). Data on the size distribution of the lipofuscin bodies revealed an increase not only in their number, but also in their size (profile area; Fig. 6).

There were good correlations between the serum gastrin concentration and the number of vacuoles per cell profile in the various experimental groups (r=0.741, P <0.0001) on one hand and between the serum gastrin concentration and the number of lipofuscin bodies per cell profile (r=0.835, P <0.0001) on the other hand (Fig. 7). Figure 8 shows the relationship between the numbers of vacuoles and of lipofuscin bodies (r=0.965, P <0.0001). At times, vacuoles and lipofuscin bodies were found to exist in close apposition (Fig. 3A–C).

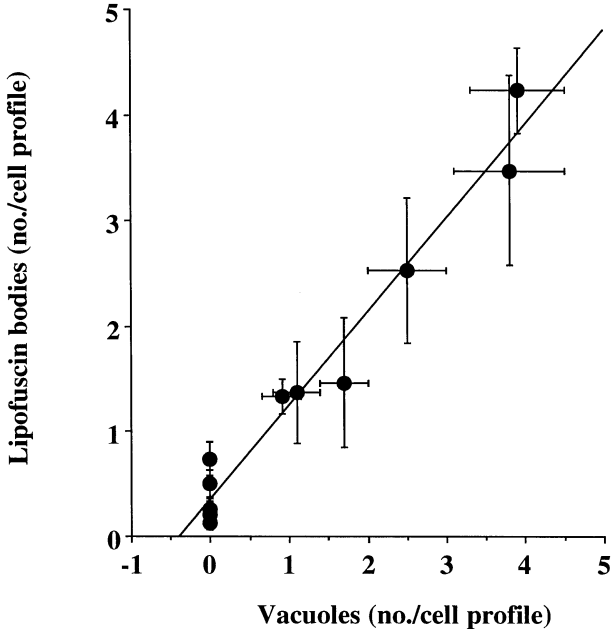

Fig. 8 The number of vacuoles *vs* the number of lipofuscin bodies per ECL cell profile. Data from rats treated with vehicle or gastrin infusion (for 1, 2 or 6 days) or omeprazole (for 2, 6 or 10 weeks) or subjected to sham-operation or antrectomy. r=0.965, P <0.0001

Vacuoles and lipofuscin after withdrawal of omeprazole

After withdrawal of omeprazole (after 2-weeks treatment), the serum gastrin concentration promptly returned to the pretreatment level (220±6 *vs* 66±10 after 5 days). Vacuoles disappeared within 5 days, whereas the lipofuscin bodies remained for at least 40 days after cessation of treatment (Figs. 2, 3D–G, 5).

Vacuoles and lipofuscin in α-FMH/omeprazole-treated rats

Combination of omeprazole with α-FMH induced the same degree of hypergastrinaemia as omeprazole alone (data not shown, see Andersson et al. 1992b). α-FMH treatment prevented the omeprazole-evoked formation of vacuoles and lipofuscin bodies (Fig. 9).

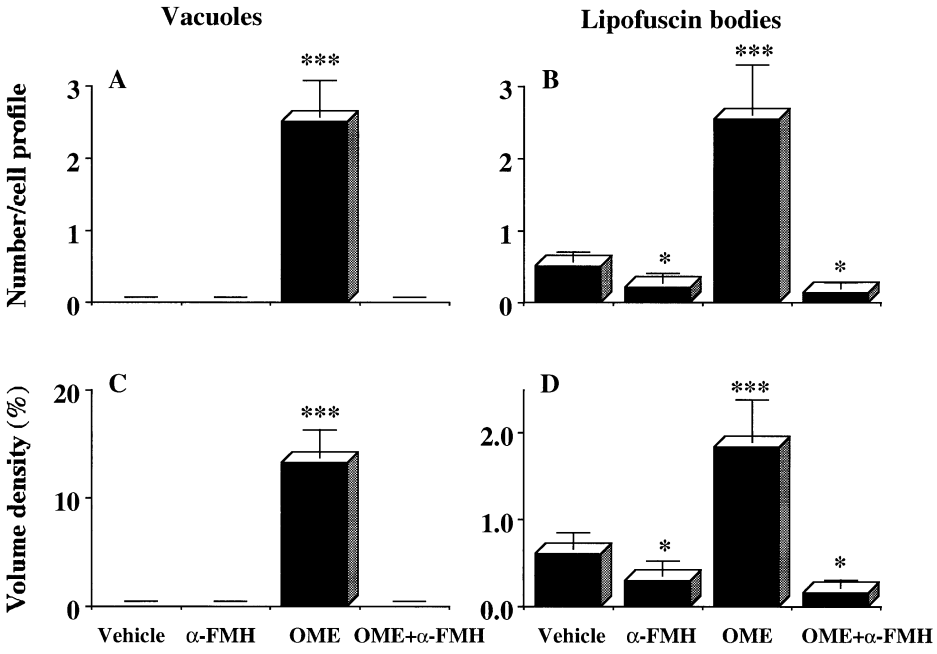

Fig. 9 Numbers and volume densities of vacuoles and lipofuscin bodies in rats treated with vehicle, α-FMH, omeprazole (*OME*), or omeprazole plus α-FMH (*OME*+α-FMH) for 6 weeks. Mean±SEM (n=30–35 cells in each group). * or *** for $P < 0.05$ or 0.001, – in **A** and **C** represents zero

Discussion

ECL cells respond to gastrin according to a precise time table (Chen et al. 1994b). Among acute effects (manifested within minutes) are the release of histamine and pancreastatin (Chen et al. 1994a). Among less immediate effects (manifested within hours) are those resulting first in an activation of the HDC and subsequently in the increased expression of HDC mRNA (Chen et al. 1994a). Long-term gastrin stimulation produces hypertrophy (after several days of hypergastrinaemia; Chen et al. 1996b), hyperplasia (after weeks of hypergastrinaemia; Håkanson et al. 1993), and dysplasia/neoplasia (after 1–2 years; Havu 1986; Håkanson and Sundler 1990). The present study reveals additional gastrin-induced changes in the ECL cells in the form of vacuoles and lipofuscin bodies.

Lipofuscin is the end product of incomplete lysosomal degradation and is thought to arise from the autophagocytotic conversion of lipids and proteins to a polymerized, non-degradable and chemically ill-defined material, usually rich in heavy metals, such as iron (Essner and Novikoff 1960; Miyawaki 1965; Brun and Brunk 1970). Lipofuscin in post-mitotic cells is thought to reflect the ageing of the cell but may also reflect enhanced autophagocytosis and/or increased oxidative stress. Vacuoles are large membrane-enclosed electron-lucent organelles that appear in the ECL cells in response to gastrin stimulation (Böttcher et al. 1989; Chen et al. 1993, 1996a, b). They are thought to arise as a result of the fusion of secretory vesicles (Böttcher et al. 1989; Chen et al. 1993, 1996a, b). In the present study, we have shown that exogenous gastrin and omeprazole-evoked hypergastrinaemia induced the formation of vacuoles and lipofuscin, whereas antrectomy-evoked hypogastrinaemia was associated with a reduced number of lipofuscin bodies. Hence, it appears that gastrin stimulates the development of both vacuoles and lipofuscin in the ECL cells. α-FMH-evoked ECL-cell histamine depletion is known to prevent the formation of vacuoles (see Chen et al. 1996a). Moreover, the development of lipofuscin was prevented, perhaps because vacuoles contribute to the formation of lipofuscin. We suggest that the development of vacuoles and lipofuscin reflects the incomplete degradation of excessive secretory products. After withdrawal of omeprazole, the vacuoles disappeared promptly, whereas the lipofuscin bodies remained (the longest time studied was 40 days), in accordance with the view that lipofuscin cannot be digested and eliminated.

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