YALE JOURNAL OF BIOLOGY AND MEDICINE 71 (1998), pp. 233-246.
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Properties of Isolated Gastric Enterochromaffin-like Cells

Ningxin Zeng and George Sachs[a]

Wadsworth Veterans Administration Hospital and UCLA, Los Angeles, California

The gastric enterochromaffin-like cell (ECL) has been studied in gastric fundic glands by confocal microscopy and as a purified cell preparation by video imaging of calcium signaling and measurements of histamine release. Regulation of gastric acid secretion is largely due to alterations of histamine activation of the H_2 receptor on the parietal cell and can be divided into central neural regulation, with direct actions of neuronally released mediators and into peripheral regulation by substances released from other endocrine cells. Gastric neuronal stimulation of acid secretion by alteration of ECL cell function is probably mediated by pituitary adenylate cyclase activating peptide (PACAP) receptors on the ECL cell, which activate calcium signaling and histamine release. Peripheral stimulation of acid secretion via the ECL cell is largely mediated by gastrin stimulation of calcium signaling and histamine release. Gastric neuronal inhibition of ECL cell function is probably mediated by galanin inhibition of calcium signaling, and histamine release and peripheral inhibition of ECL cell function is mainly due to somatostatin release from D cells.

INTRODUCTION

Acid secretion and its regulation has been a subject of intense investigation for most of this century. Until about 10 years ago, most physicians believed devoutly that peptic ulcer disease was uniquely related to acid secretion. All therapies were, therefore, focussed on inhibition of acid secretion. Early investigations, therefore, concentrated on regulation of acid secretion, perhaps stimulated by Pavlov's seminal observations on the cephalic phase of acid secretion in his trained Russian dogs in the last decade of the nineteenth century, perhaps inspired by the perceived medical need to inhibit acid secretion as a part of a therapeutic regimen [1-3].

Stimulation of acid secretion was divided into a central or cephalic phase, as envisioned by Pavlov, and a peripheral phase, as determined by injection of a putative regulator of acid secretion. The central phase results from stimulation of vagal outflow from the central nervous system and post-ganglionic release of neurotransmitters within the gastric epithelium, the peripheral phase from exoctytotic events in gastric or intestinal endocrine cells. The central post-ganglionic pathway must converge on either the gastric endocrine cells or the parietal cell or both. Therefore, a definition of neurally mediated regulation is alteration in secretion due to a direct effect of substances released from gastric nerves; peripheral regulation derives from alteration in secretion due to substances released from endocrine cells [1, 4, 5].

At the turn of the century, Edkins discovered gastrin [6], Loewi discovered acetylcholine [7] and Dale histamine [8]. So by the 1920s, many of the peripheral secretagogues that are cited today had been described although it was to take half a century for the idea of gastrin to be accepted. Then, ironically, the gastrinologists discounted any role for histamine.

[a] *To whom all correspondence should be addressed:* George Sachs, Building 113, Room 326, Wadsworth VA Hospital, Los Angeles, CA 90073. Tel.: 310-268-3923; Fax: 310-312-9478.

[b] *Abbreviations:* ECL, enterochromaffin-like; PACAP, pituitary adenylate cyclase activating peptide; ROCC, receptor operated calcium channels; VDCC, voltage dependent calcium channels; CCK-B, cholecystokinin-B/gastrin receptor; PTX, pertussis toxin; VIP, vasoactive intestinal peptide; PYY, peptide YY; dbcAMP, dibutyryl cyclic AMP; GRP, gastrin-releasing peptide; GALR, galanin receptor; SST; RT/PCR.

Also, over the next half century, therapeutic inhibition of acid secretion rested largely with the skilled hands of surgeons progressing from gastrectomy to highly selective vagotomy. Then, the synthesis of selective H_2 receptor antagonists not only provided the first effective and tolerated medication for acid inhibition but disproved the position taken by most of the researchers working on gastrin [9]. Cimetidine established histamine as a prominent player in regulation of acid secretion in the human stomach [9]. The source of gastric histamine became a topic of dispute. Were mast cells indeed the answer? Or were the enterochromaffin-like (ECL)[b] cells responsible? Was the ECL cell the sole source or was there a mixed cell population giving rise to histamine?

Whereas stimulants of acid secretion that were discovered early in gastric physiology, knowledge of the physiological inhibitors of acid secretion lagged behind. The molecules turned out to be peptides with short plasma half lives, such as somatostatin. Improvements in separation technology provided a list of possible peptide inhibitors. Still today, the word "enterogastrone" hides a fundamental ignorance as to the substances involved in the decrease of acid secretion during the intestinal phase of digestion. From laboratories experienced in peptide isolation and separation techniques, a number not only of peptide antagonists but additional peptide agonists of gastric secretion was discovered [2, 10-18].

Due to this effort, a long list of possible substances emerged as modifying acid secretion. With the advent of molecular biology, it became possible to find the structure of the receptors for these substances [19-22]. Then techniques such as *in situ* hybridization, immuno-staining and RT/PCR of RNA isolated from purified cells elucidated the location of many receptors [22-24]. They are to date, almost without exception, present in more than one cell type in the gastric mucosa. The physiological interactions of these are therefore difficult to define *in vivo*. For example, an agonist able to release both stimulatory and inhibitory factors from different endocrine cells would have an unpredictable effect following injection. The physiological role of such an agonist may be determined by the location of its release from peptidergic nerves or the presence of receptors of different selectivity in activating and inhibitory endocrine cells. For this reason, there has been increasing attention paid to less complicated models such as isolated gastric fundic glands or isolated endocrine cells. In such preparations, it is easier to decide whether a stimulatory or inhibitory action is exerted directly on the target cells, i.e., parietal cells, or whether an effect is secondary to changes in function of a specific endocrine cell. In this review, we shall focus on data derived from these *in vitro* models of regulation of gastric acid secretion focusing on the role of the ECL cell in regulation of gastric acid secretion.

ECL CELL IN FUNDIC GLANDS

Functional isolated gastric glands were first produced from the rabbit [25]. Essential to the success of this model was the ability to measure acid secretion. Since the secretory canaliculus of the parietal cell, and the lumen of the isolated gland is essentially a closed space, acid secretion was measured using the accumulation of the weak base, aminopyrine. Its pK_a of 4.0 allowed selective assessment of parietal cell acidity since other acidic spaces have a pH greater than 4.0.

This model responded well to histamine and dbcAMP. There was also a response to carbachol, but this was transient. The basis of the transient nature of this response still remains unexplained. The response to gastrin was weaker than to either histamine or carbachol and was ablated either by H_2 blockade by cimetidine or by the presence of diamine oxidase in the medium [26-31]. These latter data were explained as showing that the parietal cell response to gastrin was indirect, dependent on the release of histamine from enterochromaffin-like cells still present in the isolated gland. *In vivo* data measuring rat gastric acid secretion also showed that whereas carbachol stimulation could be only partially affected

by the presence of a H_2 receptor antagonist, gastrin stimulation was completely blocked [5, 32]. Similar data were also obtained for humans. The isolated rabbit gland data were, therefore, pharmacologically equivalent to the *in vivo* data.

The isolation and sequencing of the gastrin receptor from a parietal cell library along with binding studies proved the presence of a gastrin (CCK-B) receptor on the parietal cell [33]. There was also *in vivo* evidence that there was potentiation between gastrin and carbachol or gastrin and histamine [32]. This cluster of data was naturally interpreted as evidence for a functional gastrin receptor on the parietal cell capable of independent stimulation of acid secretion. It had been shown that gastrin was able to elevate $[Ca]_{in}$ in isolated parietal cell suspensions, proving the presence of a functional CCK-B receptor on the parietal cell correlating with the sequencing and binding studies [26, 27]. How did those data jibe with the histamine dependence of gastrin's effect as determined by H_2 blockade *in vivo* and *in vitro*?

Interaction of gastrin with the ECL cell and stimulation of histamine release from that cell type and stimulation of secretion by carbachol using different pathways could explain the potentiation seen between gastrin and carbachol. The potentiation between histamine and gastrin was more difficult to understand given that gastrin had to release histamine for stimulation of gastric secretion. However, it was shown that effective stimulation of acid secretion by gastrin (and carbachol) in isolated rat and pig parietal cells required the presence of cAMP [27]. Since histamine elevates cAMP in the parietal cell (and to a minor extent $[Ca]_{in}$), gastrin's elevation of acid secretion might well depend on elevation of cAMP, particularly if cAMP-dependent protein kinase phosphorylated a critical protein in the stimulatory cascade deriving from gastrin's binding to the CCK-B receptor. Then, in the absence of cAMP elevation, there would be no effect of gastrin on acid secretion. Superfusion of isolated gastric glands with gastrin generated a calcium signal simultaneously in the ECL cell (defined by histamine autofluorescence) and the parietal cell (defined by shape and location). Cimetidine abolished the parietal cell effect, but this was restored by the addition of dbcAMP. Even the calcium response in the parietal cell due to gastrin depended on elevation of cAMP either by histamine or by addition of dbcAMP. No such dependence was observed for the calcium signal generated by gastrin in the ECL cell. It can, therefore, be argued that cAMP elevation is essential for any effect of gastrin on the parietal cell. Elevation of cAMP in the parietal cell is consequently permissive for the action of gastrin on $[Ca]_{in}$ [27]. This fundic gastric gland model provided significant information on the mechanism of action of gastrin, carbachol and histamine but has not been used further to dissect out other ECL cell responses.

ENDOCRINE CELLS

There are at least three endocrine cells that play a major role in regulation of acid or pepsinogen secretion, the enterochromaffin-like cell, the gastrin or G cell and somatostatin or D cell. The ECL cell is found mainly in the fundic region of the stomach, the G cell in the antral gland and the D cell in both the antral and fundic region. The fundic and antral D cells may differ in some of their receptor properties, given that the antral D cell communicates with the antral gland lumen and is juxtaposed to the G cell and that the fundic D cell does not communicate with the gland lumen and is in the vicinity of the ECL cell [34, 35].

We now have considerable knowledge of the major cell types of the gastric mucosa, namely the parietal cell and the chief cell. Their function (acid and pepsinogen secretion) is relatively easy to measure *in vivo* and *in vitro*, and functional assay therefore permits a definition of the responsive elements in these cells. Purification of these secretory cells starts out with 30 percent of the cell population. Purification of the major endocrine cells of the mucosa is more troublesome, since each type represents not more than one percent of the cell population. Identification of the cell that has been enriched or purified is also

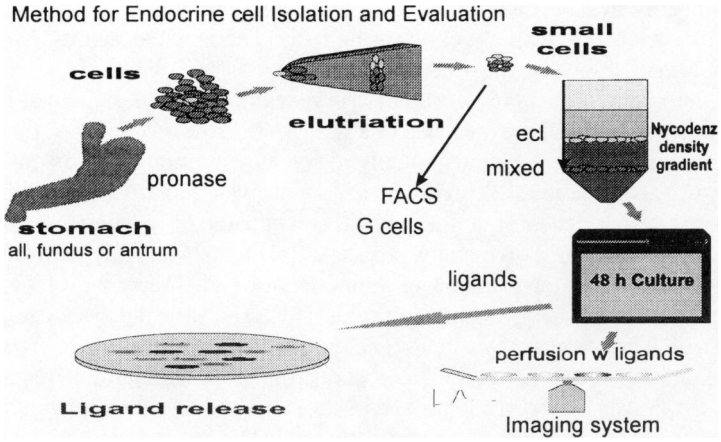

Figure 1. General methodology for purification of ECL cells and enrichment for ECL and other endocrine.

more difficult since there are multiple ligands producing different end effects. Many of these self-same ligands can also be released into the medium bathing a mixed endocrine cell population generating secondary effects. In order to identify explicitly each receptor present on a single cell type, various strategies can be used. Cells can be obtained at high purity (over 90 percent), neutralizing antibodies against possible contaminating ligands can be added to the suspension medium and superfusion used during video imaging of calcium signaling in individual cells in a population. When this method is combined with immunostaining, it is possible to identify different endocrine cell types by their characteristic ligand responses [36]. The general methods we have applied to endocrine cell purification or enrichment are shown in Figure 1.

Table 1. An abbreviated list of agonists or other agents that have been shown or postulated to react with one or other of the cells of the gastric mucosa.

Receptor	Cell Type ECL	G	D	Parietal	Chief
CCK-A	No	No	**Yes**	No	Yes
CCK-B	**Yes**	No	**Yes**	Yes	No
PACAP	**Yes**	?	**VIP**	?	Yes
M1,3,5	Yes	Yes	No	**Yes**	Yes
M2,4	No	No	**Yes**	No	No
GRP	No	Yes	Some	?	?
ST	**Yes**	**Yes**	**Yes**	Yes	Yes
Ca	?	Yes	?	?	?
Y1	**Yes**	?	?	?	?
GAL	**Yes**	?	?	?	?
Histamine	**H3**/H1	No	H3	**H2**	Species
CGRP	No	No	Perhaps	No	No
Amino acids	No	Perhaps	No	No	No
pH	No	Perhaps	Perhaps	No	No

In some instances, where effects have been described only on incubation with mixed cell populations, a question remains as to the target of these agonists or antagonists. Boldface type indicates clear significance of the receptor in target cell function. Where direct effects have not been shown, but only release studies, a question mark is put in the table.

Work to date measuring calcium signaling on five types of gastric epithelial cells in a variety of preparations is summarized in Table 1. Evidently there is significant cross-talk between the different cells of the gastric epithelium but the importance of histamine in regulation of acid secretion places regulation of the ECL cell as the most important cell controlling gastric acid secretion.

The large number of ligands that regulate secretory function of the gastric epithelium speaks to the delicate adjustment of the rate of acid secretion that occurs in order to tailor the response to meet the demand placed on the stomach by the quantity and quality of the meal. We shall however discuss only the ECL cell here, since it is the only gastric endocrine cell that we have available in high purity, making superfusion and histamine release data alike in their interpretation.

ECL CELL

The ECL cells produce and store histamine [37]. This biogenic amine is stored in vesicles to give a total content of 2.8 to 4.3 pg/cell of histamine, which is a relatively low amount compared to mast cells (12 to 20 pg/cell). As for other gastric endocrine cells, this is a small, about 10 um diameter, cell found at mostly towards the base of the fundic gastric gland. It contains acidic vacuoles with an eccentric electron dense spot [38]. Renewed interest in this cell was generated by the finding that high doses of omeprazole resulted in ECL cell hyperplasia and carcinoid formation in rats [39-41].

There are various means of identifying this cell type. Under fluorescence microscopy, the vacuoles accumulate acridine orange due to their acidity, resulting in a red fluorescence characteristic of acid spaces. The cells express histidine decarboxylase required for histamine biosynthesis [42]. Because they contain vacuoles rather than granules (such as G and D cells), they are somewhat less dense and can be purified almost to homogeneity from a gastric epithelial cell suspension. A combination of elutriation (to select a small cell population) and Nykodenz gradient centrifugation produces an ECL enriched population (about 70 percent). Forty-eight hour culture in growth medium results in a cell population containing

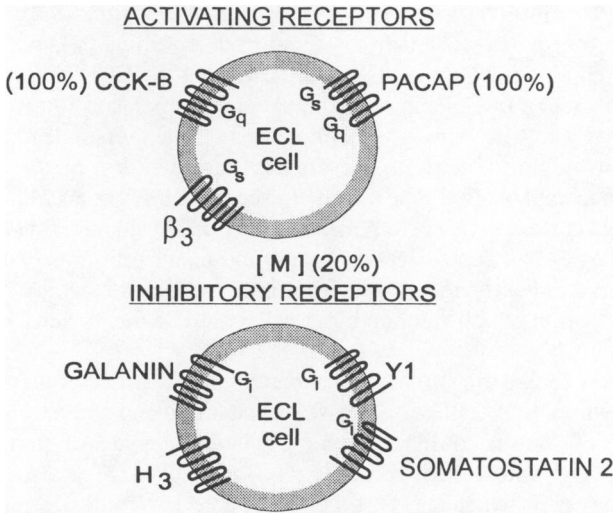

Figure 2. The various receptors of the gastric ECL cell shown to affect calcium signaling and/or histamine release.

about 90 percent ECL cells. A large number of receptors present on the ECL cell have been identified directly, by video imaging of calcium signaling, by PCR of an ECL cell cDNA library or RT/PCR of RNA isolated from ECL cells and also by measurements of histamine release from an approximately 90 percent pure population of these cells [43, 44]. Several recent reviews are available [34, 45, 46]. These receptors are summarized in Figure 2.

STIMULATION OF ECL CELLS

Peripheral

The major stimulatory ligand for histamine release from these cells is gastrin. Addition of gastrin to a perfusate results in a characteristic biphasic increase in intracellular calcium for stimulation by pituitary adenylate cyclase activating peptide (PACAP). The initial spike is due to release of calcium from intracellular stores, the steady state due to entry of calcium from the medium.

Essentially all ECL cells respond to gastrin with a similar elevation of $[Ca^{2+}]_i$. The EC_{50} for gastrin for both the calcium signal and histamine release is about 10^{-10} M. The elevation of steady state calcium is essential for release of histamine and is due to activation of receptor operated calcium channels (ROCC) by gastrin.

The ECL cell also has voltage-dependent calcium channels (VDCC). The depolarization of the ECL cell by the addition of 40 mM K^+ or 20 mM tetraethyl amine, results in a calcium signal that is inhibited by calcium channel blockers such as nifedepine or ω-conotoxin at appropriately low concentrations. The addition of the L channel activator, Bay K8644 also results in a biphasic elevation of intracellular calcium. This shows the presence of not only receptor operated calcium channels but also of depolarization activated or voltage dependent calcium channels in the ECL cell, channels similar to those found in other neuroendocrine cells [47].

These VDCCs could be activated during exocytosis due to electrical changes following fusion of the histamine containing vacuole with the plasma membrane of this cell. The histamine containing vacuole membrane has a V-type ATPase that is an electrogenic proton pump. Acidification by this pump depends on the presence of a chloride conductance that allows electrogenic proton pumping. Accumulation of histamine is driven by a histamine-proton counter-transport mechanism as found in other amine transporting vacuoles [48]. Whole cell patch clamp experiments have shown that the ECL cell has a resting voltage of about -50 mV and a low membrane conductance. Depolarization was found to activate K^+ channels that were Ba^{2+} inhibited. Stimulation of histamine release by gastrin resulted in activation of a chloride current, presumably due to fusion of the vacuole membrane with the plasma membrane [48]. The model of exocytosis that ensues from these data suggests that the chloride current derives from insertion of the Cl^- channel present in the histamine containing vacuole. The depolarization that would result from this could be counteracted by the depolarization activated K^+ channels but could also activate VDCCs. Hence, the steady-state elevation of cell calcium by gastrin could be due to activation of both ROCC and VDCC pathways

The pathway for gastrin stimulation of parietal cell acid secretion is via CCK-B receptor activation on the ECL cell with release of histamine and activation of the H_2 receptor on the parietal cell. This is consistent with the ablation of acid secretion due to pentagastrin stimulation that is found with H_2 receptor antagonists [27, 32]. It also explains the loss of gastrin responsiveness when the ECL cell is unable to synthesize histamine due to the addition of the histidine decarboxylase inhibitor, 5-α-fluoromethyl histamine [49].

Central Stimulation

Carbachol also results in a biphasic increase of intracellular calcium perhaps due to activation of a M_1 or a M_3 receptor. mRNA for both has been found by RT/PCR, and blockade by pirenzepine indicates a functional M_1 receptor [23, 24]. However, in the ECL cell population, only about 10 to 20 percent of the cells respond [36]. The parietal cell also has a M_3 receptor [24], and from these data much of the vagal cholinergic response is due to direct activation of the parietal cell. Cimetidine was relatively ineffective in inhibition of carbachol-induced acid secretion, consistent with the distribution of these activating muscarinic receptors in ECL and parietal cells.

PACAP is the most recent peptide found of the secretin/glucagon/VIP family [50]. It is found in nerve fibers and is clearly a neurotransmitter. The PACAP receptor appears to be linked to both cAMP and calcium elevation in a variety of cells. Forskolin activates adenylate cyclase and is able to stimulate histamine release from ECL cells [43]. PACAP is an effective and potent stimulant of elevation of intracellular calcium and of histamine release from the *in vitro* ECL cell preparation. The EC_{50} for PACAP is about 10^{-9} M and is about 1,000-fold less than for VIP.

The dose response to PACAP for the calcium signal is similar to the dose response for histamine release, which also illustrates that most of the ECL cells are PACAP responsive [51].

A surprising finding is that the injection of PACAP does not stimulate basal acid secretion and even inhibits gastrin stimulated acid secretion. To explain this finding, it is likely that the effect of PACAP on the fundic D cell is dominant when PACAP is present in the gastric circulation. PACAP has been shown to release somatostatin from D cells *in vitro* [51] and injection of a neutralizing somatostatin antibody results in stimulation of gastric acid when PACAP is injected [52]. Thus, the effect of PACAP on the ECL cell found *in vitro* in the absence of D cell stimulation corresponds to the effects of PACAP *in vivo*, provided that the effects of D cell stimulation are prevented. The effectiveness of PACAP as an *in vitro* stimulant of ECL cell function indicates that it is likely to be the central stimulant of the ECL cell.

Epinephrine also stimulates histamine release from ECL cells, as does the stimulant of adenylate cyclase, forskolin. Previously obtained *in vivo* data suggest that this stimulation may be mediated by activation of a ß3 adrenergic receptor, but it is unclear as to the physiological significance of this pathway of stimulation of histamine release [46].

There is, therefore, evidence for four activating receptors in the ECL cell population isolated from rat gastric mucosa. The CCK-B and PACAP receptors are likely to be dominant in positive regulation of ECL function and, therefore, histamine dependent acid secretion, as shown in Figure 2. Much of cholinergic mediation of acid secretion is due to direct effects of acetylcholine on the parietal cell.

INHIBITION OF ECL CELLS

The setting of the rate of acid secretion to a specific level requires not only stimulation of the ECL cell but also inhibition. Various inhibitors of ECL cell calcium signaling and histamine release have been described. A partial list includes somatostatin, PYY, galanin and even histamine. Two second messenger systems have been identified in ECL cells. All of the activating receptors we have studied appear to have the ability to release intracellular calcium and activate ROCC. In addition, depolarization of the cell with either high K^+ or by Bay K8644 also increases intracellular calcium levels. The action of inhibitors of ECL cell function also is exerted against calcium signaling by this cell. Inhibitors of ECL cell function apparently must inhibit calcium signaling.

Peripheral

Somatostatin inhibits ECL cell function by binding at an SST subtype 2 receptor [52]. PCR of a cDNA library based on ECL cell mRNA showed that only the SSTR type 2 was significantly enriched in ECL cDNA. Single cell video imaging of highly purified ECL cells in culture demonstrated that only the SSTR type 2 selective agonist, DC 32-87, inhibited the gastrin-induced calcium entry at concentrations reported to be selective for this subtype (10^{-11}M). Type 3 and type 4 selective agonists, DC 25-12 and DC 32-92, and also somatostatin SS-14 required 100 to 1000 times higher concentrations, namely 10^{-8} M. Similar results were obtained when the effects of selective agonists on gastrin-induced histamine release were studied [52]. The SSTR type 2 analog inhibited the gastrin-stimulated histamine release with an IC_{50} of 2 x 10^{-12} M. Somatostatin SS-14 and the type 3 and 4 analogs showed IC_{50} values of 1-5 x 10^{-9} M. The effect of somatostatin was abolished by pre-incubation with pertussis toxin (PTX) showing that the SST 2 receptor in these cells was coupled to G_i or G_o.

The proximity of the fundic D cell to the ECL cell suggests that the somatostatin involved in ECL cell inhibition is released from the fundic, not the antral, D cell. Thus, regulation of the fundic D cell is intimately involved in the peripheral regulation of ECL cell function. Somatostatin is so far the most effective inhibitory ligand of ECL cell calcium signaling and histamine release. It is the major candidate for the peripheral inhibitor of histamine release and, therefore, acid secretion. Agonists of fundic D cells, therefore, play an important role in the regulation of the ECL cell.

The peptide PYY is found in duodenal extracts and has a variety of inhibitory actions. Gastrin stimulated histamine release was partially inhibited by PYY with an IC_{50} of 2 x 10^{-9}M. Inhibition of histamine release and of calcium entry by PYY and [Pro^{34}]-PYY and no effect of PYY [3-36] identifies the inhibitory receptor as being a Y_1 receptor subtype. RT-PCR of ECL cell RNA showed that the receptor was the non-truncated Y_1 isoform. The inhibitory action of PYY and related peptides on gastrin stimulated histamine release and calcium signaling was also abolished by pre-treatment with PTX at 200 ng/ml. Additive but not synergistic inhibitory effects of PYY and somatostatin on gastrin-stimulated histamine release were observed. It is not clear, however, whether this concentration of PYY is found in the vicinity of the ECL cell *in vivo* [53, 54].

Evidence from the intact stomach, and from isolated glands has shown that there is an H_3 histamine receptor subtype present with inhibitory pharmacological actions [43, 46]. *In vivo* studies previously suggested that histamine secretion and especially histamine synthesis is under a feedback control of histamine. In the purified ECL cell preparation, the H_3 agonist, R-α-methylhistamine is able to inhibit gastrin-stimulated histamine release. The H3-antagonist, thioperamide, is able to activate histamine release. These data suggest that there is a feedback loop to prevent excessive release of histamine by activation of the H_3 receptor on the ECL cell. There is additional evidence for an H_1 receptor on this cell type, but this would result in auto-activation of this rather toxic transmitter [45].

Central Neural

Galanin is a 29-amino acid neuropeptide initially identified in the porcine intestine and now known to be widely distributed in peripheral and central neurons. In the periphery, galanin colocalizes with other neuropeptides (VIP, NPY) in nerve cell bodies and fibers of the myenteric plexus and submucosal plexus close to the mucosal epithelium mucosa [57]. A recent study also showed that a galanin receptor ($GALR_1$) was highly expressed in human gastric mucosal biopsies indicating that a target for galanin was present in the fundic mucosa [58]. A second galanin receptor has also been cloned ($GALR_2$) [59]. The former appears inhibitory, the latter was shown to elevate cell calcium when transfected into cultured cells [60].

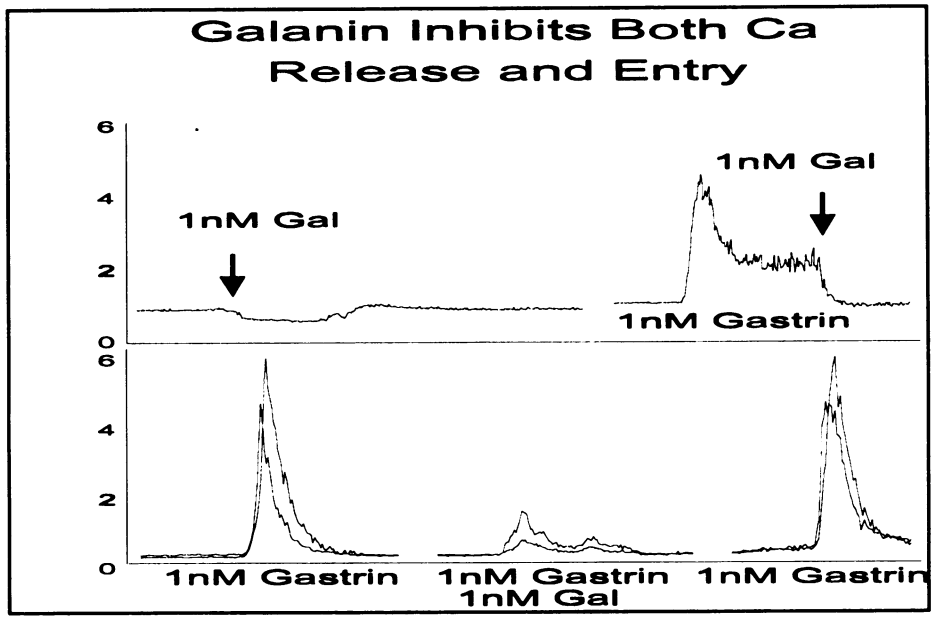

Figure 3. The effect of galanin on basal and gastrin stimulated calcium signaling in the ECL cell.

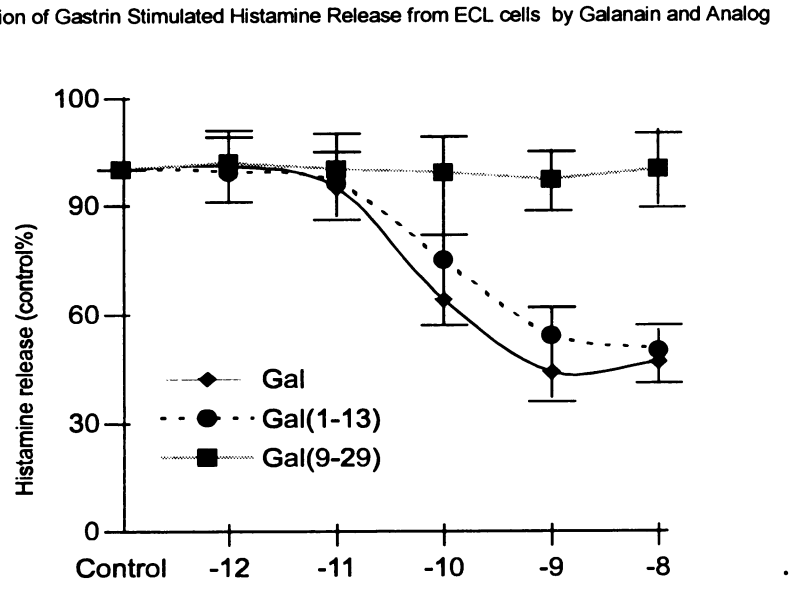

Figure 4. Dose response curve showing partial inhibition of histamine release by galanin and its N terminal fragment.

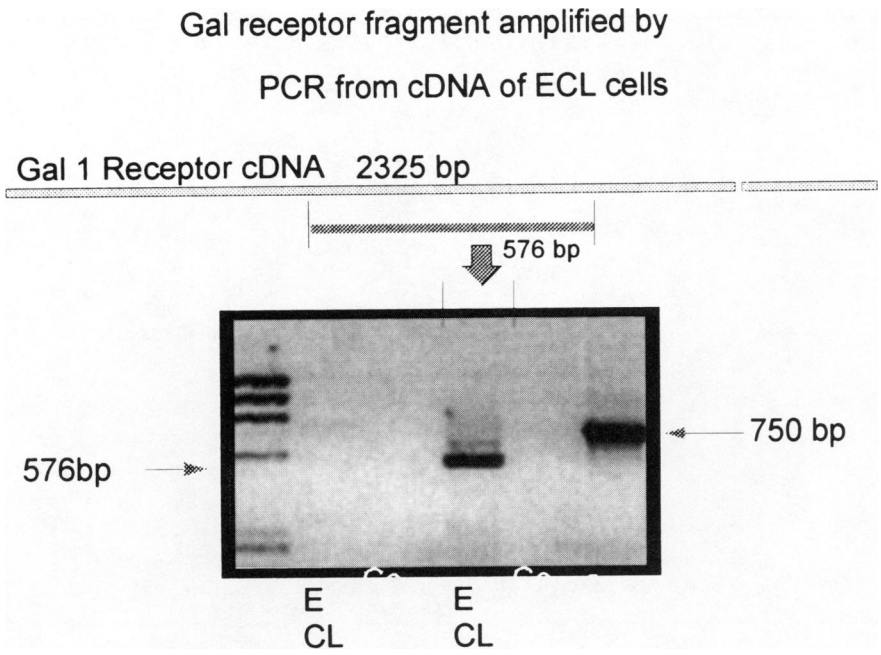

Figure 5. Nested RT/PCR showing the presence of a gal 1 receptor in the rat ECL cell.

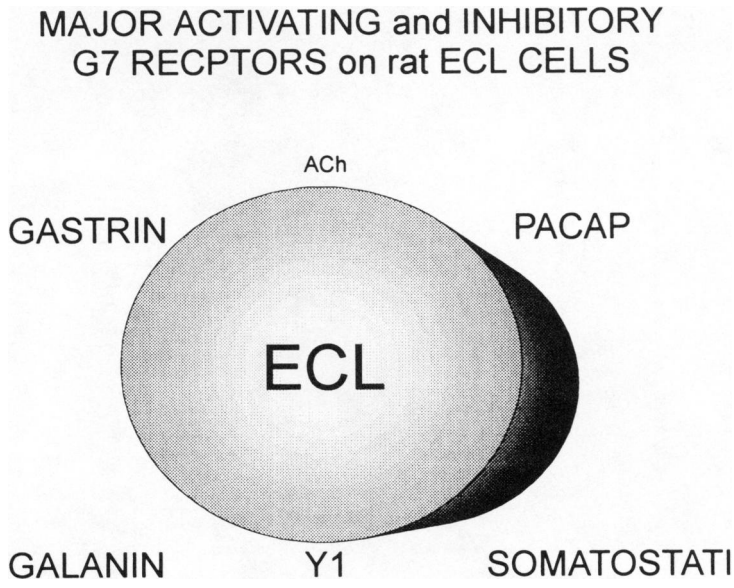

Figure 6. A model of neuronal and peripheral regulation of gastric acid secretion by the major receptors of the ECL cell.

Table 2. A comparison of the properties of the various inhibitory ligands identified thus far in perfusion studies of the isolated rat ECL cell.

	Inhibitors of ECL cells			
	Gal	PYY	SS	His3
R subtype	Gal 1	Y_1	$SSTR_2$	H3
Ca/release	Yes	No	Yes	Yes
Ca/entry	Yes	Yes	Yes	Yes
Histamine/basal	Yes	No	Yes	?
Histamine/stimulated	Partial	Partial	Complete	Complete
PTX sensitive	Partial	Complete	Complete	?

Among its many actions, galanin has been shown to influence gastric acid output [61]. A direct action on antral G cell has been suggested because galanin was found to inhibit bombesin-stimulated gastric acid secretion in rats and dogs [62, 63]. Galanin inhibited basal and GRP-stimulated gastrin release in isolated stomach preparations as well as gastrin release from *in vitro* isolated rat G cells in primary culture [64]. Since it inhibits pentagastrin-stimulated and basal acid secretion [60] and an abundance of galanin immunoreactive nerve endings are found in fundic mucosa [57], galanin must act downstream of the G cell perhaps directly on fundic ECL cells. No effect of galanin on somatostatin release was found *in vivo* and *in vitro,* hence, somatostatin is not involved in the inhibitory action of galanin [65, 66]. Because galanin had no inhibitory effect on bethanechol or histamine-stimulated gastric acid secretion, a direct inhibitory action of galanin on parietal cell is also unlikely. Morphologically, gastric mucosal nerve terminals containing galanin are found adjacent to ECL cells in gastric fundic mucosa [57].

The peptide was found to inhibit gastrin stimulated calcium signals in the ECL cell as shown in Figure 3. Here 10 nM galanin reversibly blocked gastrin effects on ECL cell $[Ca^{2+}]_i$.

The peptide also partially (60 percent) inhibits histamine release from the ECL cell with an EC_{50} of 1×10^{-10} M as shown in Figure 4.

The partial antagonist activity of galanin is due to a rapid desensitization of the galanin receptor.

Two galanin receptors have been cloned. They can be distinguished by the use of chimeric peptides containing the N-terminal sequence of galanin in combination with other peptide sequences. Nested RT/PCR shows the presence of a gal 1 receptor subtype as demonstrated in Figure 5. The actions of galantide, C7 and M40, with the first acting as an antagonist and the latter two as partial agonists, suggest that the ECL cell gal 1 receptor has properties similar to the pancreatic galanin receptor.

Pretreatment with PTX greatly reduced the inhibitory action of galanin on basal and stimulated histamine release as well as the inhibition of Ca^{2+} influx [56].

There are, therefore, a variety of inhibitory factors that influence calcium signaling and histamine release from the ECL cell, of varying potencies and efficacies. The following table summarizes their effects on calcium signaling and histamine release.

From this, and the relative efficacy of galanin and somatostatin, it is likely that somatostatin is the major down regulator of ECL cell function that is locally released. The function of PYY as a component of enterogastrone is endocrine and the affinity of the Y_1 receptor on the ECL cell for PYY is low compared to the affinity of the SST 2 receptor for somatostatin. Under *in vitro* conditions, it is difficult, perhaps, to achieve histamine concentrations sufficient to inhibit calcium signaling and histamine release. The role of the H_3 receptor is, therefore, still unclear. Galanin and galanin 1 receptor are present in the stomach. Galanin is, therefore, the only candidate identified thus far for central regulation of ECL cell function.

The role of the ECL cell in regulation of gastric acid secretion is, therefore, becoming better understood as isolated cell and gland models have become available. In summary, a model of the ECL cell as describing central and peripheral regulation of parietal cell acid secretion is shown in Figure 6. Here only the major regulatory receptors are shown, where function has been demonstrated in all isolated ECL cells with corresponding *in vivo* data.

Acknowledgments: Supported in part by NIH grants DK40615, DK41301 and DK17294 and USVA SMI grant

REFERENCES

1. Pavlov, I. The work of the digestive glands. London: Griffin; 1910.
2. Hirschowitz, B.I., Keeling, D., Lewin, M., Okabe, S., Parsons, M., Sweing, K., Wallmark, B., and Sachs, G. Pharmacological aspects of acid secretion. Dig. Dis. Sci. 40:3S-23S, 1995.
3. Hersey, S.J. and Sachs, G. Gastric Acid secretion. Physiol. Rev. 75:155-189, 1995.
4. Duke, W.W., Sachs, G., and Hirschowitz, B.I. Vagal stimulation of gastric acid secretion in man by 2 deoxyglucose. Lancet 2:871-876, 1965.
5. Tache, Y. and Yang, H. Brain regulation of gastric acid secretion by peptides. Ann. NY Acad. Sci. 597:128-145, 1990.
6. Edkins, J.S. The chemical mechanism of gastric secretion. J. Physiol. (London) 34:133-144, 1906.
7. Loewi. O. Arch. Ges. Physiol. 189:239-242, 1921.
8. Barger, G. and Dale, H.H. ß-Imidazolylethylamine, a depressor constituent of intestinal mucosa. J. Physiol. (London) 41:499, 1911.
9. Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R., and Parsons, M.E. Definition and antagonism of histamine H_2 receptors. Nature 236:385-390, 1972.
10. Walsh, J.H. Gastrointestinal hormones. In: Johnson, L.R., ed. Physiology of the Gastrointestinal Tract. New York: Raven Press; 1994, pp. 1-127.
11. Larsson, L.I., Goitermann, N., Magistris, D.E., Rehfeld, J.F., and Schwartz, T.W. Somatostatin cell processes as pathways for paracrine secretion. Science 205:1393-1395, 1979.
12. Allen, J.M., Bishop, A.E., Daly, M.J., Larsson, H., Carlsson, E., Polak, J.M., and Bloom, S.R. Effect of inhibition of acid secretion on the regulatory peptides in the rat stomach. Gastroenterology 90:309-324, 1986.
13. Anastasi, A., Esparmer, V., and Bucci, M. Isolation and structure of bombesin and alytesin, two analogous peptides from the skin of European amphibians *Bombina* and *Alytes*. Experientia 27:166-167, 1971.
14. Tatemoto, K. and Mutt, V. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. Nature 417-418, 1980.
15. Pappes, T.N., Debas, H.T., Goto, Y., and Taylor, I.L. Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. Am. J. Physiol. 248:Gl 18-Gl 23, 1985.
16. Yagci, R.V., Alptekin, N., Rossowski, W.J., Brown, A., Coy, D.H., and Ertan, A. Inhibitory effect of galanin on basal and petagastrin-stimulated gastric acid secretion in rats. Acad. J. Gastroenterol. 25:853-858, 1990.
17. Mungan, Z., Ozmen, V., Ertan, A., Coy, D.H., Baylor, L.M., Rice, J.C., and Rossowski, W.J. Structural requirements for galanin inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats. Eur. J. Pharmacol. 214:53-57, 1992.
18. DuVal, J.W., Saffouri, B., Weir, G.C., Walsh, J.H., Arimura, A., and Makhlouf, G.M. Stimulation of gastrin and somatostin secretion from the isolated rat stomach by bombesin. Am. J. Physiol. 241:G242-247, 1981.
19. Ganz, I., Munzert, M., Yashiro, T., Schaffer, M., Wang, L., Delvalle, J., and Yamada, T. Molecular cloning of the human histamine H_2 receptor. Biochem. Biophys. Res. Commun. 178:1386-1392, 1991.
20. Ganz, I., Schaffer, M., Delvalle, J., Logsdon, C., Campbell, V., Uhler, M., and Yamada, T. Molecular cloning of a gene encoding the human histamine H_2 receptor. Proc. Natl. Acad. Sci. USA 88:5937, 1991.
21. Liao, C.F., Themmen, A.P., Joho, R., Barbeds, C., Birnbaumer, M., and Birmbaumer, L. Molecular cloning and expression of a fifth muscarinic acetylcholine receptor. J. Biol. Chem. 264:7328-7337,1989

22. Lewin, M.J.M. The somatostatin receptor in the GI tract. Ann. Rev. Physiol. 54:455, 1992.
23. Wilkes, J.M., Kajimura, M., Scott, D.R., Heresy, S.J., and Sachs, G. Muscarinic receptors of gastric parietal cells. J. Membrane Biol. 122:97, 1991.
24. Kajimura, M., Reuben, M., and Sachs, G. The muscarinic receptor gene expressed in rabbit parietal cells is the M3 subtype. Gastroenterology 103:870, 1992.
25. Berglindh, T., Helander, H.F., and Obrink, K.J. Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. Acta Physiol. Scand. 97:401-414, 1976.
26. Li, Z.D., Cabero, J.L., and Mårdth, S. Gastrin and carbachol require cAMP to elicit aminopyrine accumulation in isolated pig and rat partital cells. Am. J. Physiol. 268:G82-G89, 1995.
27. Geibel, J., Abraham, R., Modlin, I., and Sachs, G. Gastrin stimulation of changes in $[Ca]_i$ in parietal cells depends on cAMP. Gastroenterology 109:1060-1067, 1995.
28. Chew, C.S. Cholecystokinin, carbachol, gastrin, histamine and forskolin increase $[Ca^{2+}]_i$ in gastric glands. Am. J. Physiol. 250:G814-G823, 1986.
29. Chew, C. and Hersey, S. Gastrin stimulation of isolated gastric glands. Am. J. Physiol. 242:G504-G512, 1982.
30. Dial, E., Thompson, W.J., and Rosenfeld, G.C. loslated parietal cells:histamine response and pharmacology. J. Pharmacol. Exp. Ther. 219:585-590, 1981.
31. Berglindh, T. The mammalian parietal cell *in vitro*. Ann. Rev. Physiol. 46:377-392, 1984.
32. Lloyd, K.C., Raybound, H.E., Tache, Y., and Walsh, J.H. Role of gastrin, histamine and acetylcholine in the gastric phase of acid secretion in anesthetized rats. Am. J. Physiol. 262:G747-G755, 1992.
33. Kopin, A.S., Lee, Y.M., McBride, E.W., Miller, .LJ., Lu, M., Lin, H.Y., Kolakowski, L.F., and Beinborn, M. Expression cloning and charaterization of the canine parietal cell gastrin receptor. Proc. Natl. Acad. Sci. USA 89:3605-3609, 1992.
34. Sachs, G., Zeng, N., and Prinz, C. Physiology of isolated gastric endocrine cells. Ann. Rev. Physiol. 59:243-256, 1997.
35. Forssmann, W.G., Orci, L., Pictet, R., Renold, A.E., and Rouiller, C. The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. An electron microscopic study. J. Cell. Biol. 40:692-715, 1969.
36. Zeng, N., Walsh, J.H., Kang, T., Helander, K., Helander, H.F., and Sachs, G. Selective ligand induced intracellular calcium changes in a population of isolated gastric endocrine cells. Gastroenterology 110:1833-1846, 1996.
37. Håkanson, R., Boettcher, G., Ekblad, F., Panula, P., Simonsson, M., Dohlsten, M., Hallberg, T., and Sundler, F. Histamine in endocrine cells in the stomach. Histochem. 86:5-17, 1986.
38. Håkanson, R., Owman, C.H., Sporong, B., and Sundler, F. Electron microscopic identification of the histamine-storing agyrophil (enterochromaffin-like) cells in the rat stomach. Z. Zellforschung 122:460, 1971.
39. Ryberg, B., Tielemans, Y., Axelson, J., Carlsson, E., Håkanson, R., Mattson, H., Sundler, F., and Willems, G. Gastrin stimulates the self-replication rate of enterochromaffinlike cells in the rat stomach. Effects of omeprazole, ranitidine, and gastrin-17 in intact and antrectomized rats. Gastroenterology 99:935-942, 1990.
40. Larsson, H., Carlsson, E., Mattsson, H., Lundell, L., Sundler, F., Sundell, G., Wällmark, B., Watanabe, T., and Håkanson, R. Plasma gastrin and gastric enterochromaffin-like cell activation and proliferation. Gastroenterology 90:391-399, 1986.
41. Lee, H., Håkanson, R., Karlsson, A., and Mattsson, H. Lansoprazole and omeprazole have similar effects on plasma gastrin levels, enterochromaffinlike cells, gastrin cells and somatostatin cells in the rat stomach. Digestion 51:125-132, 1992.
42. Dimaline, R. and Sandvik, A,K. Histidine decarboxylase gene expression in rat fundus is regulated by gastrin. FEBS Lett. 281:20-22, 1991.
43. Prinz, C., Kajimura, M., Scott, D., Mercier, F., Helander, H., and Sachs, G. Histamine secretion from rat enterochromaffin-like cells. Gastroenterology 105:449-461, 1993.
44. Prinz, C., Scott, D.R., Hurwitz, D., Helander, H.F., and Sachs, G. Gastrin effects on isolated rat enterochromaffinlike cells in primary culture. Am. J. Physiol. 267:G663-G675, 1994.
45. Sachs, G. and Prinz, C. Gastric Enterochromaffin-like cells and the regulation of acid secretion News Physiol. Sci. 11:57-62, 1996.
46. Modlin, I.M. and Tang, L.H. The gastric enterochromaffin-like cell: an enigmatic link. Gastroenterology 111:783-810, 1996.
47. Zeng, N., Kang, T., Walsh, J.H., and Sachs, G. Voltage dependent calcium channels in gastric ECL cells. Gastroenterology 110:A1136, 1996.

48. Loo, D., Sachs, G., and Prinz, C. Potassium and chloride currents on rat gastric enterochromaffin-like cells. Am. J. Physiol. 1996.
49. Zeng, N., Kang, T., Wang, H., Wen, Y., Walsh, J.H., Sachs, G., and Pisegna, J.R. PACAP type I receptor is expressed on gastric ECL cells and coupled to intracellular calcium and histamine release Ann. NY Acad. Sci. In press.
50. Andersson, K., Chen, D., Håkanson, R., Mattson, H., and Sundler, F. Enterochromaffinlike cells in the rat stomach: effect of alpha-fluoromethyl histidine evoked histamine depletion. Cell Tissue Res. 270:713, 1992.
51. Zeng, N., Bayle, D.B., Walsh, J.H., Kang, T., and Sachs, G. Localization of PACAP receptor on rat fundic ECL cell and D cells. Gastroenterology. 110:Al 131, 1996.
52. Ohning, G.V., Curi, A., Lyu, R.M., Zeng, N., Wang, H.C., Sachs, G., Walsh, J.H., and Pisegna, J.R. PACA stimulates gastric acid secretion during somatostatin immunoneutralization in rats. Gastroenterology. In press.
53. Prinz, C., Sachs, G., Walsh, J.H., Coy, D.H., and Wu, V. The somatostatin receptor subtype on rat enterochromaffin-like cells. Gastroenterology 107:1067-1074, 1994.
54. Zeng, N., Kang, T., Walsh, J.H., and Sachs, G. Peptide YY inhibition of rat gastric enterochromaffin-like cell function. Gastroenterology 112:127-135, 1997.
55. Zeng, N., Walsh, J.H., Kang, T., Wu, V., and Sachs, G. PYY and inhibition of gastric acid secretion. Physiologist 38:256, 1995.
56. Zeng, N., Kang, T., Wen, Y., Walsh, J.H., and Sachs, G. Galanin inhibition of ECL cell function. Gastroenterology 112:A340, 1997.
57. Ekblad, E., Rokarus, A., Håkanson, R., and Sundler, F. Galanin nerve fibers in the rat gut:distribution, origin and projections. Neuroscience 16:355-363, 1985.
58. Lorimer, D.D. and Benya, R. Cloning and quantification of galanin-1 receptor expression by mucosal cells lining the human gastrointestinal tract. Biochem. Biophy. Res. Commun. 222:379-385, 1996.
59. Howard, A.D., Tan, C., Shiao, L.L., Palyha, O.C., McKee, K.K., Weinberg, D.H., Feigher, S.D., Cascieri, M.A., Smith, R.G., Van Der Ploeg, L.H.T., and Sullivan, K.A. Molecular cloning and characerization of a new receptor for galanin. FEBS Lett. 405:285-290, 1997.
60. Smith, K.E., Forray, C., Walker, M.W., Jones, K.A., Tamm, J.A., Bard, J., Branchek, T.A., Linemeyer, D.L., and Gerald, C. Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover. J. Biol. Chem. 272:24612-24616, 1997.
61. Yagci, R.V., Alptekin, N., Rossowski, W.J., Brown, A., Coy, D.H., and Ertan, A. Inhibitory effect of galanin on basal and petagastrin-stimulated gastric acid secretion in rats. Acad. J. Gastroenterol. 25:853-858, 1990.
62. Mungan, Z., Ozmen, V., Ertan, A., Coy, D.H., Baylor, L.M., Rice, J.C., and Rossowski, W.J. Structural requirements for galanin inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats. Eur. J. Parmacol. 214:53-57, 1992.
63. Soldani, G., Mengozzi, G., Della Longa, A., Introrre, L., Martelli, F., and Browne, D.R. An analysis of the effects of galanin on gastric acid secretion and plasma levels of gastrin in the dog. Eur. J. Pharmacol. 154:313-318, 1988.
64. Schepp, W., Prinz, C., Tatgc, C., Håkanson, R., Schusdziarra, V., and Classen, M. Galanin inhibits gastrin release from isolated rat gastric G-cells. Am. J. Physiol. 258:G596-G602, 1990.
65. Kwok, Y.N., Verchere, C.B., McIntosh, C.H., and Brown, J.C. Effect of galanin on endocrine secretions from the isolated perfused rat stomach and pancreas. Eur. J. Pharmacol. 145:49-54, 1988.
66. Madaus, S., Schusdziarra, V., Seufferlein, T. and Classen, M. Effect of galanin on gastrin and somatostatin release from the rat stomach. Life Sci. 42:2381-2387, 1988.