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Gastrin effects on isolated rat enterochromaffin-like cells following long-term hypergastrinaemia in vivo

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Abstract

The enterochromaffin-like (ECL) cells play an important role in the regulation of gastric acid secretion. They respond to gastrin by a prompt increase in histamine secretion, an effect which is mediated by the CCK-$_B$/gastrin receptor acting through the IP_3/DAG pathway. In the rat, long-term treatment with acid secretion inhibitors induces hypergastrinaemia which, in turn, results in ECL cell hypertrophy and hyperplasia. The aim of the present study was to evaluate various functional parameters in acutely isolated rat ECL cells, following long-term hypergastrinaemia in vivo. Rats were treated with vehicle or a supramaximal daily dose of omeprazole for more than 10 weeks to ensure ECL cell hyperplasia. ECL cells were isolated from vehicle-treated animals and 24, 72 and 120 h after the last dose of omeprazole. The functional activity of the acutely isolated ECL cells was determined by measuring gastrin-and forskolin-induced histamine secretion. Changes in cytosolic free calcium upon gastrin stimulation were monitored by digital video imaging. ECL cells successively regained their ability to respond to gastrin following long-term hypergastrinaemia, reaching close to vehicle-treated levels 120 h after the last dose of omeprazole. In the rat, the response pattern of the ECL cells appears to normalise in parallel with the normalisation of plasma gastrin levels.

Keywords: Digital video imaging; Enterochromaffin-like cell; Histamine secretion; Intracellular calcium

1. Introduction

The endocrine cells play a major role in the functional regulation of the gastrointestinal tract. In the mammalian stomach they constitute approximately 1–2% of the total number of cells in the gastric mucosa [1]. The enterochromaffin-like (ECL) cells, which play a central role in the regulation of gastric acid secretion, are the predominant endocrine cell population in the oxyntic mucosa. They constitute approximately 65% of all fundic endocrine cells in the rat [1,2] and about 35% in man [3]. The ECL cells synthesise, store and secrete histamine which, in turn, stimulates the parietal cells via the histamine (H_2)-receptor to secrete hydrochloric acid. The relationship between gastric acid secretion and gastrin has been extensively studied. Gastrin, apart from stimulating acid secretion, has a general trophic effect on the oxyntic mucosa. The ECL cells are the most sensitive cells to the growth-stimulating effect of gastrin. Their acute response to gastrin is histamine release and accelerated rate of synthesis of the histamine-forming enzyme, histamine decarboxylase (HDC). If high circulating gastrin levels are sustained

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for a few days, the ECL cells become hypertrophic [4], and if the hypergastrinaemia is prolonged for several weeks, an ECL cell hyperplasia develops. The development of ECL cell hyperplasia probably reflects an accelerated rate of self replication under the influence of gastrin [5,6].

Reduction of gastric acid secretion from the parietal cells is known to increase circulating gastrin levels. Therefore, treatment with antisecretory drugs will induce hypergastrinaemia. This effect is reversible when acid secretion is normalised; upon cessation of treatment the gastrin level returns to control values [7]. Thus, inhibition of gastric acid secretion raises the level of circulating gastrin, resulting in hypergastrinaemia, which in turn results in an increase in the ECL cell number. Over time, there is a linear relationship between ECL cell density and plasma gastrin concentration [8].

The response of the ECL cells to gastrin has been extensively studied in the rat as well as in isolated ECL cell preparations [9–13]. Gastrin stimulates histamine secretion via the cholecystokinin B (CCK_B) receptor via a calcium-dependent pathway [14]. However, the studies on isolated cells were performed on cells from untreated animals. We have modified the isolation technique previously developed by Prinz et al. [11] in order to allow isolation of the hypertrophic ECL cells resulting from long-term hypergastrinaemia.

In this study, hypergastrinaemia was induced by administration of a supramaximal dose of omeprazole (400 μmol/kg per day) – which induces nearly total inhibition of gastric acid secretion for 24 h – for more than 10 weeks to ensure ECL cell hyperplasia/hypertrophy. ECL cells were then isolated at various times after the last dose of omeprazole, and the effects of gastrin on histamine secretion and on cytosolic free calcium, $[Ca^{2+}]_i$, were investigated.

2. Materials and methods

2.1. Treatment of animals

Female Sprague–Dawley rats (Møllegaard's Breeding Centre, Skensved, Denmark), weighing approximately 200–225 g at the start of the study, were used. The animals were kept in groups of two or three in Macrolon cages with free access to standard rat food pellets and tap water. One group of animals received omeprazole, 400 μmol/kg, suspended in 0.25% (w/v) buffered methyl-cellulose, orally once daily at 10 a.m. The control animals were given the vehicle only. The rats were treated for at least 10 weeks and then killed by carbon dioxide asphyxiation at various times after the last dose. All animal experiments had been approved by the local animal welfare committee.

2.2. Isolation and enrichment of rat ECL cells

2.2.1. Solutions

All solutions were freshly prepared daily. Medium A contained (in mM): 70 NaCl, 5 KCl, 1 Na_2HPO_4, 0.5 NaH_2PO_4, 20 $NaHCO_3$, 10 EDTA, 50 Hepes, 11 glucose, and 5 mg/ml bovine serum albumin (BSA), pH 7.8. Medium B contained (in mM): 70 NaCl, 5 KCl, 1 Na_2HPO_4, 0.5 NaH_2PO_4, 20 $NaHCO_3$, 100 $CaCl_2$, 15 $MgCl_2$, 50 Hepes, 11 glucose, and 5 mg/ml BSA, pH 7.4. Medium C contained (in mM): 140 NaCl, 12 $MgSO_4$, 10 $CaCl_2$, 15 Hepes, 11 glucose, 0.5 dithiothreitol, and 1 mg/ml BSA, pH 7.4. Nycodenz stock solution contained Nycodenz from the bottle supplemented with 1.2 mM $MgCl_2$, 15 mM Hepes, and 10 mg/ml BSA, pH 7.4. Histamine release medium contained Dulbecco's modified Eagle's medium F-12 Ham supplemented with 3 mg/ml BSA, 2% foetal calf serum, 0.5 mM $CaCl_2$, 1% ITS^+, 50 IU/ml penicillin, and 50 μg/ml streptomycin, pH 7.4.

2.2.2. Cell isolation

The isolation technique used was based on the method previously developed by Prinz et al. [11]. Some modifications to the isolation procedure had to be made to allow isolation of hypertrophic ECL cells. Briefly, two (omeprazole-treated) or four (vehicle-treated) non-fasting rats were used in each preparation. The stomachs were rapidly excised and placed in physiological saline. After ligation of the oesophageal–gastric junction, the stomachs were everted, ligations were thereafter performed, and the fundic sacs were filled with 3–5 ml of Pronase (0.80 mg/ml, in medium A). The gastric sacs were placed in a 250-ml E-flask containing 150 ml of oxygenated medium A and incubated for 20 min. Unless otherwise indicated, all incubations were performed

at 37°C under an oxygenated atmosphere. The medium was then discarded and 150 ml of medium B was added for 10 min. The sacs were once again placed in 150 ml of medium A and incubated for 20 min. This medium was then replaced with 150 ml of medium B and the stomachs were incubated for 10 min with gentle magnetic stirring. The sacs were then transferred to 150 ml medium A for 20 min. The medium was then replaced with 100 ml medium B and stirred for 15 min. The medium was then replaced with 75 ml fresh medium B and stirred for another 20 min. Thereafter, the medium was filtered through a 75 μm nylon mesh. After centrifugation, this fraction of gastric mucosal cells (referred to as M3) was resuspended in 10 ml of medium C containing 10 mg/ml BSA. The cell yield and viability were assessed, using Trypan blue exclusion, after which the cell concentration was adjusted to $15–16\times10^6$ cells/ml by adding medium C containing 10 mg/ml BSA. The M3 cell fraction was left to rest at room temperature for 60 min before further treatment.

2.2.3. ECL cell enrichment

The M3 suspension was subjected to counterflow elutriation using a JE-6 elutriator rotor with the standard elutriation chamber (Beckman Instruments, Palo Alto, CA, USA) run in a J-21C centrifuge (Beckman). A Masterflex pump equipped with an Easy Load pump head (Models 7550-92 and 7518-00, respectively, Cole–Parmer Instrument Co., Barrington, IL, USA) was used to regulate the flow rate of the buffer. Medium C was pumped through the system, and care was taken to remove all bubbles. About 1.0×10^8 cells were injected into the system, at a flow rate of 10 ml/min in each round. Two different elutriation protocols had to be used due to the ECL cell hypertrophy in omeprazole-treated animals (Table 1). The cells in the F2 fractions were collected by centrifugation, $150\times g$ for 5 min, and resuspended in histamine release medium. The cells were incubated at 25°C for 3 h before further treatment.

In order to obtain maximal enrichment of both normal (vehicle-treated) and hypertrophic (omeprazole-treated) ECL cells, two different density gradient systems had to be used.

In the experiments with vehicle-treated animals, the partly enriched ECL cell fraction (F2) was overlaid above two different layers of Nycodenz stock solution mixed with medium C containing 10 mg/ml BSA and 1.2 mM $MgCl_2$ (1:1 (1.058 g/ml) and 1:2 (1.046 g/ml) mix) and centrifuged at $200\times g$ for 8 min. Finally, enriched ECL cells were collected at the top interface. The collected fraction was washed with histamine release medium.

For the omeprazole-treated animals, a linear density gradient ranging between 1.044 g/ml and 1.078 g/ml (9 ml of each) had to be used. The gradient was prepared using a Bio-Rad gradient former, model 385. The partly enriched ECL cell fraction (F2) was laid over the linear density gradient and centrifuged at $200\times g$ for 8 min. The finally enriched ECL cells were then collected as a 5 ml-fraction, 3 ml from the top. The collected fraction was then washed with histamine release medium.

2.3. Immunocytochemistry

ECL cell density was determined by means of immunocytochemical staining. Staining was performed using a polyclonal primary antibody raised in rabbit against HDC. Alkaline phosphatase-conjugated, goat anti-rabbit IgG was used as the secondary antibody. Cell suspensions were left to dry on poly-L-lysine-coated glass slides at room temperature overnight. Cells were then prefixed in ice-cold acetone and thereafter fixed in 1% formaldehyde and 0.25% glutaraldehyde. Cells were incubated with the primary antibody (dilution 1:400), diluted with phosphate-buffered saline containing 2% (w/v) BSA and 0.25% (v/v) Triton, for 2 h and then washed. The secondary antibody was added for 2 h, and a commercially available substrate-kit (DAKO New Fuchsin) was used to visualise specific primary antibody binding in cells. Non-specific binding was blocked by preincubation with 25% goat serum. At least 200 cells per preparation were counted in different visual fields at a magnification of 400×. Data are expressed as positive cells per total number of cells.

2.4. Histamine release experiments

The histamine content and the histamine released were determined in the finally enriched ECL fraction as follows. Cells were incubated in Eppendorf vials (5×5000 cells/vial, total volume 200 μl) at 37°C under an oxygenated atmosphere under the indicated

experimental conditions. DMSO was used as a vehicle for gastrin and forskolin, at a final concentration of less than 0.1%. At these concentrations, DMSO did not alter histamine release. The incubation was stopped after 60 min by centrifugation (1500×*g*, 3 min) and the supernatant was removed. The histamine content was determined at time zero, after removal of the supernatant for measurement of initial extracellular histamine, the cell suspension was homogenised by sonication, and the histamine content was determined. All samples were diluted and stored at −20°C until histamine analysis. The histamine concentration was measured with a commercially available radioimmunoassay kit.

2.5. Video imaging of intracellular calcium

The finally enriched ECL fraction was resuspended in histamine release medium and allowed to rest for 3 h at room temperature. The cells were then placed on poly-L-lysine-coated glass cover slips (∅ 22 mm) in an open chamber for 15 min at 37°C, to ensure attachment, before addition of medium C containing 2 μM Fura-2 AM. After 30 min incubation at 37°C, the chamber was placed on the stage of an inverted microscope (Nikon Diaphot 300, Tokyo, Japan) equipped for epifluorescence. The chamber was perfused with medium C at 37°C, at a flow rate of 1 ml/min, for 5–10 min before stimulation. The stimulant was then added for 3 min and replaced with medium C until the cells had returned to their prestimulated state. Then a second gastrin concentration (10 times higher than the first) was added for 3 min, after which it was replaced with medium C. Cells were allowed to return to prestimulated values, after which ionomycin (10 μM) followed by EGTA (10 mM) was added in order to obtain maximum and minimum fluorescence levels. Fura-2 fluorescence, as well as background fluorescence, was imaged using a Nikon UV-Fluor objective (40×, N.A. 1.3). Fluorescent emission was monitored by an intensified video camera (Dage-MTI CCD-72) and expressed as the ratio (R_{340}/R_{380}) of the fluorescence intensity of selected cells, under the control of Ratio-Tool software (Inovision Corporation, Raleigh, NC, USA). $[Ca^{2+}]_i$ was calculated according to Grynkiewicz et al. [15], assuming a dissociation constant of 224 nM. Cells were excluded if they did not remain visible throughout the experiment.

2.6. Presentation of the data and statistical analysis

The data presented correspond to the mean ± standard error of the mean (S.E.M.). Data corresponding to video imaging of intracellular calcium were expressed as mean peak $[Ca^{2+}]_i \pm$ S.E.M. Effective concentrations 50% (EC_{50}) were calculated using linear regression analysis. Differences between omeprazole-treated groups and vehicle-treated groups were compared with the Mann–Whitney U-test. $P < 0.05$ was considered to be significant.

2.7. Chemicals

All chemicals were of analytical grade and obtained from the indicated sources: bovine serum albumin, Fraction V (BSA), carbamylcholine (carbachol), dithiothreitol, Dulbecco's modified Eagle's medium F12 Ham, ethylenediamine tetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-

Table 1
Elutriation protocols for the enrichment of ECL cells from vehicle-treated and omeprazole-treated animals

	Vehicle-treated animals			Omeprazole-treated animals		
	Rotor speed (rpm)	Flow rate (mg/ml)	Volume collected (ml)	Rotor speed (rpm)	Flow rate (mg/ml)	Volume collected (ml)
F0	2000	10	50	2000	15	50
F1	2000	15	50	2000	23	100
F2[a]	2000	21	100	2000	27	100
F3	2000	50	100	2000	50	100

Elutriation at 25°C.
[a]The ECL cell-rich fraction.

Table 2
ECL cell density in the finally enriched fraction by means of histidine decarboxylase immunocytochemistry

	Vehicle-treated animals (% ECL cells)	Omeprazole-treated animals (% ECL cells)
Vehicle-treated	69.3 ± 2.4	n.a.
Omeprazole 24 h	n.a.	66.2 ± 4.6
Omeprazole 72 h	n.a.	62.8 ± 3.0
Omeprazole 120 h	n.a.	67.2 ± 3.5

Values are mean ± S.E.M. of five independent preparations. n.a., not available.

N,N,N′,N′-tetraacetic acid (EGTA), and *N*-[2-hydroxy ethyl]piperazine-*N*′-[2-ethanesulfonic acid] (Hepes) were from Sigma Chemical Co. (St. Louis, MO, USA). Cell-Tak and ITS$^+$-culture supplement (6.25 μg/ml insulin, 6.25 μg/ml transferrin, 6.25 ng/ml selenous acid, 1.25 mg/ml BSA, 5.35 μg/ml linoleic acid) were from Collaborative Biomedical, Bedford, MA, USA. Pronase E was from Boehringer Mannheim, Mannheim, Germany. Rat gastrin-17 was from Peninsula Laboratories, Belmont, CA, USA. 7β-Acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-ene-11-one (forskolin) was from Calbiochem, La Jolla, CA, USA. Methocel was from Dow Corning Corp., Midland, MI, USA. Nycodenz (NycoPrep 1.150) was from Nycomed, Oslo, Norway. Normal goat serum and DAKO New Fuchsin Substrate System were from DAKO, Glostrup, Denmark. Alkaline phosphatase goat-anti-rabbit IgG was from Vector Laboratories, Burlingame, CA, USA. Omeprazole was from AstraZeneca, Mölndal, Sweden. The histamine radioimmunoassay kit was from Immunotech, Marseilles, France. Foetal calf serum, penicillin/streptomycin solution, and Trypan blue were from Life Technologies, Paisley, UK. The polyclonal rabbit-anti-HDC antibody was a generous gift from Dr. Lo Persson, Department of Molecular and Cellular Physiology, Lund University, Sweden. All other chemicals were of the highest grade commercially available.

3. Results

The ECL cell density was about 1–2% in the initial cell suspension. After elutriation, regardless of elutriation protocol, the ECL density increased up to about 20–30%. Two different density gradients were used, one step-gradient for cells from vehicle-treated animals and another linear one for the cells from omeprazole-treated rats. The numbers of HDC-positive cells in the finally enriched fractions were similar, regardless of the isolation procedure employed (Table 2). The average ECL cell density in the finally enriched cell fraction was 66 ± 2%. (Thus, the total enrichment with respect to the gastric mucosa was about 70-fold.) The histamine content was measured just before the start of the histamine release experiment. The histamine content ranged from 95–127 nmol per million ECL cells (Table 3). The histamine content appears to be somewhat higher following omeprazole treatment; this increase was, however, not significant. Forskolin, a direct activator of adenylate cyclase, was able to trigger histamine secretion in cells isolated from vehicle-treated animals.

Table 3
Histamine content and forskolin (10 μM)-stimulated histamine secretion from ECL cells isolated from vehicle-treated animals and from omeprazole-treated animals 24, 72, and 120 h after the last dose of omeprazole

	Histamine content (nmol/10^6 ECL cells)	Histamine secretion (nmol/10^6 ECL cells)
Vehicle-treated	95 ± 8	11.3 ± 2.9
Omeprazole 24 h	121 ± 16	2.7 ± 0.4*
Omeprazole 72 h	127 ± 12	8.9 ± 2.4
Omeprazole 120 h	111 ± 26	6.3 ± 1.1

Values are mean ± S.E.M. of five independent preparations.
*$P < 0.05$ as compared to vehicle-treated cells (Mann–Whitney U-test).

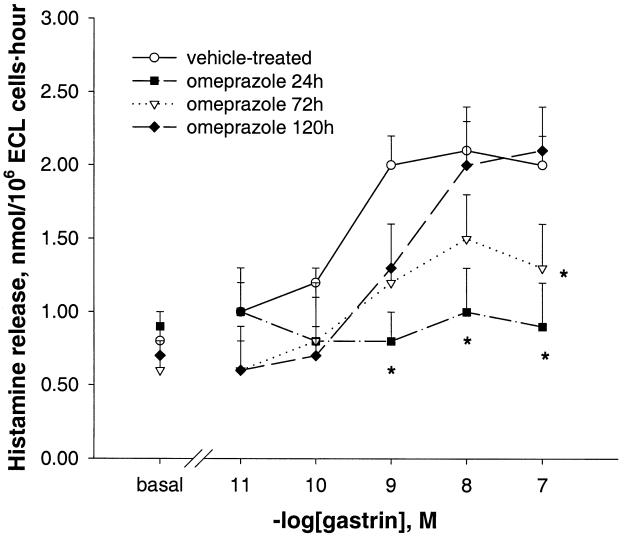

Fig. 1. Histamine secretion from isolated ECL cells in response to gastrin. Cells were isolated from vehicle-treated animals and 24, 72 and 120 h after the last dose of omeprazole. Following 1 h of incubation with gastrin, at the indicated concentrations, the supernatant was harvested and assayed for histamine. Mean ± S.E.M. of five independent preparations. $^{*}P < 0.05$ as compared to vehicle-treated cells (Mann–Whitney U-test).

Thus, increases in cyclic AMP alone were able to trigger histamine secretion. In cells isolated from omeprazole-treated rats, 24 h after the last dose, there was a significant decrease in forskolin-induced histamine secretion. The response to forskolin in cells isolated 72 and 120 h after the last dose of omeprazole was, however, not significantly different from that in controls. Carbachol failed to stimulate histamine secretion from ECL cells isolated from vehicle-treated animals (data not shown).

3.1. Effects of gastrin on histamine secretion

Rat gastrin-17 stimulated histamine secretion in a concentration-dependent manner. In all experiments with cells isolated from vehicle-treated animals, the maximal response was obtained at a concentration of 10^{-8} M. Fig. 1 shows the concentration-dependent histamine secretion after 1 h of incubation under basal conditions and after addition of 10^{-11}–10^{-7} M gastrin. The EC_{50} for gastrin was calculated to be 0.2 nM in cells isolated from vehicle-treated animals. The same experiments with hypertrophic ECL cells were repeated at three different time points, 24, 72 and 120 h, after stopping the omeprazole administration. Twenty-four hours after the last dose of omeprazole, there was no functional response in terms of increased histamine secretion above basal levels. At the other time points, the ECL cells progressively regained their ability to respond to gastrin stimulation, reaching close to control levels 120 h after last dose. EC_{50} values 72 and 120 h after the last dose of omeprazole were 0.4 nM and 1 nM, respectively.

3.2. Gastrin effects on cytosolic free Ca^{2+} in single ECL cells

Changes in cytosolic free calcium upon gastrin stimulation (10^{-11}–10^{-7} M) were monitored in single acutely isolated ECL cells using the fluorescent dye Fura-2 and digital video imaging. ECL cells isolated from vehicle-treated animals responded to a gastrin challenge with a prompt increase in cytosolic free calcium, whereas the cells isolated 24 h after ending the omeprazole treatment showed a weaker response.

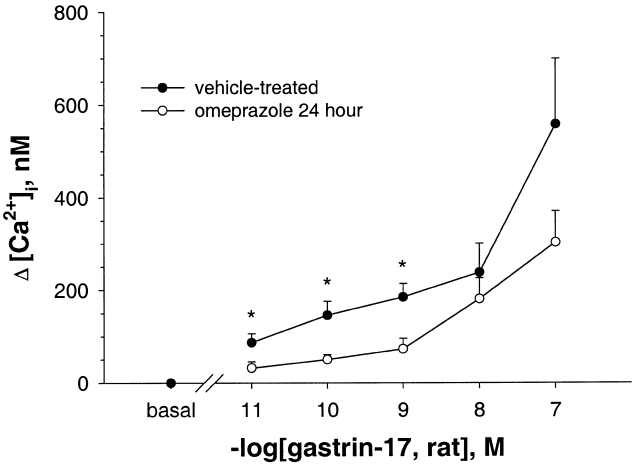

Fig. 2. ECL cells were isolated from vehicle-treated animals and from animals 24 h after stopping omeprazole administration. Changes in intracellular calcium, $[Ca^{2+}]_i$, in single acutely isolated ECL cells were measured using Fura-2 and digital video imaging. Basal $[Ca^{2+}]_i$ were 86 ± 4 and 50 ± 4 nM in cells from vehicle-treated and omeprazole-treated animals, respectively. Data are expressed as peak mean $[Ca^{2+}]_i$ ± S.E.M. from basal levels following addition of gastrin from 6–46 individual cells from 12 different preparations. EC_{50} values were calculated using the response obtained with 0.1 μM gastrin as the maximum for vehicle-treated and omeprazole-treated, respectively. $^{*}P < 0.05$ as compared to cells from vehicle-treated animals (Mann–Whitney U-test).

At the higher gastrin concentrations, however, the increments in $[Ca^{2+}]_i$ in these cells were similar to those observed in cells isolated from vehicle-treated animals (Fig. 2). Yet the cells isolated from the omeprazole-treated animals 24 h after last dose of omeprazole did not secrete histamine in response to gastrin (Fig. 1). The estimated EC_{50} values for the effect of gastrin on $[Ca^{2+}]_i$ were 3.7 nM in cells from vehicle-treated animals and 4.2 nM in cells isolated 24 h after ending the treatment with omeprazole.

4. Discussion

Gastrin is a potent stimulant of gastric acid secretion. The ECL cells are known to be under both functional and trophic control of gastrin. Their role, and that of histamine, in the regulation of gastric acid secretion has been the focus of numerous studies. In the present study, daily administration of a supra-maximal dose of omeprazole (400 μmol/kg per day) was used to induce hypergastrinaemia (a consequence of the profound inhibition of gastric acid secretion). The hypergastrinaemia was sustained for more than 10 weeks to ensure both ECL cell hypertrophy and hyperplasia. Thereafter, the readiness of the ECL cells to secrete histamine in response to a gastrin challenge was determined. The CCK_B receptor function was assessed by monitoring gastrin-induced changes in cytosolic free calcium.

The modified isolation procedure enabled us to harvest both normal and hypertrophic ECL cells with similar ratios of enrichment. ECL cells isolated from vehicle-treated animals secreted histamine in response to gastrin in a concentration-dependent manner. This response was nearly identical to that previously reported [9,11]. The vehicle-treated cells also responded with a prompt increase in cytosolic free calcium. This is also in agreement with previous observations [11,16,17]. In ECL cells isolated 24 h after withdrawal of omeprazole, gastrin induced a concentration-dependent increase in the cytosolic free calcium level. The response was somewhat lower than in cells isolated from vehicle-treated animals but reached levels that induced near maximal secretion of histamine in control cells. The ability of the ECL cells to secrete histamine in response to gastrin gradually returned, reaching close to vehicle-treated levels 120 h after discontinuation of omeprazole treatment. Increases in cytosolic free calcium are both necessary and sufficient for the initiation of secretion [18]. It seems clear that additional signals, such as the activation of protein kinase C [19] or one or more G proteins, are often required for the initiation of non-excitable cells [20].

The ECL cells act as intermediate regulatory cells, secreting histamine in response to food (gastrin) and perhaps also to neural stimulation. The effect of gastrin is mediated though the CCK_B receptor. The CCK receptors belong to the superfamily of G-protein-coupled receptors, many of which undergo internalisation within minutes after agonist exposure [21]. Both CCK_A- and CCK_B-receptors have been shown to undergo ligand-induced internalisation in pancreatic acini [22] or transfected NIH 3T3 [23] cells, respectively. During sustained hypergastrinaemia the ECL cells might undergo a process of adaptation resulting in down-regulation of gastrin receptors in response to abnormally high gastrin levels. This could explain the responses observed following long-term treatment. In terms of the calcium response 24 h after withdrawal of omeprazole compared to that in cells from vehicle-treated animals, the EC_{50} value remained unchanged ($\approx$4 nM). The unaffected EC_{50} value supports a decrease in receptor number, perhaps through internalisation or receptor recycling during agonist pressure, rather than receptor desensitisation.

The cytoplasm of the ECL cells contains characteristic granules and vesicles of varying size: secretory vesicles, granules, microvesicles, and vacuoles. Histamine is produced in the cytoplasm, then accumulated and stored in the secretory vesicles until release [24]. Long-term hypergastrinaemia is known to increase not only the ECL cell density but also the ECL cell size. The hypertrophy is maximal after 2 weeks of sustained hypergastrinaemia [4]. Ultrastructural changes occur and, perhaps most importantly, the number of secretory vesicles decreases. Furthermore, there is also the introduction of large vacuoles thought to be the result of fusion between a number of secretory vesicles [25]. These vacuoles may play a role in the degradation of overproduced secretory products (including histamine). Their ability, compared to the secretory vesicles, to respond to a gastrin stimulus is thought to be impaired. The fact

that forskolin, a direct activator of adenylate cyclase, failed to release histamine supports this view. Upon withdrawal of omeprazole, the serum gastrin concentrations promptly returns to the pretreatment level [26]. The ECL cell function should therefore return to normal upon withdrawal of omeprazole, parallel to the gastrin concentration. The mechanisms behind the reversibility of the gastrin-evoked changes are far from clear. The hypertrophy of the ECL cells is completely reversed within 40 days. Within 5 days of ending omeprazole administration it was found that the vacuoles had completely vanished and that a marked increase in the number of secretory vesicles has occurred [25,27]. Five days appear to be sufficient for the ECL cells to normalise cell functions, to form new functional secretory vesicles, and/or regulate receptor function before being able to respond to gastrin.

In summary, a method has been optimised which enables enrichment of ECL cells from both normal and hypertrophic gastric mucosa. The histamine release experiments showed that ECL cells respond to gastrin challenge in a concentration-dependent manner ($EC_{50} = 0.2$ nM). Twenty-four hours after the last dose of omeprazole, there was no significant increase in histamine secretion following a gastrin challenge. The ECL cells successively regained their ability to respond to gastrin, reaching vehicle-treated levels after 5 days. The weak but significant increments in $[Ca^{2+}]_i$ in response to gastrin, observed 24 h after stopping the omeprazole treatment, indicate that the ECL cells are capable of responding to gastrin by increasing $[Ca^{2+}]_i$. Why that does not result in a significant increase in histamine secretion could be due to a combination of receptor internalisation and/or post-receptor modulation and an impaired vesicle function.

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