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The Biology and Physiology of the ECL Cell

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The enterochromaffin-like (ECL) cells, which are the predominant endocrine cell type in the acid-producing part of the vertebrate stomach, are characterized by numerous, electron-lucent vesicles and few electron-dense granules in the cytoplasm. The biological and physiological significance of the ECL cells remains poorly understood. They produce and store histamine and pancreastatin and are thought to produce an as yet unidentified peptide hormone. The most important clue to their function is their willingness to respond to changes in circulating gastrin. The present review presents current knowledge of the biology and physiology of the rat stomach ECL cells.

Examination of serially sectioned ECL cells has revealed that the cytoplasmic vesicles almost invariably contain an electron-dense core, suggesting that perhaps the distinction between granules and vesicles is artificial. We propose a life cycle of the secretory organelles in the ECL cells with a progressive development from granules to vesicles. The results showed that the gastrin-evoked release of histamine and pancreastatin was accompanied by loss of vesicles, and that synthesis of histamine and pancreastatin was accelerated by sustained infusion of gastrin, a treatment that was associated with renewal of vesicles. The events described are instrumental in bringing about a change in the "steady state" or "equilibrium" of the ECL cells, from a non-stimulated, resting state to a gastrin-stimulated, active state. This change is attained within six to eight hr. The next "steady state" change is that from "normal-sized" but active ECL cells to "hypertrophic" ECL cells. The increase in cell size is complete after about one week. The gastrin-evoked increase in the ECL cell self-replication rate is maximal after about 10 days, after which time there is a gradual return back to pre-stimulation values. The ECL cell density increases fairly slowly and does not reach maximum (four-fold increase) until after 20 weeks of hypergastrinemia. The activity of the histamine-forming enzyme, histidine decarboxylase, is elevated by gastrin and remains elevated for as long as the gastrin stimulus is maintained (the longest time studied was 20 weeks).

The physiological significance of the ECL cells is probably related to their capacity to produce and store histamine and an as yet unidentified peptide hormone. The ECL cells are thought to be the source of histamine necessary for the gastrin-evoked acid response. In addition, preliminary evidence suggests that the ECL cells and the anticipated ECL cell hormone play a role in bone formation.

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[d]*Abbreviations:* ECL, enterochromaffin-like (cells); HDC, histidine decarboxylase; 5-HT, 5-hydroxytryptamine; α-FMH, α-fluoromethylhistidine; LI, labelling index.

INTRODUCTION

The so-called enterochromaffin-like (ECL)[d] cells have attracted much attention in recent years. Nonetheless, their physiological significance remains poorly understood. They are histamine-producing and peptide-hormone-producing cells that occur exclusively in the acid-producing gastric mucosa [1-5]. Several different endocrine cell types can be demonstrated in this location [6-9]. Together they represent about two percent of the cells in the acid-producing mucosa of the rat; the corresponding figure in man is 0.5 to one percent. This means that the endocrine cells in the acid-producing mucosa constitute an endocrine organ that is about equal in size to the endocrine pancreas. In the rat, the ECL cells comprise at least 65 percent of the endocrine cells [5, 7], the corresponding figure in man is 30-35 percent [7]. The ECL cells can be demonstrated—albeit not selectively—by silver-staining techniques and also by immunocytochemistry, taking advantage of the fact that they contain histamine and the histamine-forming enzyme, histidine decarboxylase (HDC). In mammals, they occur predominantly in the basal half, sometimes in the basal third, of the acid-producing mucosa in the chief-cell-rich region.

ULTRASTRUCTURAL FEATURES OF THE ECL CELL

The ECL cells are best defined by their characteristic ultrastructure [1, 2, 6, 8, 9]. They are irregular in shape, often displaying prominent extensions, and they contain electron-dense granules and electron-lucent vesicles (Figure 1). The ultrastructure of the ECL cells distiguishes them from all other endocrine cell types in the gastric mucosa. Besides histamine and the anticipated peptide hormone, the ECL cells produce chromogranin A, which is a member of the chromogranin/secretogranin family and occurs in many peptide hormone-producing cells [10-15]. Chromogranin A and its cleavage products are co-stored with biogenic amines and regulatory peptides and are thought to play a role in the formation and stabilization of secretory granules [15]. Chromogranin A is proteolytically processed to yield fragments, one such fragment is pancreastatin, which has been demonstrated in the ECL cells [10].

The cytoplasmic vesicles in the ECL cells are numerous and of varying size, often quite large; they are empty-looking or contain a small, eccentrically-located dense core [16-18]. The granules are few and small in size and have a dense core that is separated from the enclosing membrane by a thin electron-lucent halo. Examination of serially sectioned ECL cells has revealed that the vesicles almost invariably contain an electron-dense core (Figure 2), suggesting that perhaps the distinction between granules and vesicles is artificial. We have argued previously that granules probably develop into vesicles as a result of progressive accumulation of pre-formed histamine from the cytoplasm and that they grow in size during this process, possibly through osmotic forces generated by the resulting high local concentration of histamine [17-18] (Figure 3).

If the histamine-containing ECL cells are exposed to exogenous 5-hydroxytryptophan or DOPA, they are able to take up and decarboxylate these amino acids and to store the resulting amine, 5-hydroxytryptamine (5-HT) or dopamine, in the cytoplasm [19]. Histochemistry at the electron microscopic level suggested that the newly formed amine was in fact confined to the dense core of the granules and vesicles [1, 20]. Rubin and Schwartz [21] used autoradiographic technique to show labelling of secretory organelles in the ECL cells with ^{3}H-5-HT after incubating tissue specimens with ^{3}H-5-hydroxytryptophan. In a subsequent study, Rubin and Schwartz [22] labelled ECL cells with ^{3}H-histidine. However, the labelling in the cytoplasm, which reflected the presence of ^{3}H-histamine, could not be convincingly associated with the vesicles. Using immunocytochemistry at the electron microscopic level, Delwaide et al. [23] recently demonstrated histamine in ECL cell granules, while Nissinen and Panula [5] failed to do so. The failure to

demonstrate histamine label or histamine immunostaining over granules and vesicles may reflect diffusion of histamine from its subcellular storage site because of inadequate fixation and processing of the specimens. Although direct evidence for the storage of histamine in the secretory organelles is still missing, available information seems to favour the view that amine storage in the ECL cell is associated with granules and vesicles (Figure 3).

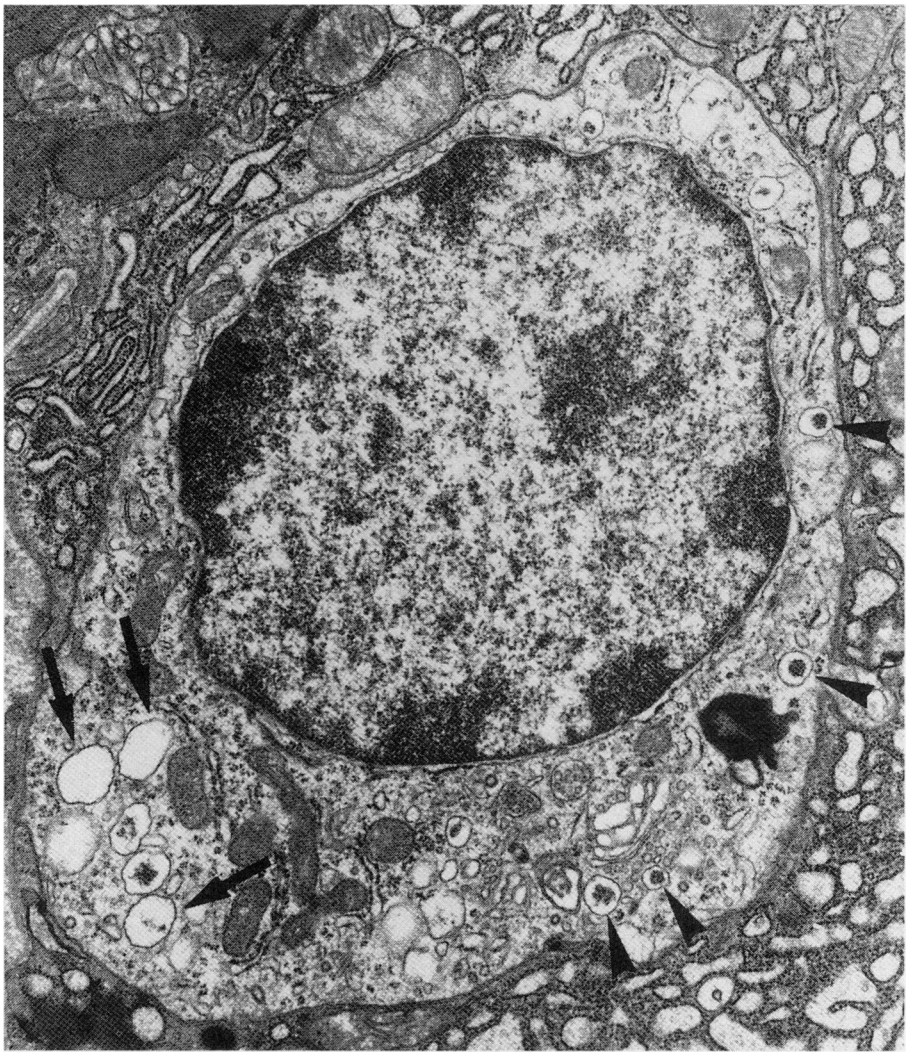

Figure 1. Electron micrograph showing an ECL cell in rat stomach. Numerous electron-lucent vesicles (indicated by arrows) and less numerous electron-dense granules (indicated by arrowheads) are typical features of the ECL cells. Vesicles are defined as cytoplasmic membrane-enclosed organelles without dense core or possessing a small, often eccentrically-located dense core, the diameter of the dense core being only a fraction of the diameter of the organelle. The granules are defined as cytoplasmic membrane-enclosed organelles, furnished with an electron-dense core and with a thin electron-lucent halo between the membrane and the dense core; the diameter of the dense core represents 50 percent or more of the diameter of the entire organelle. It cannot be excluded that granules and vesicles represent separate entities, but there have been growing suspicions for some time that there is a gradual transition from granules to vesicles (see text). 10,000 X.

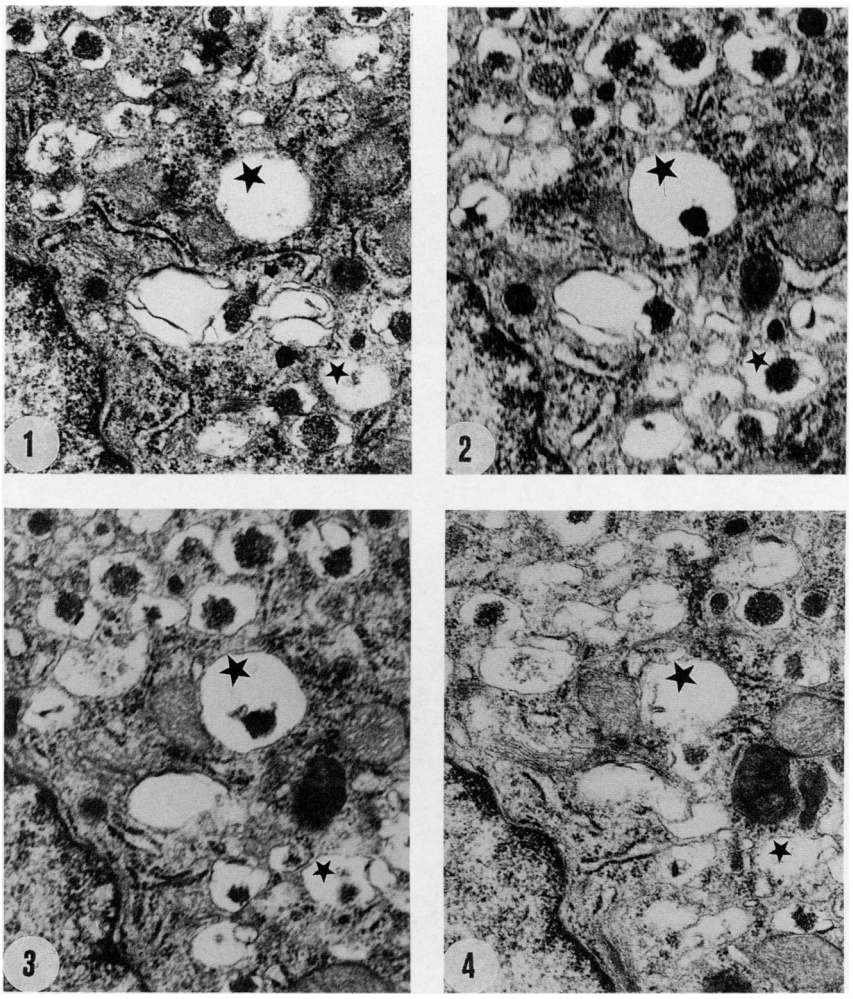

Figure 2. A series of four consecutive electron micrographs (1-4) of an ECL cell. Serially sectioned vesicles are indicated by asterisk. Note that apparently "empty" vesicles usually turn out to have an electron-dense core. 24,000 X.

IMMEDIATE RESPONSE OF THE ECL CELL TO GASTRIN

The ECL cells respond to gastrin according to a preset time table. An acute challenge with maximal doses of gastrin in the form of a sustained intravenous infusion to conscious rats results in a prompt reduction of the number of vesicles per cell profile, lowering of the concentration of histamine in the oxyntic mucosa (reflecting the release of histamine [24]) (Figure 4) and release of pancreastatin [25] (Figure 5). Gastrin-evoked histamine release has been observed also in isolated ECL cells [26]. Half an hour after the start of gastrin infusion, approximately 50 percent of the vesicles had disappeared. It is likely that the process of exocytosis explains the reduced number of vesicles and the release of histamine and pancreastatin (Figure 3). Signs of exocytosis (omega figures) were difficult to find, probably because the actual process of exocytosis is very brief

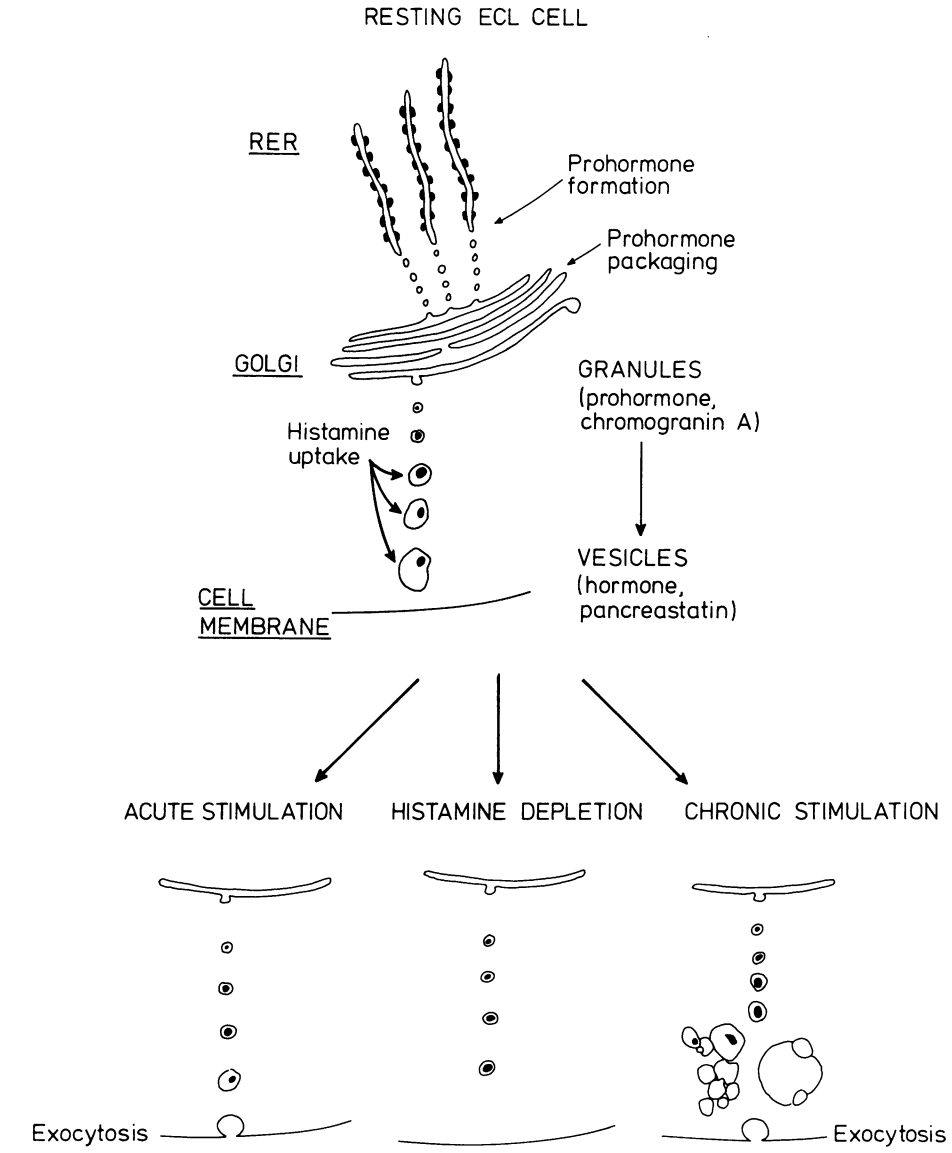

Figure 3. Proposed life cycle of ECL cell granules and vesicles. The prohormone of the ECL cell is synthesized in the rough endoplasmic reticulum (RER) together with chromogranin A and other granular proteins. They are then transported to the Golgi region for packaging into the prosecretory granules. The granules are small and electron-dense, and as they are transported away from the Golgi area, they actively take up preformed histamine from the cytoplasm to become vesicles. At the same time, chromogranin A is being hydrolyzed into small fragments, such as pancreastatin. The vesicles grow in size during the process of histamine accumulation, possibly through osmotic forces generated by the resulting high local concentration of histamine and peptide fragments. When the ECL cells are activated by acute gastrin stimulation, histamine and pancreastatin (and the anticipated hormone) in the vesicles are released via exocytosis. Following blockade of histamine synthesis by α-FMH, the ECL cells are depleted of histamine, and the granules cannot accumulate histamine. Hence, they cannot develop into vesicles. When histamine synthesis (and release) is being continuously stimulated (e.g., by omeprazole-evoked hypergastrinemia), the vesicles become large and numerous. In fact, very large vesicles are sometimes observed; they appear to be formed by fusion of smaller vesicles.

indeed. Within the first hour after the start of gastrin infusion the ECL cells begin to adapt to the sustained stimulus. HDC and chromogranin A are now being synthesized at an accelerated rate and the number of vesicles is increasing. The number of vesicles was back to pre-stimulation values about four hr after start of gastrin infusion. The histamine concentration was back to normal after three to four hr, and the pancreastatin concentration had returned to normal after four to six hr [26] (Figures 4 and 5). At this stage, a new "steady state" seems to have been attained; the short-term responses have been dealt with, and the cells have adjusted to the new situation of a maintained gastrin stimulus.

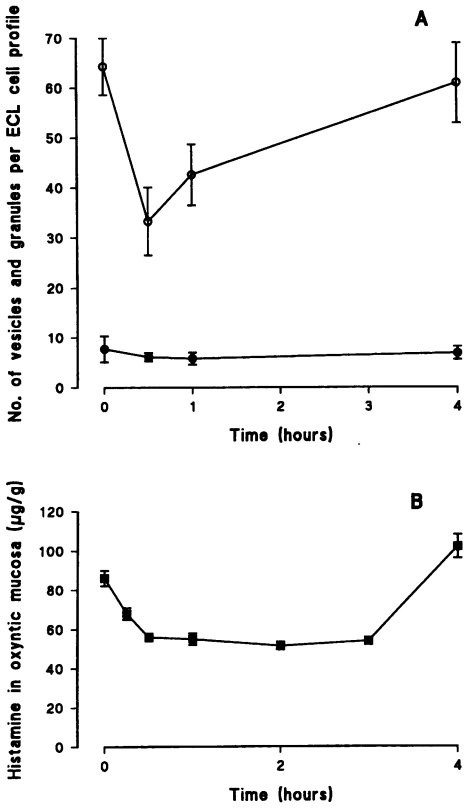

Figure 4. Time course of the changes in (A) number of vesicles (open circles) and granules (filled circles) (expressed as number per ECL cell profile) and in (B) histamine concentration (µg/g) in the oxyntic mucosa in response to Leu^{15}-gastrin-17 infusion (5 nmol/kg/hr, i.v.). Mean ± SEM (vertical bars, n = 30-35 cells from 4-9 rats) [25].

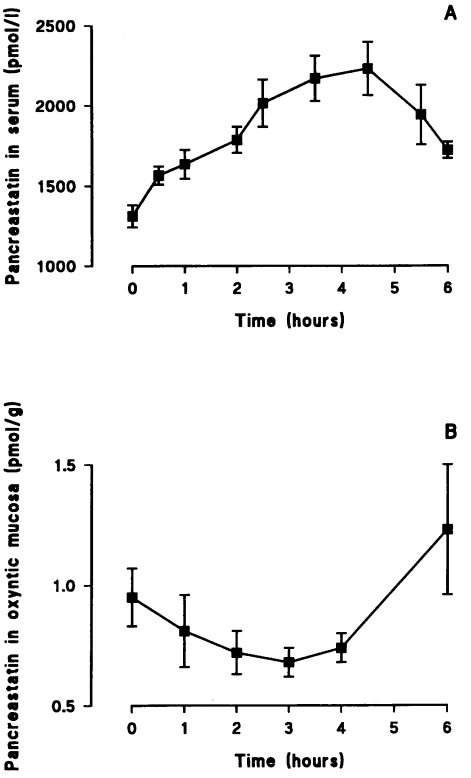

Figure 5. Time course of (A) the rise in the concentration of circulating pancreastatin (pmol/l) and of (B) the reduction in the oxyntic mucosal pancreastatin concentration (pmol/g) in response to Leu^{15}-gastrin-17 infusion (5 nmol/kg/hr, i.v.). Mean ± SEM (vertical bars, n = 4-9 rats). The concentration of pancreastatin was measured by radioimmunoassay using antibodies against the C-terminal decapeptide end of rat pancreastatin (chromogranin A 264-314-amide) [25].

The association between gastrin-stimulated degranulation on one hand and secretion of histamine and pancreastatin on the other supports the view that the two secretory products are stored together in granules and/or vesicles. However, the reduction in mucosal histamine did not completely parallel that of pancreastatin, in that the drop in histamine occurred much sooner after start of gastrin infusion than did the drop in pancreastatin, and in that mucosal histamine was replenished much faster than was pancreastatin [26]. Also, the release of pancreastatin into the circulation seemed to occur more slowly than what has been described for histamine [24, 27]. These discrepancies may favour the view that histamine and pancreastatin do not occur together in the same secretory organelles, or that the two secretory products are co-stored but not necessarily in the same compartment and/or not in fixed proportions. Secretory products in the resting ECL cell may occur in two pools, one close to the cell membrane and immediately available for release ("mature" vesicles) and another restrained from contact with release sites in the cell membrane, either by sheer distance or by the organization of the cytoskeleton (granules and "immature" vesicles) (Figure 3). Conceivably, the readily releasable pool contains proportionately more histamine than pancreastatin while the opposite is the case for the reserve pool. It can be assumed that chromogranin A is deposited in the secretory organelle at the time of its formation in the Golgi apparatus, whereas histamine is being taken up preformed from the cytoplasm at a much later stage. Hence, it is to be expected that the proportion of histamine versus chromogranin A-derived peptides (for instance pancreastatin) may vary depending upon the age of the organelle.

Histamine is rapidly replenished as a result of the increased HDC activity. It has been suggested that the release of histamine and the activation of HDC are coupled so that the release of histamine triggers the activation of HDC and that the HDC activity is turned off when the histamine stores have been replenished [28]. The present results show the HDC activity to increase quite slowly at first; after 30-60 min of infusing gastrin the enzyme activity started to increase more rapidly until reaching a plateau at six hr (Figure 6A). It should be noted that although the increase in HDC activity was initiated when the histamine concentration in the oxyntic mucosa was lowered, it continued long after histamine replenishment, suggesting that the activation of HDC is independent of the concentration of histamine, or perhaps more likely, that the activation of HDC is initiated by the lowering of histamine (or by the combination of gastrin stimulation and lowering of histamine) and maintained by gastrin stimulation.

The activation of HDC appears to be associated with a progressive increase in the level of HDC mRNA (Figure 6B). Interestingly, the gastrin-evoked increase in HDC mRNA was slower and less marked than the increase in HDC activity [25]. The activation of HDC is generally thought to reflect de novo synthesis of enzyme [29]. In view of the relatively slow accumulation of HDC mRNA in the oxyntic mucosa in response to gastrin, it may be argued either that preformed HDC may be transformed from inactive to active enzyme or that existing HDC mRNA is rapidly and effectively translated into enzyme. Conceivably, gastrin stimulates transcription as well as translation of the HDC gene in the ECL cells.

LONG-TERM EFFECTS OF GASTRIN ON THE ECL CELL

Six hr after the start of gastrin infusion, the ECL cells are in an activated "steady state," where nothing very dramatic seems to be going on. However, this impression is probably deceptive since it is possible to detect an increase in the cell size and an increased amount of endoplasmic reticulum and Golgi after about four to eight days of continuous gastrin infusion [16, 18]. The cells remain enlarged for at least 28 days, the longest time studied. The cytoplasmic vesicles display an altered appearance following a

period of sustained hypergastrinemia. Many of them become very large, giving the impression that they result from fusion of several smaller vesicles [16] (Figure 3).

In the time course of events that takes place in the ECL cells in response to sustained hypergastrinemia (whether of endogenous or exogenous origin) the next step is stimulated self replication [30]. The ECL cells are capable of dividing, and although their mitotic activity is low, it appears to be sufficient to maintain the ECL cell population. Sustained elevation of the circulating gastrin levels, induced by gastrin infusion, by pharmacological treatment with inhibitors of acid secretion (omeprazole, ranitidine) or by surgery, e.g., partial removal of the acid-producing part of the stomach (partial fundectomy) or antrum exclusion, stimulates mitosis in the ECL cells and induces hyperplasia [31-39]. The labelling index (LI), i.e., the proportion of ECL cells that incorporate ^{3}H-thymidine in preparation for mitosis, is elevated almost 10-fold after about 10 days of omeprazole-evoked hypergastrinemia, but this effect is transient; after 10 and 20 weeks, the LI is back to pre-stimulation values in spite of the maintained hypergastrinemia [30] (Figure 7A). The actual increase in the number of ECL cells in the oxyntic mucosa progressed quite slowly by comparison and reached a maximum of about four-fold increase after 20 weeks and up to 52 weeks of hypergastrinemia [30] (Figure 7B). Interestingly, the HDC activity, which is thought to reflect the functional activity of the ECL cells, still remained elevated after 20 weeks (Figure 7C). The decline in ECL cell LI during long-term omeprazole-evoked hypergastrinemia might reflect an impaired ability to respond to gastrin because of receptor down-regulation or because of local accumulation of growth inhibitors [40]. The fact that the ability of gastrin to activate HDC was not impaired indicates that gastrin retains its ability to activate the ECL cells (reflected in HDC activities well above control levels) at a stage when it had a reduced effect as a growth-stimulating factor (reflected in a LI at control level). This could be interpreted to mean either that the gastrin receptor responsible for the trophic response is distinct from the receptor responsible for activating the cells or that the gastrin-induced responses in the ECL cells are mediated via two independent post-receptor pathways, one of which is off-set by a long-lasting gastrin challenge while the other is not.

PHYSIOLOGICAL SIGNIFICANCE OF THE ECL CELLS

It has turned out to be difficult to define what physiological role the ECL cells fulfill. The most important clue to the function of the ECL cells is their willingness to respond to changes in circulating gastrin. This feature has made the ECL cells the prime suspect for a role as local supplier of histamine to stimulate the parietal cells. The significance of ECL cell histamine can be studied by the use of α-fluoromethylhistidine (α-FMH) an irreversible inhibitor, a so-called suicide inhibitor, of HDC. Treatment of rats with α-FMH results in irreversible inhibition of HDC and reduces the histamine concentration in the oxyntic mucosa by about 80 percent [17, 41]. It could be shown by immunocytochemistry that α-FMH depletes histamine from the ECL cells but not from the mast cells, which are few in the oxyntic mucosa of the rat. Interestingly, histamine depletion was associated with a marked loss of cytoplasmic vesicles, and the few that remained were quite small [17, 41]. This observation supports the contention that granules develop into vesicles as a result of intragranular accumulation of histamine. Hence, it might be speculated that granules are "young and immature" and that vesicles are more "mature" (Figure 3). ECL cell histamine depletion has important functional consequences. Basal and gastrin-stimulated acid secretion is suppressed while histamine- and vagally-stimulated acid secretion is unaffected [42]. Histamine-depleted ECL cells were found to respond to the growth-stimulating effects of gastrin much like normal ECL cells. Also, the gastrin-evoked growth of the oxyntic mucosa was unaffected by depletion of ECL cell histamine [41].

In a discussion of the biology of the ECL cells, it may be pertinent to point out that the ECL cell hormone still remains to be identified. Hormone deprivation (i.e., gastrectomy) should be one way to identify the type of hormone produced by the ECL cells. Gastrectomy has a number of quite dramatic consequences, some of which concern impairment in bone architecture and a deficient calcium homeostasis. Thus, gastrectomy

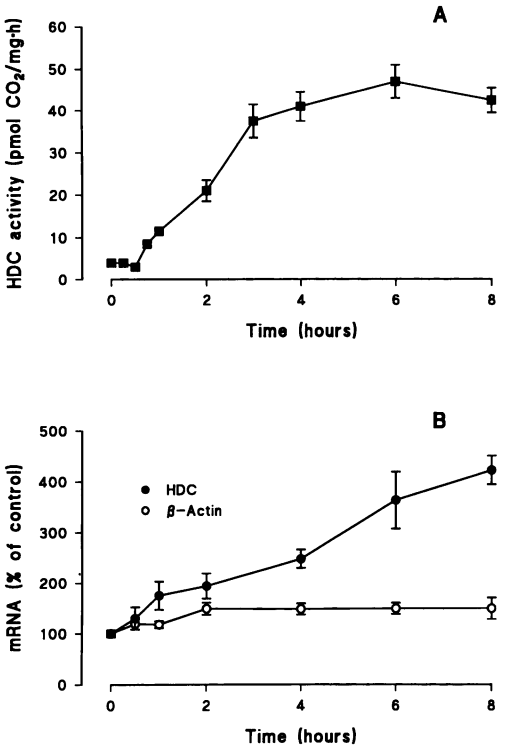

Figure 6. Time course of the rise in (A) HDC activity (pmol CO_2/mg hr) and in (B) HDC mRNA level (percent pre-stimulation) and ß-actin mRNA (a housekeeping gene) level (percent pre-stimulation) in the oxyntic mucosa in response to Leu[15]-gastrin-17 infusion (5 nmol/kg/hr, i.v.). Mean ± SEM (vertical bars, n = 4-9 rats). The mRNAs for HDC and ß-actin were analyzed by Northern blot using ^{32}P-labelled cRNA probes; the levels were determined (semi-quantitatively) by densitometric scanning after autoradiography [25].

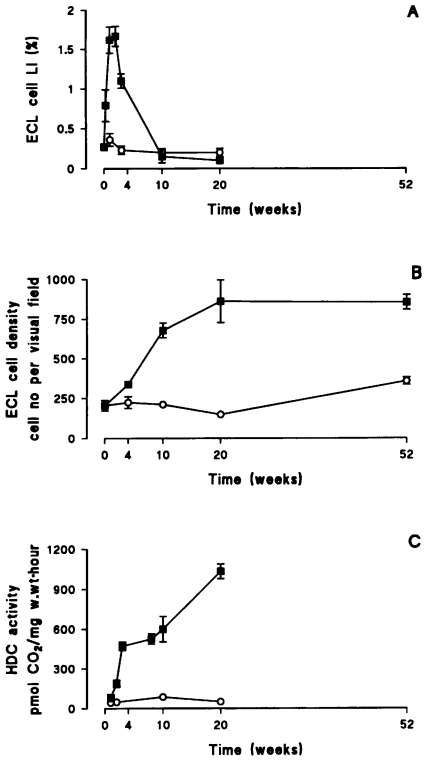

Figure 7. Time course of the changes in (A) ECL LI (percent), (B) ECL cell density (number of cells per visual field) and (C) HDC activity (pmol CO_2/mg hr) in the oxyntic mucosa of omeprazole-treated(400µmol/kg/day, p.o.) (filled square) and vehicle-treated (open circle) rats. Mean ± SEM (vertical bars, n = 7-10 rats). The ECL cells were visualized by immunocytochemistry using histamine antibodies [30].

caused rapid bone loss and a marked reduction in trabecular bone volume [43-45]. These effects do not reflect the loss of gastric acid since omeprazole-induced blockade of gastric acid secretion did not reproduce the efffects of gastrectomy. The gastrectomy-evoked osteoporosis could be prevented only partly or not at all by supplementation with calcium [44], and it cannot be excluded that the bone loss reflects a gastric hormone deficiency rather than calcium deficiency. Moreover, gastrectomized rats respond to an oral $CaCl_2$ load with a marked hypercalcemia, unlike intact rats that do not display any perturbation of blood Ca^{2+} levels under identical circumstances (Håkanson, in preparation). Conceivably, the acid-producing part of the stomach harbours an anti-hypercalcemic signal that can be released by gastrin. In addition, gastrectomy greatly aggravates the hypocalcemia that ensues following parathyroidectomy [46]. The hypocalcemia evoked by parathyroidectomy is thought to reflect a reduced turn-over of bone. Perhaps the combination of gastrectomy and parathyroidectomy aggravates the impairment in bone turnover. Finally, gastrin stimulates the uptake of Ca^{2+} into bone and evokes a transient hypocalcemic response, possibly reflecting the uptake of Ca^{2+} [47-50]. These effects of gastrin are abolished by gastrectomy or fundectomy, suggesting that gastrin acts to release a hypocalcemic agent from the acid-producing part of the stomach [49, 50]. This agent, which has been referred to provisionally as gastrocalcin, may originate from the ECL cells.

CONCLUDING COMMENTS

The histamine-producing ECL cells that are confined to the acid-producing part of the stomach represent an endocrine organ of impressive size. It appears likely that the ECL cells, which are highly responsive to gastrin, represent the local source of histamine that participates in controlling the activity of the parietal cells. The peptide hormone of the ECL cells has not yet been identified, but indirect evidence suggests that the ECL cells (and the anticipated ECL cell hormone) play a role in controlling or directing bone modelling and re-modelling.

Gastrin is said to have two physiologically important actions: stimulating acid secretion and stimulating growth of the oxyntic mucosa, notably the ECL cells. The acid-stimulating effect of gastrin seems to be largely mediated by the gastrin-induced release of histamine from the ECL cells. In view of the powerful stimulating effect of gastrin on the ECL cells, it is tempting to suggest that the primary effect of gastrin is to stimulate the ECL cells and to cause secretion of histamine and the ECL cell hormone.

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