The mechanism of histamine secretion from gastric enterochromaffin-like cells

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The mechanism of histamine secretion from gastric enterochromaffin-like cells. Am. J. Physiol. 277 (Cell Physiol. 46): C845–C855, 1999.—Enterochromaffin-like (ECL) cells play a pivotal role in the peripheral regulation of gastric acid secretion as they respond to the functionally important gastrointestinal hormones gastrin and somatostatin and neural mediators such as pituitary adenylate cyclase-activating peptide and galanin. Gastrin is the key stimulus of histamine release from ECL cells in vivo and in vitro. Voltage-gated K⁺ and Ca²⁺ channels have been detected on isolated ECL cells. Exocytosis of histamine following gastrin stimulation and Ca²⁺ entry across the plasma membrane is catalyzed by synaptobrevin and synaptosomal-associated protein of 25 kDa, both characterized as a soluble N-ethylmaleimide-sensitive factor attachment protein receptor protein. Histamine release occurs from different cellular pools: preexisting vacuolar histamine immediately released by Ca²⁺ entry or newly synthesized histamine following induction of histidine decarboxylase (HDC) by gastrin stimulation. Histamine is synthesized by cytoplasmic HDC and accumulated in secretory vesicles by proton-histamine countertransport via the vesicular monoamine transporter subtype 2 (VMAT-2). The promoter region of HDC contains Ca²⁺-, cAMP-, and protein kinase C-responsive elements. The gene promoter for VMAT-2, however, lacks TATA boxes but contains regulatory elements for the hormones glucagon and somatostatin. Histamine secretion from ECL cells is thereby under a complex regulation of hormonal signals and can be targeted at several steps during the process of exocytosis.

Histamine decarboxylase; exocytosis; neuroendocrine; high-voltage-activated calcium channels; vesicular monoamine transporter

ENTEROCHROMAFFIN-LIKE (ECL) cells are endocrine cells of the gastric mucosa, constituting ~1–3% of the fundic epithelial cell volume. ECL cells display typical features of neuroendocrine cells and can be detected by staining with bivalent cations using the Grimelius staining protocol (52, 94). Ultrastructural studies have determined that this spherical cell type 8–10 µm in diameter contains numerous secretory vesicles with electron-dense cores (1, 2, 8, 11, 13, 15, 27, 28, 31). In 1986, the presence of histamine was detected in gastric ECL cells using immunohistochemistry. In this study, ECL cells were visualized at the basis of the fundic mucosa using antibodies against histamine and the endocrine marker chromogranin A (26). Further immunohistochemical studies have confirmed that this cell type is typically located in the lower third of the gastric glands, often in close contact to chief or parietal cells (78). Localization, cellular products, and morphology of this cell type had been defined earlier by in vivo studies, yielding clear evidence for the function and importance of this cell type in the regulation of acid secretion (8, 25). In vivo studies investigating the secretory process in ECL cells, however, have been complicated by numerous neuronal, hormonal, and paracrine signals present in the gastric mucosa. This review intends to elucidate the mechanism of histamine secretion by recent observations made in isolated ECL cells and ECL cell cultures, lacking numerous interactions and thereby allowing to test direct effects of added substances.

Histamine is a functionally important product stored in high concentrations in gastric ECL cells. It is the most important stimulus of gastric acid secretion in humans and most animals (33). Blockade of histamine receptors on gastric parietal cells is one modality of therapy of acid-related disorders like reflux esophagitis and ulcer disease of the upper gastrointestinal tract.
It was thought for some time that mucosal mast cells constituted the main source of histamine in the stomach and therefore represented the major histamine pool. Studies using isolated mast cells, however, did not detect any effect of gastrointestinal hormones on this cell type.

Meanwhile, in vivo studies have determined that ECL cells play a pivotal role in the peripheral regulation of acid secretion. The importance of ECL cells was accentuated when hyperplasia of these cells was observed under therapy with proton pump inhibitors in humans. Antisecretory therapy with proton pump inhibitors or H$_2$-receptor antagonists resulted in an increased proliferation of ECL cells in rats and mice and even carcinoid formation in female rats after years of treatment. Although several in vivo studies determined an increased density of the ECL cells under therapy, they were unable to show a direct effect of hormones and cytokines present in the gastric mucosa. In vivo, there are complex interactions between neural, paracrine, and endocrine mechanisms. Therefore, a model of highly enriched cells was developed to test the direct effects of added substances.

This model of isolated ECL cells is based on the typical morphology of this neuroendocrine cell. ECL cells are small cells that show a characteristic electron microscopic appearance. Histamine is accumulated in secretory vesicles. Two arguments support the localization of histamine within these vesicles. First, histamine release can be stimulated from permeabilized cells by the addition of Ca$^{2+}$, indicating that histamine must be stored inside membrane-surrounded structures within the cytoplasm. Any histamine present in the cytoplasm would be eluted using this technique. Ca$^{2+}$-induced histamine release from permeabilized cells is a typical criterion for regulated exocytosis, implying that histamine is stored in secretory vesicles, as shown in previous reports investigating exocytosis from adrenal chromaffin cells. Second, secretory vesicles can be defined as a subgroup of vesicles in ECL cells with special ultrastructural characteristics. They contain a small electron-dense core surrounded by a large halo. The loss of histamine following treatment with α-fluoromethylhistidine plus omeprazole is associated with a greatly reduced size of this secretory vesicle compartment, whereas gastrin stimulation increases number and size.

The model of isolated ECL cells uses the relatively small density of the cells to enrich this cell type to a purity of 85–95% (62). A combination of elutriation, density gradient centrifugation, and short-term culture has been applied. The decisive advantage over in vivo studies lies in the high grade of enrichment, which allows conclusions on direct effects of test substances and intracellular steps of activation.

Figure 1 illustrates the localization of the two key components for histamine synthesis and storage in isolated ECL cells. Histamine secretion from ECL cells is of critical importance for gastric acid secretion. In vivo studies have shown that the antral hormone gastrin is the main stimulus for acid secretion and histamine release. This hormone is released from gastrin (G) cells of the antrum after food uptake and binds to specific gastrin receptors on ECL cells. Gastrin stained with a specific antibody against the histamine-synthesizing enzyme, histidine decarboxylase (HDC). In Figure 1b, ECL cells were incubated with a specific antibody against the vesicular monoamine transporter subtype 2 (VMAT-2), which is responsible for the storage of histamine in secretory vesicles. Previous studies have demonstrated the presence of the vesicular monoamine transporter subtype 2 (VMAT-2) in the gastric mucosa (14, 20, 61, 92). The percentage of HDC-positive and VMAT-2-positive cells was similar with a slightly lower percentage of HDC-positive cells. These findings were obtained in isolated ECL cells after 48 h of culture.
reaches the ECL cells via the systemic circulation. Gastrin-induced histamine release starts after 5 min and reaches its maximum after 60 min. This corresponds to in vivo data that show increased histamine secretion in the stomach 10 min after addition of gastrin (25). Isolated ECL cells respond to gastrin stimulation already after 3–5 min of incubation (62–65). Binding to the receptor leads to Ca\(^{2+}\) release from cytoplasmic stores and to influx of extracellular Ca\(^{2+}\) across the plasma membrane, which activates exocytosis of histamine vesicles. Therefore, gastrin-induced histamine secretion can be divided into three successive steps: 1) binding of gastrin to the receptor, 2) Ca\(^{2+}\) release from cytoplasmic stores and Ca\(^{2+}\) entry across the membrane, and 3) assembly of specific soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins and exocytosis of histamine.

Receptor Activation: Gastrin-Induced Histamine Release From Isolated, Highly Enriched Rat Gastric ECL Cells

Studies on isolated ECL cells showed that gastrin potently induces histamine secretion from these cells within minutes of incubation. This gastrin effect is hence classified as an acute effect on ECL cell function. In enriched, uncultured ECL cells gastrin-induced stimulation was two- to threefold. After 48 h of short-term culture, basal histamine release was 3–4% of the content, and histamine secretion was stimulated three- to sevenfold after gastrin addition, corresponding to 15–20% of the histamine content (65). Statistically significant differences of histamine in the medium were observed at 5 min after stimulation. Gastrin stimulated histamine release in a time- and dose-dependent manner after 48 h of short-term culture. The maximal effect was found to be at a concentration of 10\(^{-9}\) M and the EC\(_{50}\) was 10\(^{-10}\) M. After short-term culture of 72 h, basal histamine release ranged from 1 to 2% of the ECL cell content and gastrin stimulation gave a fourfold increment with an EC\(_{50}\) of 10\(^{-10}\) M (65).

Histamine secretion could also be stimulated by cholecystokinin octapeptide (CCK-8). The maximal effect was achieved at a concentration of 10\(^{-9}\) M, with an EC\(_{50}\) value of 5 \times 10\(^{-9}\) M. The equipotent effect of gastrin and CCK-8 shows that a CCK-B receptor mediates the gastrin effect. Gastrin/cholecystokinin receptors are divided into CCK-A (alimentary tract) and CCK-B (brain) according to the effect of selective receptor antagonists (89, 90). CCK-A receptors are responsible for the impact of cholecystokinin on gall bladder contraction, whereas CCK-B receptors mediate fear in the brain (90). CCK-B receptors were identified in the gastric mucosa on isolated ECL and parietal cells (3, 62, 90). The gastrin effect could be inhibited in a dose-dependent manner by the CCK-B antagonist L-365260 (IC\(_{50}\) = 2 \times 10\(^{-8}\) M), confirming that ECL cells express membrane-bound CCK-B receptors mediating gastrin-induced stimulation (65). Maximal inhibition was observed at an L-365260 concentration of 10\(^{-6}\) M. The CCK-A antagonist L-364718 had no effect on gastrin-induced histamine secretion at concentrations of 10\(^{-8}\) and 10\(^{-7}\) M (62).

Signal Transduction: Single Cell Analysis of Intracellular Ca\(^{2+}\) Levels in Isolated ECL Cells

Cells in 48–96 h primary cultures can be utilized to observe Ca\(^{2+}\) signals of single ECL cells with superfusion. At a chamber exchange rate of about five times per minute, cross talk from the small number of contaminating cells is prevented. Hence this approach allows direct assessment of effects of test substances added to the perfusion medium. The basal Ca\(^{2+}\) level in ECL cells was \(\sim 60\) nM, and the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{i}\)]) showed a characteristic biphasic response on gastrin stimulation: an initial peak of \(\sim 800\) nM followed by an intracellular plateau of \(\sim 250\) nM. By addition of CCK-B antagonists (such as L-365260), or the hormones somatostatin or galanin during the Ca\(^{2+}\)-entry phase, the [Ca\(^{2+}\)\(_{i}\)] could be lowered to basal values in a dose-dependent manner, which corresponds to the dose-dependent inhibition of histamine secretion by these ligands (65, 97). Addition of the gastrin receptor antagonist L-365260 (10\(^{-7}\) M) during the plateau phase reduced [Ca\(^{2+}\)\(_{i}\)] to 150 nM, and at a concentration of 10\(^{-6}\) M L-365260, [Ca\(^{2+}\)\(_{i}\)] was back at basal levels. This effect corresponded to the inhibition of histamine secretion.

Addition of EGTA (10 mM) during the plateau phase of gastrin-induced signal transduction decreased [Ca\(^{2+}\)\(_{i}\)], to basal values corresponding to the results for histamine secretion (62). These observations suggest that the plateau phase is of functional importance for stimulation of secretion and is mediated through Ca\(^{2+}\) entry along the plasma membrane. Ca\(^{2+}\) influx during the plateau phase is probably triggered by Ca\(^{2+}\) released from intracellular stores during the initial peak phase. This event has been described as “Ca\(^{2+}\) release activated Ca\(^{2+}\) current” or “Ca\(^{2+}\)-induced Ca\(^{2+}\) release” (60).

Exocytosis in ECL Cells: Involvement of SNARE Proteins

The presence of specific exocytotic proteins in highly enriched ECL cells was initially determined by Western blotting. The t- and v-SNARE proteins synaptosomal-associated protein of 25 kDa (SNAP-25), syntaxin, vesicle-associated membrane protein (VAMP or synaptobrevin), synaptotagmin, and synaptophysin were found in ECL cells based on immunoreactivity for SNAP-25 at 25 kDa, synaptobrevin at 18 kDa, syntaxin at 35 kDa, synaptotagmin at 65 kDa, and synaptophysin at 38 kDa (36, 96). Evidence for the presence of the SNARE proteins SNAP-25 and synaptobrevin in ECL cells of rat gastric mucosa was also obtained by immunohistochemistry and immunocytochemistry. The colocalization of these two proteins could be shown by double staining with an antibody against HDC in consecutive thin sections of the gastric mucosa as well as in isolated ECL cells (36).

For analysis of the functional importance of exocytotic proteins in ECL cells, a newly developed model of...
permeabilization of Ca²⁺ entry along the plasma membrane plays a pivotal role in histamine secretion. After permeabilization, histamine secretion should be directly induced by addition of Ca²⁺ bypassing the receptor-associated steps of activation. Cultured ECL cells were incubated with digitonin (8 µM) and made permeable for extracellular substances like Ca²⁺ and high-molecular-weight substances such as the clostridial neurotoxins. This technique allows an analysis of intracellular mechanisms of exocytosis in ECL cells after Ca²⁺ influx. Permeabilization with digitonin was found to be the optimal procedure to render the cells permeable to both Ca²⁺ and the neurotoxins tetanus toxin light chain (TeTxL) and botulinum neurotoxin A (BoNTA). These neurotoxins inhibit exocytosis in neuronal and chromaffin cells by specific cleavage of the exocytotic proteins synaptobrevin [TeTxL (5, 35, 74)] and SNAP-25 [BoNTA (4, 6, 29, 37, 82)]. In this study, highly enriched ECL cells were permeabilized with digitonin (8 µM) after 48 h of short-term culture and then preincubated with the neurotoxins TeTxL (1 µM) or BoNTA (100 nM) for 15 min. Histamine release was stimulated by addition of Ca²⁺ (30 µM) after 10 min of incubation. ECL cells, which had not been incubated with Ca²⁺, showed a basal release of 6–15% of cellular histamine content. Incubation with Ca²⁺ increased the release three- to fourfold. Preincubation of permeabilized ECL cells with 1 µM TeTxL resulted in a distinct inhibition of Ca²⁺-induced secretion. These data show that the exocytotic apparatus of ECL cells can be influenced by TeTxL. The impact of BoNTA was tested with an identical procedure. After preincubation of 100 nM BoNTA for 15 min, histamine secretion was tested by addition of 30 µM free Ca²⁺. BoNTA completely inhibited Ca²⁺-induced histamine release (Fig. 2). The cleavage of the exocytotic proteins SNAP-25 and synaptobrevin was also examined (36). TeTxL cleaved the exocytotic protein synaptobrevin (47, 74), whereas BoNTA split SNAP-25 (4, 37). Other exocytotic proteins assayed were not affected by these toxins in ECL cells (36).

The increase of intracellular Ca²⁺ levels in ECL cells and other neuroendocrine cells leads to the association of different exocytotic proteins during interaction of secretory vesicles with the plasma membrane (36, 82). While synaptobrevin was identified on sections of the gastric mucosa, SNAP-25 was only detectable in protein fractions derived from purified parietal cells (59). The present immunoblots as well as the colocalization in sections and isolated cells, however, give clear evidence for the presence of these proteins in ECL cells. TeTxL completely cleaved synaptobrevin in ECL cells but partially inhibited Ca²⁺-induced histamine secretion. In chromaffin cells on the other hand, TeTxL totally blocks secretion but cleaves synaptobrevin only partially (35). Ca²⁺-induced histamine secretion from ECL cells is totally inhibited by BoNTA, although SNAP-25 is only partially cleaved. Similar results have been found in isolated chromaffin cells (37, 44). Thus ECL cells are similar to other neuroendocrine cells by the presence of the functionally important SNARE proteins SNAP-25 and synaptobrevin, as well as the vesicle-associated proteins synaptophysin and synaptotagmin, which can be found both in adrenal chromaffin and neuronal cells.

**ELECTROPHYSIOLOGICAL CHARACTERIZATION OF ECL CELLS**

Using patch-clamp techniques, it is possible to analyze ionic contributions to resting and stimulated cells. Whole cell currents were measured by patch-clamp techniques (7, 50). ECL cells have a negative resting membrane potential of about −60 mV (50). Depolarization does not result in an action potential showing that ECL cells are electrically nonexcitable. In the presence of K⁺, Na⁺, and Ca²⁺, depolarization of the plasma membrane leads to an inward current of 400 pA that has a reversal potential of −0 V and can be blocked by Cs⁻. Under these circumstances, depolarization of the ECL cell membrane causes an outward rectifier K⁺ current (73), suggesting the existence of a K⁺ channel in ECL cells that is important for the maintenance of the resting membrane potential. Stimulation of the cells with 12-O-tetradecanoylphorbol 13-acetate (TPA; 10⁻⁶ M), gastrin (10⁻⁹ M), or CCK-8 (10⁻⁷ M) does not provoke an increased current of K⁺ but leads to an additional outward current of negative ions. These ions are probably Cl⁻, according to the reversal potential. This membrane current generated by stimuli probably originates from membrane fusion of histamine vesicles. Histamine is accumulated in secretory vesicles of ECL cells as a consequence of a proton gradient generated by a V-type ATPase (21). Additional transport of Cl⁻ and Na⁺ into the vesicles must be present to maintain electroneutrality. During exocytosis of the vesicles, Cl⁻ channels are incorporated into the plasma membrane, and stimulation therefore results in a Cl⁻ current (50).

Observations of Ca²⁺ currents were also carried out by patch-clamp techniques. During these experiments, K⁺ and Na⁺ were substituted by Cs⁻ and choline chloride in the solution to block the currents carried by these ions and to determine the presence of other ion...
that an influx of Ca\textsuperscript{2+} release. Whole cell patch-clamp experiments revealed is of critical importance for the activation of histamine secretion from isolated and cultivated ECL cells leads to a potent inhibition of gastrin-induced histamine secretion. Activation of SSTR-2 receptors are present since \textsuperscript{1}L-type Ca\textsuperscript{2+} channels are suggested by the mechanisms of activation. The current-voltage relationship in the hyperpolarized range (in which L-type channels remain open) suggests that additional N-type channels are present since ~50% inactivation was observed at a holding potential of ~100 mV. The functional importance of the N-type channels in ECL cells, however, remains to be determined. Preliminary studies of our own group investigating the effect of N- and L-type channel blockers on ECL cell function have revealed that both inhibit histamine secretion and L-type blockers are more effective (66).

Receptor binding is followed by Ca\textsuperscript{2+} influx along the plasma membrane within 20–30 s, probably through voltage-activated L-type Ca\textsuperscript{2+} channels (65). This latter Ca\textsuperscript{2+} influx is capacitative, regulated by Ca\textsuperscript{2+} release from intracellular stores, as known from other nonexcitable cell systems (60). Voltage-gated L- and N-type channels were discovered in ECL cells as well as in chromaffin cells (7, 22, 55, 60, 87). Besides the stimulus-secretion coupling, they could be responsible for the activation of K\textsuperscript{+} outward rectifier currents to maintain the membrane potential (50). Similar observations were reported from other research groups (73).

**INFLUENCE OF SOMATOSTATIN ON HISTAMINE RELEASE FROM ECL CELLS**

Somatostatin inhibits gastrin-induced histamine secretion from isolated and cultured ECL cells in a dose-dependent manner. Experiments on isolated ECL cells show that somatostatin totally inhibits gastrin-induced histamine secretion at a concentration of \textit{10}^{-7} M. The selective somatostatin receptor agonist DC-3287 inhibits gastrin-induced histamine release at concentrations 1,000-fold below that of somatostatin, indicating the functional importance of the somatostatin receptor subtype 2 (SSTR-2) in ECL cells (64). Video imaging studies on fura 2-AM-loaded ECL cells showed that addition of somatostatin \textit{10}^{-7} M or the somatostatin receptor agonist DC-3287 \textit{10}^{-10} M during the plateau phase resulted in a decrease of [Ca\textsuperscript{2+}]\textsubscript{1}, back to basal levels corresponding to the observed inhibition of histamine secretion. Activation of SSTR-2 receptors leads to a potent inhibition of gastrin-induced histamine secretion from isolated and cultivated ECL cells by preventing the entry of Ca\textsuperscript{2+} along the plasma membrane.

**OTHER STIMULI OF HISTAMINE SECRETION FROM ECL CELLS**

Besides gastrin, other stimuli of ECL cells were tested to provide evidence for further receptors on these cells. Therefore, both receptor-dependent and receptor-independent steps of activation were determined. Histamine release was determined in acutely isolated cells. Carbachol (\textit{10}^{-5} M) significantly stimulated histamine secretion from acutely isolated cells (65% enrichment) (62). Carbachol had no consistent effect on highly enriched and cultured cells, which was also observed by other investigators (46). Therefore, ECL cells may lack muscarinic receptors. Epinephrine (\textit{10}^{-5} M) caused significant stimulation. Especially effective were direct activators of intracellular steps of activation like TPA (\textit{10}^{-6} M), dibutyryl adenosine 3’;5’-cyclic monophosphate (\textit{10}^{-3} M), and forskolin (\textit{10}^{-5} M) (46, 62). Besides gastrin, pituitary adenylate cyclase-activating peptide (\textit{10}^{-9} M) has been determined as a decisive stimulus for histamine secretion (46, 96), which is probably of critical importance for neural stimulation of histamine in the cephalic phase of acid secretion.

**REGULATION OF HISTAMINE SECRETION: DIFFERENT HISTAMINE POOLS IN THE EXOCYTOTIC APPARATUS**

Histamine secretion from intact, highly enriched ECL cells was also determined after 60 min of preincubation with the HDC inhibitor, \textit{\omega}-FMH (\textit{10}^{-6} M), the translation blocker cycloheximide (\textit{10}^{-6} M), the transcription blocker actinomycin D (\textit{10}^{-6} M), or the VMAT inhibitor reserpine (IC\textsubscript{50} = \textit{5 \times 10}^{-7} M) (66). Sixty minutes of pretreatment with each of these substances and subsequent stimulation of histamine secretion with 1 nM gastrin attenuated the response by 40–60%, indicating that gastrin-induced release within 60 min of stimulation depends largely on secretion of de novo synthesized histamine. Similar observations regarding the effect of \textit{\omega}-FMH were also made using isolated perfused rat stomachs (9). Furthermore, 4 h of preincubation with \textit{10}^{-6} M reserpine resulted in complete inhibition of gastrin-induced histamine release (66). Therefore, exocytosis of histamine is separated into different pools of histamine either immediately released by Ca\textsuperscript{2+} entry or secreted following gastrin-induced de novo synthesis due to HDC stimulation.

**SYNTHESIS OF HISTAMINE BY HDC IN ECL CELLS**

Stimulation of histamine synthesis has been classified as an intermediate gastrin effect. HDC is the only enzyme performing histamine synthesis in ECL cells (62), which has been detected immunohistochemically on sections of the gastric mucosa and immunocytochemically on isolated cells by specific antibody staining (65). Thirty to sixty minutes after addition of gastrin (\textit{10}^{-9} M), an increased activity of HDC occurs. After 60 min of incubation, HDC activity was increased twofold in acutely isolated ECL cells and three- to fourfold in...
cultured cells. The EC50 value of gastrin for stimulation of HDC activity corresponded to that found for induction of histamine secretion. These results are in accordance with the data obtained in vivo where HDC activity was increased after 60–120 min of incubation with gastrin (16). Similar kinetics regarding the stimulation of histamine synthesis were also observed in basophilic leukemia cells (53).

Besides direct enzyme activation, translation of present HDC mRNA and posttranslational regulation of HDC also seem to be of great importance for gastrin-induced activation. Preincubation with the protein synthesis inhibitor cycloheximide (10^{-6} M) inhibited gastrin-stimulated HDC activity and thereby histamine synthesis (12, 38), indicating that activation of HDC in the cytoplasm is of critical importance for gastrin-induced secretion. Furthermore, recent observations suggest that mammalian HDC may be synthesized as an inactive proenzyme that requires posttranslational processing to become active (95). Molecular cloning of the mammalian HDC has revealed that the cDNA encodes a protein with a molecular mass of 74 kDa (40, 95), whereas purified fetal liver protein subunits [relative mol wt (M, r) = 54,000] or Sf9 mastocytoma cells (M, r = 53,000) contain a protein of different size and molecular weight (83). Furthermore, the 74-kDa isoform appears to be enzymatically inactive (95). These discrepancies might be explained by the fact that HDC is posttranslationally processed.

Gastrin stimulates gene expression of HDC mRNA after 2 h of incubation (16). These reports are in accordance with the observation that preincubation with the HDC inhibitor α-FMH (10^{-6} M) inhibited gastrin-stimulated histamine secretion (1, 2). In previously published studies, transcription of HDC was stimulated after 120 min of incubation in transfected cells and was found to involve both Ca^{2+}- and cAMP-dependent transcription factors (34). Gastrin therefore plays an important role for histamine synthesis by HDC activation and gene expression within hours of incubation. Gene regulation of the HDC promoter has been investigated by molecular techniques (34). The HDC gene promoter contains two Ca^{2+}-responsive TATA boxes and is under complex regulation. The upstream promoter sequence was cut out and inserted into a cell line stably transfected with the gastrin receptor (34). Promoter analysis showed that this promoter sequence was stimulated by gastrin addition and was controlled by Ca^{2+}- and cAMP-responsive elements. Furthermore, protein kinase C (PKC) activation resulted in stimulation of HDC gene expression, whereas downregulation of PKC activity decreased the enzyme activity (34).

### HISTAMINE TRANSPORT VIA THE VMAT

ECL cells show close similarity to certain neuronal cells of the brain stem as well as chromaffin cells of the adrenal medulla because of their ability to accumulate biogenic amines in secretory vesicles using specific transporters. Two subtypes of VMAT have been characterized. Chromaffin, enterochromaffin, and neuronal cells store epinephrine, norepinephrine, serotonin, or dopamine via activation of VMAT-1. Studies on human basophilic leukocytes and ECL cells revealed that these cells express VMAT-2 (20, 92). In contrast to the ligands discussed above, histamine is a diamine. Mast cells, in contrast, do not contain VMAT-2.

In rat ECL cells, gene sequence of VMAT-2 was detected by RT-PCR (24). Cloning and sequencing of the PCR product in ECL cells revealed a 1,345-bp open reading frame.
reading frame, corresponding to 515 amino acids. The VMAT-2 sequence obtained from ECL cells was 98% homologous to previously published sequences obtained in rat basophilic leukemia (RBL-2) cells (19). The presence of VMAT-2 in isolated ECL cells was also shown by immunocytochemistry, as seen in Fig. 1 and consistent with sections performed in the gastric mucosa (61).

The VMAT-2 promoter region has recently been cloned by different research groups using the Genome Walker kit combined with nested PCR, and identical results were achieved (24, 91). Interestingly, translation of the VMAT-2 protein starts at exon 2, as shown in Fig. 3. Exon 1 and intron 1 are incorporated into the promoter sequence and seem to have regulatory functions. As shown in Fig. 3, analysis of the promoter revealed no TATA boxes within the upstream sequence. However, binding sites for AP-2, Sp1, NFκB, Ca^{2+}, and a cAMP-responsive element motif, as well as somatostatin- and glucagon-responsive elements were detected (24, 91). The physiological stimulants of histamine storage remain to be determined.

There is close similarity between the mechanisms of vesicular uptake in ECL cells and basophilic leukocytes. The transporters in these cell types show ~100% homology (19, 48). In analogy to basophilic and chromaffin cells, there is a countertransport of the amine with protons in ECL cells. Vesicle acidification can be visualized in vitro by staining of the cells with acridine orange, which accumulates in acidic compartments.

Fig. 4. Mechanism of histamine secretion from rat ECL cells. A: 3 consecutive steps in the process of exocytosis: gastrin receptor binding, Ca^{2+} influx, and assembly of SNARE proteins. B: model of 5 different exocytotic proteins present in ECL cells. Localization of synaptophysin and synaptotagmin is not definitely known, but most studies support the localization in dense core vesicles (45, 51, 54, 75, 93). C: accumulation of histamine in vesicles dependent on V-type ATPase and VMAT-2. The vesicular H^{+} gradient is established by a V-type ATPase, pumping H^{+} into the vesicles. To maintain electroneutrality, VMAT-2 transfers H^{+} outside. Therefore, accumulation of histamine via VMAT-2 has been described as H^{+} countertransport (32). D: different histamine pools can be detected in ECL cells: gray vesicles represent histamine that is immediately released after Ca^{2+} entry; black vesicles represent histamine that is stored after de novo synthesis by activation of histamine decarboxylase (HDC) and VMAT-2; and empty vesicles (blue) lack histamine inside. Small circles outside the cell represent histamine released from the cell. NSF, N-ethylmaleimide-sensitive factor.
(21). The stimulus of histamine uptake is currently unknown. Apparently, there is no direct involvement of gastrin in the gene regulation of the transporter, and a feedback mechanism seems possible (24). The mechanism of histamine uptake into vesicles can be inhibited by reserpine (66) and could build a basis for alternative methods for blocking gastric acid secretion (48).

**CHRONIC GASTRIN EFFECTS**

The chronic gastrin effect on isolated ECL cells consists of stimulation of cell proliferation within 2-4 days of incubation corresponding to in vivo studies (43, 47, 84–86). Gastrin stimulated ECL cell mitosis, after an incubation of 24–96 h, with an EC50 value of 4 x 10^{-11} M (65). Bromodeoxyuridine, a thymidine analog, which is incorporated in single-stranded DNA during the S phase of mitosis (39), was used as a marker for increased DNA synthesis (65). Gastrin probably stimulates the transition from G0 to G1 phase in ECL cells (39, 58), while ECL cells from the rat are obviously able to divide according to these data, this feature could not be shown in human cells. However, no appropriate technique has yet been established for enrichment of human cells.

**CONCLUSIONS**

Figure 4 summarizes the mechanism of histamine secretion from rat ECL cells. Regulation of histamine secretion from ECL cells occurs at different cellular levels (Fig. 4A). At the plasma membrane, receptor expression and receptor affinity for the hormone gastrin can be targeted by specific receptor antagonists. Receptor affinities for stimulation of secretion and proliferation show a remarkable difference. After receptor activation, plasmalemmal Ca^{2+} channels appear to be of great importance for stimulation of histamine release. High-voltage-activated Ca^{2+} channels have been detected in ECL cells that may be involved in stimulus-secretion coupling and also as function as activators of outward rectifier K^{+} currents to maintain the membrane potential of -50 mV. Ca^{2+} entry across the membrane induces a specific assembly of the SNARE proteins SNAP-25 and synaptobrevin (Fig. 4B). These proteins are cleaved by dosidrial neurotoxins such as TeTxL and BoNTA, inhibiting exocytosis. After extrusion of histamine, the biogenic amine is restored by the action of HDC. This enzyme is stimulated by gastrin-dependent signal transduction steps but can be inhibited by histidine analogs. Finally, histamine is transported into the secretory vesicles, presenting another cellular level at which histamine secretion can be blocked using substances such as reserpine (Fig. 4C). Different histamine pools can be detected following gastrin stimulation, since histamine is quickly synthesized de novo and accumulated in secretory vesicles (Fig. 4D). All of these different cellular levels may present a tool in the future to inhibit histamine secretion from ECL cells, enabling us to treat acid-related disorders of the upper gastrointestinal tract with novel therapeutic agents.

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