Gastric Acid Secretion: Activation and Inhibition

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Peripheral regulation of gastric acid secretion is initiated by the release of gastrin from the G cell. Gastrin then stimulates the cholecystokinin-B receptor on the enterochromaffin-like cell beginning a calcium signaling cascade. An exocytotic release of histamine follows with concomitant activation of a Cl− current. The released histamine begins the H2-receptor mediated sequence of events in the parietal cell, which results in activation of the gastric H+/K+-ATPase. This enzyme is the final common pathway of acid secretion. The H+/K+-ATPase is composed of two subunits: the larger α–subunit couples ion transport to hydrolysis of ATP, the smaller β-subunit is required for appropriate assembly of the holoenzyme. Both the membrane and extracytoplasmic domain contain the ion transport pathway, and therefore, this region is the target for the antisecretory drugs of the post-H2 era. The 100 kDa α-subunit has probably 10 membrane spanning segments with, therefore, five extracytoplasmic loops. The 35 kDa β-subunit has a single membrane spanning segment, and most of this protein is extracytoplasmic with the six or seven N glycosylation consensus sequences occupied. Omeprazole is an acid-accumulated, acid-activated, prodrug that binds covalently to two cysteine residues at positions 813 (or 822) and 892, accessible from the acidic face of the pump. Lansoprazole binds to cys321, 813 (or 822) and 892; pantoprazole binds to cys813 and 822. The common binding site for these drugs (cys813 or 822) is responsible for the inhibition of acid transport. Covalent inhibition of the acid pump improves control of acid secretion, but since the effective half life of the inhibition in man is about 48 hr, full inhibition of acid secretion, perhaps necessary for eradication of Helicobacter pylori in combination with a single antibiotic, will require prolongation of the effect of this class of drug.

INTRODUCTION

Once the secretion of acid was discovered, it became evident that somehow the presence of this noxious substance in the stomach was necessary for most of the diseases affecting that organ and its immediate neighbors, the esophagus and the duodenum. The challenge faced by physicians was how to regulate acid secretion, how much to regulate acid secretion and when to regulate acid secretion in order to heal the disease. The mechanism by which the acid wrought its havoc remained speculative until quite recently.

Until the 1970s, the only effective interventionist treatment of the acid-related diseases was surgical. Much of the early understanding of secretory regulation was, therefore, due to surgeons such as Lester Dragstedt, to whom this symposium is dedicated. His contributions are discussed elsewhere in this symposium.

Progress in medical treatment of acid-related diseases depended largely on first, an understanding of the physiology of acid secretion, and subsequently, on an understanding of the biochemistry of acid secretion. The therapeutic advances made in the last few decades have also relied heavily on the insight and ingenuity of industrial pharmaceutical science.

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†Abbreviations: CCK, cholecystokinin; ECL, enterochromaffin-like (cell).
Treatment of acid-related diseases has already been transformed and will be changed further since we now are becoming more familiar with the pathogenesis of these diseases.

**CELLULAR BASIS OF REGULATION OF ACID SECRETION**

The first 25 years of the twentieth century defined the major mechanisms of stimulation of acid secretion. Gastrin, a hormone released from the antrum [1], and acetylcholine, released from the vagus [2], were early upon the scene, dividing regulation of acid secretion into a peripheral and central phase, respectively. Although it was recognized that histamine was a potent stimulus of acid secretion [3], histamine remained for many years a controversial actor on this stage.

Therapeutic regulation of acid secretion until 1973 depended either on surgical intervention or on the use of vagal blockade by extract of belladonna or atropine. In 1973, the introduction of the first H2-receptor antagonist, cimetidine, not only changed medical therapy but also changed our understanding of gastric regulatory physiology [4].

This H2-receptor antagonist not only blocked histamine-induced acid secretion, but also gastrin-induced acid secretion and blocked much, but not all, of the vagally-mediated acid secretion [4]. Since the histamine molecule bears no resemblance to either gastrin or acetylcholine, evidently the release of histamine is a major regulatory event in the stimulation of acid secretion.

![Diagram of cellular elements involved in regulation of gastric acid secretion](image)

**Figure 1:** The cellular elements involved in regulation of gastric acid secretion. E is the ECL cell D the fundic or antral somatostatin containing cell, G the gastrin containing cell. The parietal cell is illustrated as having three activating receptors: that for gastrin CCK-B, acetylcholine M3 and histamine. The ECL cell is activated by gastrin and other ligands. Somatostatin inhibits G and ECL cell function.
In vivo experiments suggested that a histamine-containing cell in the gastric mucosa, the enterochromaffin-like (ECL) cell, was the cell stimulated by gastrin or acetylcholine to release histamine. This histamine release mediated all or most of the stimulation of gastric acid secretion [5]. This cell type, not the mast cell, was in the right location and showed the right responses for an intermediary in secretory stimulation.

Gastrin release from the antral G cell accounts for most of the stimulation of histamine release from the ECL cell in vivo, and therefore, the G cell plays a vital role in stimulation of acid secretion. The somatostatin-containing D cell is located both in fundus and antrum, and somatostatin inhibits both G and ECL cell function.

Regulation of acid secretion by gastric endocrine cells involves, therefore, positive and negative interactions between a triumvirate, G, ECL and D cells as shown in the model of Figure 1. Of these cells, the ECL cell has been the most intensively studied in the isolated, purified state.

The enterochromaffin-like cell

Purification of the ECL cell. The rat gastric mucosa has few mast cells, and the major histamine content of the mucosa of this species is due to the presence of ECL cells [6]. The mucosa of this species is thus an appropriate tissue for purification of the ECL cell.

The first step is production of a viable cell suspension. This is achieved by pronase digestion of everted gastric sacs to allow the dissociated cells to fall off into pronase free solution [7]. This mixture of cells differs in cell size and density. The ECL cell has a small size, 10 µm, and is of low density. Fractionation methods, therefore, which depend on size and density differences, appeared appropriate for isolating the cell at a sufficient purity for physiological and biochemical studies.

The use of a counterflow elutriator rotor allows separation of cells largely according to size, thereby enriching an ECL cell population. The low density of these cells allows further purification of the ECL cell to between 65 and 80 percent by using a Nycodenz gradient [8].

If these cells are now placed in short-term culture, enrichment to greater than 90 percent is achieved, allowing for specific measurement of the physiological properties of the pivotal regulatory cell of the fundic gastric mucosa [9].

Criteria of purity. There has been a recent controversy as to the location of the stimulatory receptors in the gastric mucosa using in situ hybridization methods. It is claimed that it is the immunocytes of the lamina propria that express the important receptors for histamine, acetylcholine and gastrin. Data, suggesting a direct stimulation of the parietal cell by these transmitters or hormones, were said to be an artifact due to contamination by these immunocytes releasing a yet unknown transmitter [10]. These in situ methods used did not consider a specific accumulation within immunocytes of the cobalt used to purify the RNA probes, nor took into account data obtained on single perfused parietal cells [11]. High purity preparations are essential to counteract such arguments and a routine method of establishing purity equally essential. Data obtained on single perfused parietal cells and ECL cells show that these cell types do indeed contain the receptors involved in stimulation and inhibition of acid secretion.

Accumulation of histamine within the granules of the ECL cell depends on an acid gradient generated by a V type ATPase [8, 9]. The presence of a vacuolar acid gradient can be visualized directly in a fluorescent microscope using the accumulation of acridine orange. The presence of red fluorescent granules in a small cell fraction is a rapid means of assessing ECL enrichment in a gastric cell preparation [8].

Purity is established further by immunostaining and electron microscopy, which takes advantage of the characteristic morphological appearance of the ECL cell. This is a small
cell, with a large central nucleus and a rim of cytoplasm rich in hollow vacuoles, many containing a central, dense core. With these methods, the 90 percent purity following 48 hr culture can be established.

**Histamine release by the ECL cell-activation.** Stimulation of histamine release by the addition of gastrin can be detected within five minutes of addition. Release is linear for up to 60 min, and there is a three- to five-fold increase of histamine liberation over baseline.

The EC50 for gastrin stimulation of histamine release is $10^{10}$ M. Cholecystokinin (CCK) is about equipotent in stimulation of histamine release. Histamine release is blocked by CCK-B antagonists and requires non-specific levels of CCK-A antagonists for such blockade. An ECL cell library is also enriched in CCK-B receptor cDNA. Therefore, the ECL cell has a gastrin/CCK-B receptor coupled to histamine release. This conclusion is confirmed when looking at $[\text{Ca}^2+]_i$ changes due to the addition of gastrin or CCK-A.

Other agonists also release histamine. For example, acetylcholine and epinephrine are effective stimulants. Peculiarly, whereas gastrin stimulates all cells, acetylcholine simulates about 20-30 percent of the cells in a field. The receptor mediating the action of epinephrine is of the β subtype [8]. Beta-adrenergic stimulation of this receptor on the ECL cell and the D cell may explain some confusing results obtained in the rat in vivo [12].

**Inhibition.** Stimulation of a histamine 3 receptor subtype inhibits histamine release. Apparently there is inhibitory feedback regulation by histamine of its own release [8, 9]. Somatostatin also inhibits ECL release of histamine [8]. The somatostatin receptor on the ECL cell has been shown to be of the ST2 subtype [9]. This hormone, therefore, interferes with acid secretion at at least three sites: the G cell, the ECL cell and the parietal cell.

**Second messengers for histamine release.** Addition of gastrin results in a biphasic increase in intracellular $\text{Ca}^{2+}$ studying ECL cells in a perfusion chamber [8]. This shows that the receptor is present on the ECL cell and not elsewhere. The early transient is due to release of calcium from intracellular stores, and the steady state increment due to calcium entry. Blockade of calcium entry by $\text{La}^{3+}$ prevents histamine release. Increase of cell calcium is likely to activate a variety of calcium-dependent signaling systems including protein kinase C. The C-kinase activator, the phorbol ester, tetradecanoyl-13-phorbol acetate, stimulates histamine release putting this protein kinase in the calcium dependent pathway.

Forskolin, an intracellular stimulant of adenylate kinase, is also an effective stimulant of histamine release from the ECL cell. This is consistent with the finding of a β adrenergic receptor on the ECL cell’s surface. The ECL cell thus utilizes both cAMP and intracellular $\text{Ca}^{2+}$ to stimulate histamine release from the vesicles present in the ECL cell cytoplasm. Both somatostatin and the $H_3$ agonist, R-α-methylhistamine, inhibit the gastrin induced $\text{Ca}^{2+}$ signal.

**Electrophysiology of histamine granule exocytosis.** The loss of ECL cell granule content to the medium depends on exocytosis. The granule membrane contains a V type ATPase. This class of ATPase generates an electrochemical gradient of protons at the expense of ATP. The pump is electrogenic and, therefore, requires an ionic conductance in parallel to generate an acid gradient. The histamine granule is acidic, as shown by the uptake of acridine orange. Therefore, there must be either a $K^+$ or $\text{Cl}^-$ conductance in its membrane. Since the cytoplasm of the cell has a high $K^+$ concentration, the $K^+$ gradient would be in the wrong direction for $K^+$ to serve as an effective co-ion. It is, therefore, likely that the chloride ion serves as the co-ion for the $H^+$ transported by the V-ATPase in the membrane of the secretory granule [13]. With exocytosis the granule membrane fuses with the plasma membrane. The plasma membrane should, therefore, acquire a $\text{Cl}^-$ conductance as a function of histamine release. Electrophysiological methods applied to the isolated ECL cell preparation are able to analyze the properties of the plasma membrane relevant to exocytosis.
Whole cell current analysis of the ECL cell has shown a resting potential of about -60 mV, in contrast to the mast cell, which has a potential close to zero. This potential is due largely to the activity of a depolarization activated K+ current, which also maintains the potential difference after stimulation of exocytosis. Gastrin, CCK-B and TPA, which all stimulate exocytosis, activate a Cl- current, which we believe is due to fusion of the vesicle with the plasma membrane [14].

**Histamine biosynthesis.** The only enzyme involved in histamine biosynthesis is histidine decarboxylase. This enzyme is stimulated in vivo by apparently a gastrin induced increase in gene transcription [15]. Gastrin also stimulates ECL cell HDC activity in vitro [16]. Its rapid action in vitro suggests that perhaps, besides increase of gene transcription, there is also activation of pre-existing enzyme, similar to the regulation of other decarboxylases [17].

**Growth of the ECL cell.** Prolonged hypergastrinemia in the rat results in ECL cell hyperplasia and eventually, carcinoid formation. It has been suggested that this may be due to direct drug effects. Measurement of bromodeoxyuridine incorporation into ECL cells in culture has shown that gastrin can stimulate this incorporation up to three-fold within 48 hr. Whereas the EC$_{50}$ for histamine release was about $10^{-10}$ M, the EC$_{50}$ for growth stimulation was 50-fold less, namely $2 \times 10^{-12}$ M. This suggests either a receptor variant or differential coupling of the CCK-B receptor to secretion and growth. Omeprazole, lansoprazole and pantoprazole are without effect in this model showing that these drugs are without direct effect on ECL cell growth [16].

**A model for the ECL cell.** From the above, it has been possible to develop a model for regulation of ECL cell function in the gastric mucosa as related to acid secretion or growth as summarized in Figure 2. This model is consistent with data obtained on acid secretion in vivo and with what has been deduced for the ECL cell in vivo [5, 6, 15].

The important role of histamine in stimulation of acid secretion places regulation of ECL cell function as central in determining the rate of acid secretion. The activation pathways due to gastrin release from G cells, acetylcholine and epinephrine release from nerve terminals and perhaps other mediators converge on the ECL cell to release histamine. On the other hand inhibition of ECL cell histamine release by somatostatin released by the fundic D cell is of major importance in inhibition of acid secretion.

**Receptor Pharmacology of the Parietal Cell**

Inhibition of activation of the histamine 2 receptor is a reasonably effective and safe means of regulating acid secretion. Regulation of acid secretion by the parietal cell requires activation of both the ECL cell and the parietal cell. Cholinergic ligands not only stimulate release of histamine from the ECL cell, but also stimulate the parietal cell directly by activation of an M3 receptor on the surface of this cell [18, 19]. There is also a CCK-B receptor on the surface of this cell [20]. It may seem unlikely, therefore, that a single receptor antagonist could fully inhibit acid secretion, whether stimulated centrally or peripherally, since there is no common pathway of stimulation of the parietal cell.

Histamine activation of adenylyl cyclase may be permissive for the action of gastrin on the parietal cell (J. Geibel, in preparation). If histamine stimulation is also permissive for other Ca-dependent pathways, it may be possible to fully inhibit acid secretion with a potent H$_2$-receptor antagonist. Equally, if cell calcium elevation is also a permissive requirement for histamine stimulation of acid secretion, suppression of cell calcium may also have pleiotropic effects on acid secretion stimulated by cAMP pathways [21]. Receptors are subject to regulation, by internalization or changes in rate of synthesis. Tolerance has been found for H$_2$ receptor antagonists, perhaps due to upregulation of alternate pathways of stimulation of acid secretion [22], illustrating the constancy of function principle.
THE MECHANISM OF ACID SECRETION

The activation of the gastric acid pump, the H⁺/K⁺-ATPase is the common target of all stimulatory pathways for acid secretion by the gastric mucosa. This enzyme is an integral membrane protein. In the resting cell, it is found largely in smooth membranous cytoplasmic tubules. Stimulation of the cell results in large changes in cytoskeletal organization and rearrangement of the pump membrane so that now it forms the microvilli of an infolding at the apex of the cell called the secretory canaliculus [23, 24, 25]. This morphological change is essential for acid secretion.

It is only in the canaliculus that the pump secretes acid [26]. In the tubules, it does not secrete acid. Along with the change in pump location there occurs activation of the pump. The ATPase exchanges cytoplasmic H⁺ for canalicular K⁺ [27]. Activity of the ATPase requires K⁺ on the luminal surface, and part of the activation process is to provide a K⁺/Cl⁻ permeability of the membrane associated with the ATPase [28]. With activation of acid secretion, KCl effluxes out of the parietal cell into the secretory canaliculus, and K⁺ is reabsorbed by the enzyme in exchange for H⁺. This results in a highly acidic (pH ca 0.8) space forming in the canaliculus. The KCl gradient into the canaliculus is maintained by the H⁺/K⁺ ATPase. This gradient maintains an osmotic driving force for fluid secretion out of the canicular lumen into the lumen of the gastric gland and thence into the lumen of the stomach.

ION TRANSPORT BY THE ATPASE

The catalytic subunit of the enzyme undergoes a cycle of phosphorylation and dephosphorylation as a function of ionic ligands asymmetrically distributed across the membrane. On the cytoplasmic side, the presence of H⁺ and Mg²⁺, besides ATP, results in the transient

**Figure 2.** A model illustrating the receptors, the ionic conductances and the vesicle pump probably present in the ECL cell. The plasma membrane contains K channels, whereas the histamine granule membrane contains a V type ATPase and a Cl conductance. With exocytosis the pump and the Cl conductance is inserted into the plasma membrane.
formation of a conformation that is phosphorylated where the H⁺ ion binding site is cytoplasmic [Mg.E₁⁻P·H⁺]. This conformation rapidly changes to the form with the ion binding site facing the exterior, where the H⁺ is now bound in its hydrated form, the hydronium ion, H₃O⁺ [Mg.E₂⁻P·H₃O⁺]. This form can release H₂O⁺ to the exterior and hydrated K⁺ now binds to the extracytoplasmic region previously occupied by hydronium ion forming the Mg.E₂⁻P·K⁺ conformer. The presence of the large K⁺ species at the extracytoplasmic site allows the enzyme to dephosphorylate, resulting in the E₂⁻K⁺ form. This allows K⁺ to move through the membrane domain to form the E₁⁻K⁺ form, from which K⁺ is released back into the cytoplasm in the presence of ATP. Mg²⁺ remains bound to the enzyme in an occluded form not released until phosphorylation [29, 30, 31]. The above is summarized in the model of Figure 3.

**STRUCTURE OF THE ATPASE**

The ATPase is a member of a large family of ion motive ATPases known as the P type ATPases, since transport is coupled to a cycle of phosphorylation and dephosphorylation of the protein [32,33]. Like the Na⁺/K⁺-ATPase, the H⁺/K⁺-ATPase is a heterodimer [32]. One part of this enzyme is formed of a large, 114 kDa catalytic α-subunit, which is responsible for conversion of the scalar energy of ATP hydrolysis into vectorial ion transport. The other, the smaller β-subunit, of 35 kDa protein mass, is glycosylated and is required for correct assembly and membrane targeting of the mature enzyme.

Crystal structure of this pump, as well as that of the Na⁺/K⁺- and Ca²⁺-ATPases has shown that it is a membrane-embedded molecule with three domains. These are a large cytoplasmic domain accounting for 80 percent of the volume of the molecule, a membrane domain accounting for 15 percent of the molecular volume and the extracytoplasmic domain...
domain which accounts for five percent of the volume of the pump [34]. Based on calcium ATPase structure, the cytoplasmic domain is further divided into a head and membrane connecting stalk [35]. Conformational changes in the cytoplasmic domain due to ion and ATP binding result in H⁺ being driven into the lumen and K⁺ being reabsorbed. The ion pathway seems to be contained within the membrane and the extracytoplasmic domains.

**THE MEMBRANE DOMAIN OF THE CATALYTIC SUBUNIT OF THE H/K ATPASE**

Analysis of the amino acids and their arrangement in the membrane domain of this pump enables better understanding of mechanisms of inhibition of the gastric acid pump. The most frequent means of defining the composition of the membrane domain of a protein is to analyze the primary sequence in terms of hydrophobic stretches of amino acids. Hydrophobic sequences containing about 15 amino acids are potentially membrane spanning. If such an analysis is applied to the primary sequence of the α-subunit of the H⁺/K⁺-ATPase, the N-terminal one third appears to have four such membrane spanning sectors. In the middle of the enzyme, there is a stretch of about 430 amino acids that does not contain a hydrophobic sequence long enough to act as a membrane spanning α-helix. In the C terminal one third, hydrophobic analysis suggests the presence of perhaps five such segments.

Both the N-terminal and C-terminal regions of the enzyme are on the cytoplasmic surface [36]. Therefore, there can only be an even number of membrane-spanning segments. Thus from hydropathy, eight, not nine, membrane-spanning segments are predicted, as was done with the original sequencing of the enzyme [37]. However, experimental evidence is not in full agreement with hydropathy analysis.

The gastric H⁺/K⁺-ATPase is readily prepared as a 90 percent homogeneous population of cytoplasmic side out vesicles. Digestion with trypsin will cleave only on the cytoplasmic side, leaving within the membrane the membrane embedded segment pairs connected by an extracytoplasmic loop. It was thus possible to prove the existence of four pairs of membrane-spanning segments. M1 through M4 were consistent with hydropathy predictions as were M7 and M8. However, where a single M5 segment was predicted by hydropathy, the biochemical data shows the presence of a pair of segments M5 and M6. Using this method no evidence could be found for the last two pairs of membrane spanning segments predicted by hydropathy [38].

The presence of the large cytoplasmic region was confirmed using side reagents. Fluorescein isothiocyanate bound to lys517 in the H⁺/K⁺-ATPase as well as to lys497 and 792 in the Na⁺ pump and pyridoxal phosphate binds at lys497 in the H⁺/K⁺-ATPase [39, 40, 41]. The phosphorylation from ATP occurs at asp385 [42]. These amino acids are found in the major loop between M4 and M5 and, therefore, define this region as cytoplasmic.

The general reaction mechanism of the substituted benzimidazoles is shown in Figure 4. Under acid transporting conditions, these compounds act exclusively on the extracytoplasmic side of the secreting membrane. Omeprazole binds to cysteines 813 (or 822) and 892, lansoprazole to cys321, 813 (or 822) and 892 and pantopozole to cys813 and 822 [37, 43, 44]. These cysteines must be in or close to the extracytoplasmic region of the pump. These are predicted to be at the end of M3, between M5 and M6 and between M7 and M8. Hence, these binding sites define the presence of membrane spanning pairs at M3/M4, M5/M6 and M7/M8. In addition, the photolytic K⁺-competitive reagent, [³H]Me-DAZIP covalently binds in a saturable manner to the M1/M2 region [45]. These reagents, therefore, confirm the presence of at least eight membrane-spanning segments.

Folding of a protein into a membrane is determined by sequences in the protein that contain information for membrane insertion, namely signal anchor sequences and signal transfer sequences [46]. In vitro translation using a constructed protein provided additional
Figure 4. The mechanism of activation of the substituted benzimidazole, omeprazole. The compound reaches the parietal cell cytoplasm via the blood and is accumulated in the active secretory canaliculus. There it is transformed by acid into a cationic sulfenamide which then reacts with available cysteines on the catalytic subunit of the gastric H⁺/K⁺-ATPase.

Information on the membrane spanning properties of that protein. Sequences M1 through M4 acted as signal anchor or stop transfer sequences, as does M8. Additionally, two regions not defined biochemically, M9 and M10, act as signal anchor and stop transfer sequences respectively [47]. Hence, by a combination of techniques, there are 10 transmembrane segments in the α-subunit of the H⁺/K⁺-ATPase. A model based on these is shown in Figure 5. It may be noted that the hydrophilic regions of the membrane domain are contained within the TM4-8 transmembrane segments. Presumably one or more of these bound the ion transport pathway.

Binding of the substituted benzimidazole drugs to cysteines in the M5/M6 region not only abolishes transport [38, 43, 44] but also increases proton leak [48], suggesting that these cysteines are directly within the ion transport pathway.

The association between the two subunits of the enzyme is of importance in maintaining structural integrity and for membrane targeting and assembly. Mapping of an epitope of a monoclonal antibody shared between the α- and β-subunits indicated association between the extracytoplasmic face of TM7 and a region of the β-subunit between cys161 and 178 [49, 50]. Retention of TM7/8 with the β-subunit in solubilised digested enzyme on a wheat germ agglutinin column also showed strong association between TM7/8 and the β-subunit [51]. Under different digestion conditions, TM5/6 also remained associated with the β-subunit. The α:β association is also illustrated in Figure 5.
Figure 5. The two-dimensional arrangement of the membrane domain of the gastric H⁺/K⁺-ATPase. This is derived from a combination of trypsin hydrolysis, site specific labelling, epitope mapping, β subunit binding and in vitro translation. The hydrophilic amino acids are highlighted.

THE ION TRANSPORT PATHWAY OF THE H/K ATPASE

The ion transport pathway across proteins such as the H⁺/K⁺-ATPase must be hydrophilic, able to bind the transported cations, H⁺, O²⁻ and K⁺. In terms of carboxylic amino acids, these are present in TM4, TM5, TM6 and TM8. Most of these are conserved across the mammalian P type ATPases. Mutation of the carboxylic acids in TM5/TM6 affects calcium transport or a partial reaction of the Ca-ATPase of the sarcoplasmic reticulum as well as transport and ATP hydrolysis by the Na⁺/K⁺-ATPase [52,53]. Other hydrophilic amino acids are serine, threonine and cysteine. These are more widely distributed but again concentrate towards the C-terminal sector of the protein. A characteristic signature of the H⁺/K⁺-ATPase is the presence of four cysteines in the ninth hydrophobic sector. Their role in its function is unknown, but perhaps they are derivatized in the mature protein making detection of TM9 and TM10 difficult by routine biochemical methods [38].

A part of the binding site for the K⁺-competitive imidazopyridines and aryl quinolines is in the M1/loop/M2 region of the enzyme [45, 54]. A region responsible for high affinity ouabain binding in the sodium pump may be in the same general area [55], and a mutation in M3 also affects ouabain binding [56]. Ouabain is a partially K⁺-competitive inhibitor of the Na⁺/K⁺-ATPase. Thus, binding of these larger molecules in this region of these two pumps excludes K⁺ from participation in the enzyme reaction. It is also of interest to note that the binding of SCH28080 to the protein prevents inhibition of the enzyme by the benzimidazole, omeprazole [57]. Since omeprazole binds to TM5/TM6 and TM7/TM8, it appears that there is close physical association between the first loop and these regions of the enzyme, suggesting a very compact arrangement of the extracytoplasmic domain of the H⁺/K⁺-ATPase.
Covalent reaction with the substituted benzimidazoles occurs at cys321, cys813 (822) and cys892. The only common cysteine is cys813 (822). It seems likely, therefore, that this region predicted to be loop between TM5 and TM6 is on the proton pathway. It is also of interest to note that when both cysteines of TM5 and TM6 are reacted, the conformation of this region of the protein changes [44]. Inhibitors of gastric acid secretion that target the H⁺/K⁺-ATPase have, therefore, yielded not only effective therapy of acid related disease but also some insight into the structure and function of the target molecule.

**PHYSIOLOGICAL CONSEQUENCES OF INHIBITION OF ACID SECRETION**

In the first section of this review, we considered some aspects of the peripheral regulation of acid secretion. The hormone gastrin not only stimulates acid secretion, but its own secretion is subject to feedback inhibition by the presence of acid in the antrum [58]. Any means of reducing acid secretion, vagotomy, H₂ receptor antagonists, the substituted benzimidazoles and even partial fundectomy, inevitably results in elevation of serum gastrin. Since gastrin not only releases histamine from the ECL cell but is also trophic for the mucosa and the rat ECL cell in particular, treatment of rats with the substituted benzimidazoles has resulted in ECL cell hyperplasia, and after two years, carcinoids [59]. Many different experiments showed that hypergastrinemia was essential for this effect on ECL cells [59,60]. Recently it has been shown in vitro that whereas gastrin elevation is a sufficient condition for DNA synthesis in isolated purified ECL cells, neither omeprazole, lansoprazole nor pantoprazole induce DNA synthesis directly in the ECL cell in vitro [16].

**THE PATHOGENESIS OF ULCER DISEASE**

Early in this century, the inevitable association between the presence of acid and disease was recognized [61]. However, since almost everyone secretes acid and few get sick, clearly this was not the full answer. Resistance to acid is a property of this region of the gastrointestinal tract. Apparently the apical facing membranes of the cells, other than the parietal cell, have no proteins able to transport protons, and therefore, are essentially proton impermeable. Since the hydrophilic mucous gel layer cannot form a barrier to proton back diffusion and the rate of HCO₃⁻ secretion cannot exceed more than 10 percent of acid secretion, the defense against acid back diffusion must lie in the tight junctions between the epithelial cells.

In 1983, it was suggested that a bacterial infection by an organism, *Helicobacter pylori*, was essential for the development of duodenal and gastric ulcer [62]. Subsequent studies have shown that this seminal observation was correct. *H. pylori* does not play a role in esophagitis but does also result in gastritis [63]. Eradication of *H. pylori* has been shown to greatly reduce the incidence of ulcer recurrence [64].

Both acid and *H. pylori* are essential for peptic ulcer disease. It is tempting to speculate that the contribution of *H. pylori* is mediated via tight junction disruption. As the tight junctions become progressively leakier, in the presence of luminal acid, acid back diffusion increases. In contrast to the apical surface, the basal lateral membranes of the gastric and duodenal epithelium have the normal high proton permeability of mammalian cell membranes. The cell cytoplasm will then acidify, the cell die and increasing acid back diffusion result. Besides acid back diffusion, other molecules probably can back diffuse, such as the bacterial urease. This, in turn, will set up an immune response such as gastritis. Since normally the esophageal epithelium is not exposed to acid, neither the cells nor the tight junctions are constructed to resist acid. Thus *H. pylori* does not contribute to acid related disease in this area of the body.

A yet unresolved problem is why so many people with both acid secretion and *H. pylori* infection do not get gastric and duodenal ulcers. Perhaps different strains of *H. pylori*
differ in their ability to induce tight junction damage. Perhaps other factors predispose to \textit{H. pylori} induced tight junction damage.

**PHARMACODYNAMICS OF PUMP INHIBITION**

Major research efforts are being made to develop effective protocols for eradication of \textit{H. pylori}, as recommended by consensus in Europe and America [e.g., 65]. One such is combination of omeprazole and one or two antibiotics. \textit{H. pylori} grows better at neutral than acidic pH. In the antrum, the pH is significantly higher than in the fundus, and full inhibition of acid secretion is required to generate neutral pH in the fundic wall. If this is necessary to improve efficacy of growth-dependent antibiotics such as amoxicillin (cell wall biosynthesis target) or clarithromycin (protein synthesis), it is worth considering the inhibitory effect of single dose pump inhibitor. The effective half-life of inhibition of acid secretion in man is 48 hr [66] and of the protein in the rat is 50 hr [67]. These pump inhibitors are also effective only against the functional pump. Following a meal, when the drug is present in the plasma with a half-life of about 60 min, perhaps not all pumps are active. On the assumption that 75 percent of the pumps are active, and that about 25 percent of the pumps are synthesized or recover in a 24 hr period, the data of Figure 6 can be calculated.

This three-dimensional plot illustrates the percent pump capacity and the percent of time during the day intragastric pH is elevated as a function of single daily dosing of a covalent pump inhibitor. The row labeled before indicates pump capacity before the first dose of drug, and pump capacity immediately before the next dose. The second row, labeled after, shows pump capacity immediately after the daily dose of drug. The third row shows the expected degree of elevation of intragastric pH to a value of greater than 3. This value has been suggested to be optimal for duodenal ulcer healing [68]. The average percent time that pH > 3.0 in untreated people is 20 percent as illustrated in the Figure. Following the first dose, 25 percent pump capacity remains, increasing to 50 percent over the first 24 hr. However, the inhibition is sufficient to elevate intragastric pH to a value above 3.0 for about 50 percent of the day. With the next dose, only 12 percent pump capacity remains and returns to a level of 37 percent after 24 hr. This inhibition elevates pH > 3.0 for 58 percent of the day, and thereafter to 60 percent and 64 percent with the assumptions made above.

**Figure 6:** The postulated pharmacodynamics of single daily dosing of a substituted methylpyridyl benzimidazole acid pump inhibitor. The first row shows pump capacity immediately before dose, the second pump capacity immediately after dose and the last row the calculated percentage of time intragastric pH is elevated to above 3.0.
Close to steady-state elevation of intragastric pH is achieved by the second day of dosing according to these calculations. It is clear however that single daily dosing will not elevate gastric wall pH to neutrality, since a significant fraction of pump activity is returning over 24 hr. To achieve true neutrality in this region of the stomach, divided doses of these inhibitors will be required.

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