INTERACTION OF ANTI-ULCER DRUGS WITH THE GASTRIC PROTON PUMP

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SUMMARY

In this review consideration is given to anti-ulcer drugs interaction with the gastric H^+-K^+ ATPase. The review has been divided into three sections. First, properties of the gastric proton pump are described in terms of structure, biological activity and ions transport activity, followed by an account of interactions involving antisecretory agents. Emphasis is given to a new class of drugs (substituted benzimidazole) that shows a unique antisecretory action and is safe and effective for short-term treatment of patients with duodenal or gastric ulcers. The final section briefly examines future directions for the production of more selective inhibitors of the gastric proton pump.

I. INTRODUCTION

Therapeutic approaches to the management of peptic ulcer disease and reflux esophagitis have as a common goal the reduction of gastric acidity. Although a cause-effect relationship has not been established between acid and lesions of the upper gastrointestinal tract, the empirical observation of "no-acid-no-ulcer" still prevails. Current medical methods for reducing gastric acidity involve three major classes of agents: antiacids, receptor antagonists, and proton pump inhibitors. While only the last class interacts directly with the proton pump, the first two classes influence its activity through indirect regulatory factors. Recent advances in our understanding of the cellular mechanism of gastric acid secretion provide us with new insights of how these agents interact with the proton pump and raise the possibility of designing new drugs to interact with this clinically important system.

This review summarizes what is known about the structure, the kinetic mechanism and the transport activity of the gastric proton pump and examines the influence that antisecretory agents interactions have on the H^+-K^+ ATPase.

II. PROPERTIES OF THE GASTRIC PROTON PUMP

2.1 Identification of the proton pump as an H^+-K^+ ATPase

The discovery in the 1930's that formation of ATP was the primary means of energy conservation during carbohydrate oxidation led naturally to the proposal that ATP hydrolysis might supply the energy for various forms of cellular work. Thus, a gastric ATPase model was postulated linking H^+ translocation to the hydrolysis of ATP. In the search for a H^+-transporting ATPase, several possible candidates have been investigated. Na^+-K^+ ATPase, which is located in the basolateral membrane of the parietal cells /5/ appeared to play a crucial but indirect role in gastric acid secretion by maintaining a high cellular K^+ level /1/. A thiocyanate-sensitive, HCO_3^--stimulated ATPase was also implicated, based on the inhibitory property of SCN^- on HCl secretion, but later, this system was proven to be of mitochondrial origin /2, 3/. Finally, this search led to the establishment of a H^+- and K^+-transporting ATPase as the gastric H^+ pump /4, 5/. The enzyme was first identified by Forte et al. /6/ in the form of a K^+-stimulated phosphatase activity. Isolated in a light microsomal fraction from tissue homogenates, the phosphatase activity is associated with a K^+-stimulated ATPase activity which is enhanced by membrane treatment with ionophores or low concentration of non-ionic detergent. The relationship between the K^+-ATPase activity and gastric H^+ secretion was noted by Lee et al. /7/ who observed that addition of ATP to a microsomal preparation from dog mucosa results in alkalinization of the medium, which in turn indicates that the microsomal fraction consists mainly of inside-out vesicular structures of the membrane generating HCl in the intact cells. In 1977, Sachs et al. /8/ and Chang et al. /9/ finally demonstrated that H^+ accumulation in these vesicles was the product of an ATP-driven electroneutral exchange of intravesicular K^+ for H^+.

Substantial evidence now has accumulated to identify the H^+-K^+ ATPase as the gastric proton pump. Antibodies against the purified H^+-K^+ ATPase show specific staining of the gastric parietal cell /10, 11/. This cell type has been shown to be the sole source of gastric acid secretion /12/. Within the parietal cell, antibody against the H^+-K^+ ATPase is localized at the apical secretory surface and at intracellular membrane structures, known as tubulovesicles /11/. This localization is consistent with these membrane structures being the source of

H^+-K^+ATPase-enriched vesicles obtained from gastric tissue. Using preparations of gastric cells which had been selectively permeabilized, it was possible to demonstrate that ATP was a necessary and sufficient energy source to drive K^+-dependent proton transport in situ /13/. Measurement of H^+-K^+ ATPase activity in situ shows excellent correlation with the properties of this enzyme found in vesicular preparations /14/, particularly in being K^+-dependent and enhanced by K^+-ionophores. Finally, with the recent-development of specific inhibitors for the H^+-K^+ ATPase it has been possible to correlate inhibition of acid secretion with inhibition of ATPase activity /15/. Thus, it seems fairly certain that gastric acid secretion is a direct result of the H^+-K^+ ATPase.

2.2 Structure of the H^+-K^+ ATPase

The H^+-K^+ ATPase is an intrinsic membrane protein that has been isolated and purified from fundic mucosa of various species by differential and density-equilibrium centrifugation and by free-flow and zonal electrophoresis techniques /16-21/. The purified enzyme consists of about 90% of one polypeptide strongly associated with the membrane and requiring lipids for catalytic activity /19, 22-25/. The molecular weight of this protein has been determined by SDS gel electrophoresis to be 90.000-105,000 /19, 25/. Because carbohydrate makes up approximately 8% of the mass of the gastric enzyme /25/, the uncertainty in molecular weight may be partially due to variabilities in sugar compositions.

Very recently the primary structure of rat and pig gastric ATPase has been deduced from the nucleotide sequence of the cDNA /26, 27/. The enzyme consists of 1,033 amino acids and has a molecular weight of 114,012. Hydropathy analysis indicates that there are four major hydrophobic domains in the N-terminal third of the protein and there is sufficient evidence to conclude that each of these protein domains represents a single transmembrane pass. The transmembrane organization in the C-terminal region is unclear. However, it has been estimated that four transmembrane domains are likely to be present in this region, two of them exceptionally hydrophobic. As shown in Figure 1, a model for the path of the main subunit of the gastric enzyme in the membrane bilayer can be drawn, based on the structural evidence accumulated to date.

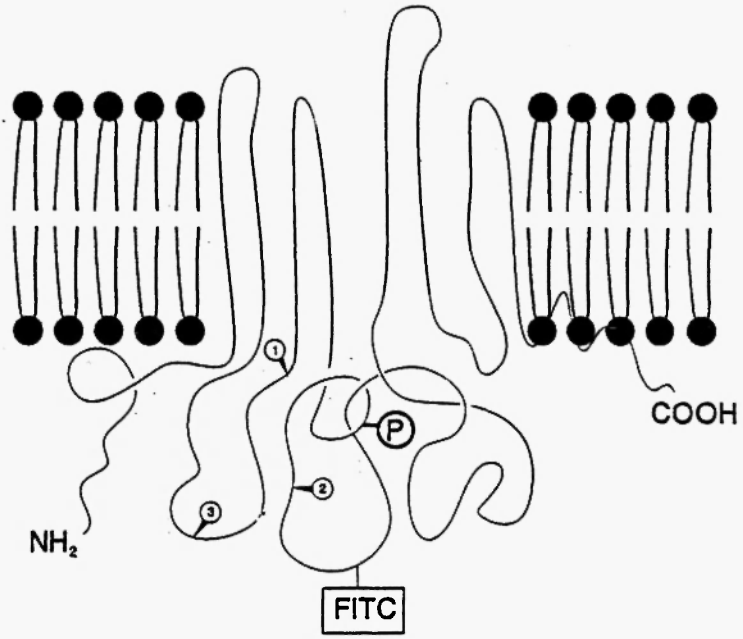


Fig. 1: Possible model for the path of the catalytic subunit of the gastric H^+-K^+ ATPase in the membrane bilayer. Hydropathy analysis suggests eight major hydrophobic domains, each one representing a single transmembrane pass /26/. The numbered arrows on the model mark the sites of tryptic cleavage in the presence of ATP, site 1, and in the presence of K^+, site 2 /42/. The primary papain split is indicated by, site 3 /47/. The phosphorylation site, Asp-385, is indicated by P while FITC shows the binding site for fluorescein isothiocyanate, Lys-517.

The molecular weight of the functional H^+-K^+ ATPase has been estimated by several techniques. Radiation inactivation gave molecular masses between 230,000-270,000 or 444,000 /28-30/, indicating a functional dimer or tetramer. Examination of two-dimensional crystalline arrays of membrane-bound enzyme show a P_2 symmetry (1) with a

(1) The basic molecular unit of the H^+-K^+ ATPase in two dimensional crystal arrays is arranged in a regular pattern. P_2 symmetry means that a 180° rotation of the unit brings the crystal arrays into a pattern indistinguishable from the original one.

unit cell consisting of two monomers /31/. Chemical crosslinking studies of functional enzyme also indicate the existence of a dimer /32/. While significant evidence suggests that the H^+-K^+ ATPase functions in a dimeric form, there is no consensus as to whether the subunits are identical or heterogeneous /24, 25/.

2.3 Enzymatic Activity of the H^+-K^+ ATPase

In terms of its enzymatic activity, the H^+-K^+ ATPase belongs to a class of ATPases which form a covalent phosphoenzyme intermediate. It shares this property with certain other transport ATPases, notably the Na^+-K^+ ATPase and the Ca^{2+} ATPase of sarcoplasmic reticulum and plasma membrane /33, 34/. These three enzymes show a number of similarities including the size of the catalytic subunit /35, 36/ a high degree of homology in primary amino acid sequence /37, 38/, and similar partial reactions. Based on kinetic data from all three enzymes, a general scheme (Figure 2) has been developed to describe the catalytic mechanism.

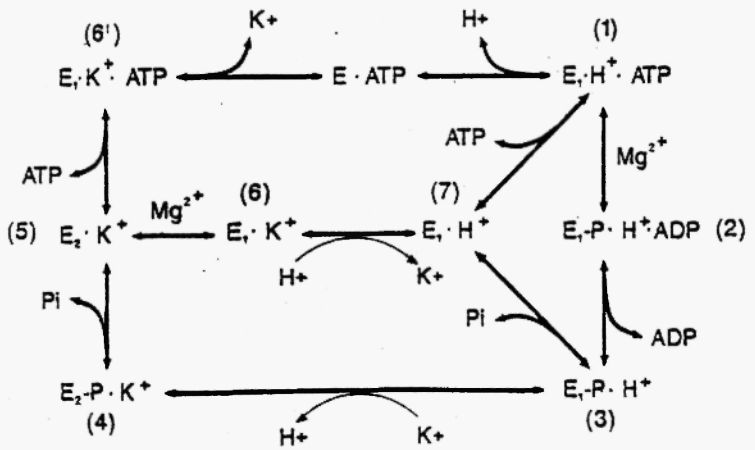


Fig. 2: Model summarizing the kinetic mechanism for the ATP-driven H^+ and K^+ transport of the gastric ATPase. E_1 and E_2 designate the principal conformations of the protein that favor binding of cytosolic H^+ or cytosolic K^+ respectively. Much of the evidence for this model has been published elsewhere /39-41/.

The scheme proposes that the H^+-K^+ ATPase can exist in two major conformational states designated as E_1 and E_2. The initial step in the catalytic cycle is the binding of cytosolic ATP to the E_1 form of the enzyme. In the presence of Mg^{2+} and cytosolic H^+, the $E_1 \cdot$ ATP complex breaks down to form an acid-stable acyl-phosphate bond. It is assumed that a transportable proton is attached to this complex at this stage. The next step in the cycle involves a conformational change in the phosphoenzyme to the E_2-P form. This conformational change, likely, results in the translocation of the proton and exposes a high affinity K^+ binding site at the lumenal surface. The binding of lumenal K^+ to the enzyme promotes the dephosphorylation of the enzyme. This dephosphorylation occurs without the release of bound K^+ or a conformational change of the enzyme, leaving an $E_2 \cdot K^+$ complex. The conversion of the enzyme to the E_1 conformation with translocation and release of K^+ is likely the rate limiting step for the overall cycle /39, 41/. This step requires binding of ATP to form $E_1 \cdot K^+ \cdot$ ATP which has a low affinity, cytosolic site for K^+. Alternatively, it has been suggested /42/ that Mg^{2+} can promote the conversion of $E_2 \cdot K^+$ to $E_1 \cdot K^+$. The K^+ is released in exchange for H^+ and a new cycle begins Although this scheme is oversimplified, it does provide a satisfactory model for the major aspects of a normal catalytic cycle.

In addition to the complete ATPase reaction, this H^+-K^+ ATPase also catalyzes several partial reactions, including a K^+-dependent phosphatase activity. This partial reaction often is used to identify the H^+-K^+ ATPase /6/. It is characterized as a neutral phosphatase (p-nitrophenyl phosphatase) requiring Mg^{2+} and K^+ but insensitive to ouabain. The phosphatase activity does not form a stable phosphoenzyme nor does it catalyze transport activity. Kinetic evidence indicates that the phosphatase activity involves only the E_2 conformation of the enzyme without cycling between conformations /41/. It seems likely that the K^+ binding site for the phosphatase activity is the same as that for the ATPase reaction but this has not been proven.

Current knowledge regarding the various binding sites associated with the catalytic cycle is rather meager. Attempts to identify regions of the enzyme associated with binding of ATP, H^+ and K^+ have relied on site-specific labeling and partial digestion. Only a few facts have emerged from these studies. Walderhaug, et al. /43/ demonstrated that enzyme phosphorylation occurs at an aspartyl residue in a sequence

-Cys-(Ser/Thr)-Asp(P)-Lys. The Asp(P) is identified in the primary sequence as residue 385 /26/. Presumably an ATP binding site exists at or near this sequence as well. 5'-fluorescein isothiocyanate (FITC) binds selectively to a lysine residue in a cytosolic segment of the enzyme (residue 517) and inhibits the ATPase activity /44, 45/ but not the phosphatase activity /44/. Addition of K^+ to the lumenal surface results in a quench of the FITC fluorescence indicating a K^+-dependent conformational change of the enzyme /44/. These results indicate that FITC binds at, or near, the ATP binding site. A carboxyl group selective agent, N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline (EEDQ), binds to the enzyme from the lumenal surface and this binding is prevented by lumenal K^+ /46/ suggesting that the high affinity K^+ site involves a carboxyl group.

Partial enzymatic digestion of the H^+-K^+ ATPase has provided some insights in mapping the positions of the binding sites. As shown in Figure 3, tryptic digestion yields two different phosphopeptides

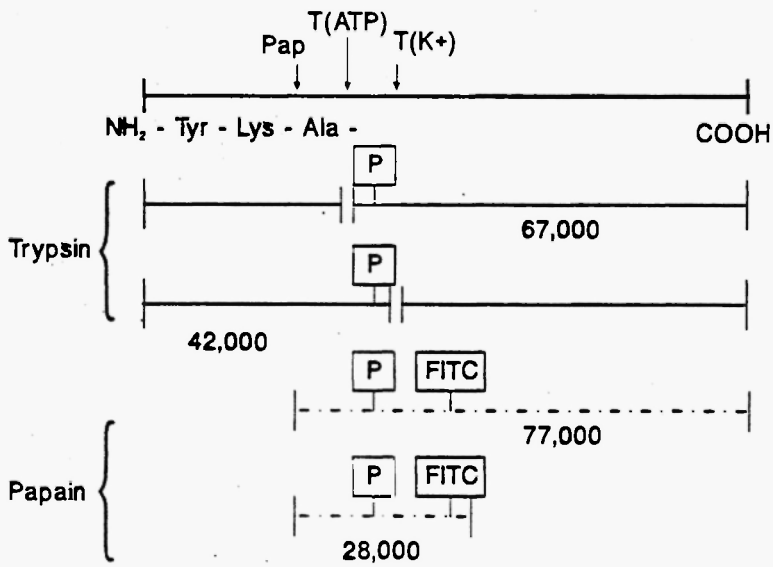


Fig. 3: Linear map of the catalytic subunit of hog gastric H^+-K^+ ATPase with position of sites of tryptic cleavage in the presence of K^+, T(K^+) and ATP, T(ATP) and papain cleavage (Pap). NH_2-terminal residues from Lane et al. /48/. Labeling with [^{32}P]-ATP and FITC are described in references 42 and 47.

depending on the presence of ATP or K^+ /42/. These observations indicate that ATP and K^+ induce significant conformational changes allowing trypsin access to different cleavage sites. Papain digestion /47/ initially results in a 77 kDa peptide containing both the phosphorylation site and the ATP-binding site (FITC site). Continued digestion with papain yields a 28 kDa peptide which retains both sites. The smaller peptide is water soluble suggesting that it originates from a hydrophilic cytoplasmic domain. These observations are summarized in Figure 3. Primary sequences involving the K^+ and H^+ binding sites have not been identified.

2.4 Transport Activity of the Proton Pump

The transport activity of the H^+-K^+ ATPase has been studied primarily in gastric microsomes. These membrane structures are isolated from gastric homogenates as small (0.1-0.2 micron in diameter) sealed vesicles, oriented with the cytosolic surface outward (toward the bath). The normal transport mode for these vesicles is an ATP-driven electroneutral exchange of lumenal K^+ for H^+ derived from the medium /4, 49/. This transport is usually measured as an acidification of the vesicle interior using pH sensitive fluorescent probes /50-52/. Optimal transport requires the presence of K^+ ionophores, e.g., valinomycin /4, 50, 53/ because the microsomes have relatively low permeabilily to K^+. This requirement indicates that K^+ is acting at the lumenal surface as would be expected from both the transport mode and the catalytic mechanism of the H^+-K^+ ATPase. While the use of K^+ ionophores has been helpful in defining the transport properties of microsomes, this requirement raises a serious question concerning the function of the H^+-K^+ ATPase in situ where ionophores are not required for full activity. This question has been answered recently by observations indicating that the H^+-K^+ ATPase represents only part of the in situ proton pump mechanism. The other part consists of a parallel KCl permeability pathway in the secretory membrane of the parietal cell.

Studies of the transport activity of the H^+-K^+ ATPase in situ indicate that the access of K^+ to the lumenal exchange site is much greater than for the microsomal preparations. Recent observations have provided evidence that the H^+-K^+ ATPase, in situ, exists in at least two

different membrane environments. One type of membrane exhibits low permeability to KCl similar to the microsomes while another type contains a relatively high KCl permeability /54, 55/. In both cases the ATPase is fully functional but limited, to a greater or lesser extent by the K^+ permeability. Moreover, the distribution of the ATPase between the two functional membrane types has been shown to depend upon the state of activity of the parietal cells. Thus, for resting or non-secreting cells, most of the ATPase is associated with microsomal type membranes. Upon stimulation of the parietal cell to secrete, the ATPase is found associated with a membrane containing an endogenous KCl permeabilily /14, 55, 57/. The exact nature of the KCl permeability associated with the active membranes has not been defined, nor is it known how the permeability pathway becomes associated with the ATPase. However, it is generally accepted that introduction of the KCl permeability represents the major mechanism for regulating the H^+-K^+ ATPase in situ /14, 66-68/. Therefore, the action of various stimuli on the parietal cell results in the introduction of a KCl permeability in the secretory membrane rather than a direct action on the ATPase.

Stimulation of the parietal cell is known to activate a variety of intracellular events. Stimulation is coupled to secretion by either an increase in cellular cyclic AMP /59/ or an increase in cytosolic calcium /60/. The major stimulus for acid secretion is histamine /61-63/, acting at an H_2-type receptor /64/. The H_2-histamine receptor is coupled to adenylate cyclase, and the binding of histamine leads to an increase of cellular cyclic AMP /59/. The rise of intracellular cyclic AMP results in activation of the cyclic AMP-dependent protein kinase /65/. The protein kinase is postulated to phosphorylate several endogenous proteins /66, 67/, but the identification of these proteins has not been determined as yet. Direct stimulation of the parietal cell by cholinergic agents, and, possibly gastrin, also has been proposed /61, 62/. In the case of these stimuli, the response is believed to be mediated by a rise of intracellular calcium /60/. The mechanism by which calcium mediates acid secretion is essentially unknown. Although the exact mechanism of action of the intracellular mediators has not been determined, one dramatic result of stimulation is a change in the morphology of the parietal cell /68-71/. Accordingly, in the resting, or non-secreting state, parietal cells exhibit only small amounts of secretory canaliculi, but they contain abundant intracellular membrane

structures, termed tubulovesicles. Following stimulation the active cells show a reduction in tubulovesicles and a marked expansion of the secretory canaliculi. Based on these observations, it is postulated that stimuli lead to the incorporation of tubulovesicles into the secretory membrane. Since the tubulovesicles contain the H^+-K^+ ATPase /11/ but do not appear to form acid /12, 68/, it is reasonable to assume that these membrane structures represent the microsomes isolated from resting tissue and do not contain a functional KCl permeabilily. The secretory membrane would then correspond to the active membrane containing a KCl permeability. It is not known whether the KCl permeability is incorporated with the ATPase into the secretory membrane or is activated simultaneously. Nevertheless, the overall process of acid secretion by the parietal cell and its regulation are seen as a very complex set of cellular events. Figure 4 summarizes the major steps in this process as they are currently understood. From the view of pharmacology, each of these major processes represents a potential site for drug interaction. At present, available drugs interact at only two of these sites leaving the possibility open for additional modes of pharmacological intervention.

III. INTERACTION OF ANTI-ULCER AGENTS WITH THE H^+-K^+ ATPASE

The general goal of current medical treatment for gastric and duodenal ulcer as well as reflux esophagitis is to reduce the acidity of the gastric contents. Pharmacological approaches to this goal fall into three major categories: antacids, receptor antagonists, and proton pump inhibitors.

3.1 Antacids

Antacids continue to have widespread use in symptomatic relief of ulcer disease and esophagitis. These agents do not interact directly with the acid secretory ability of the pariatal cell, but neutralize the resultant hydocholoric acid and thus reduce gastric acidity. Antacids at the usual dosage, result in only a short-lived reduction in gastric acidity.

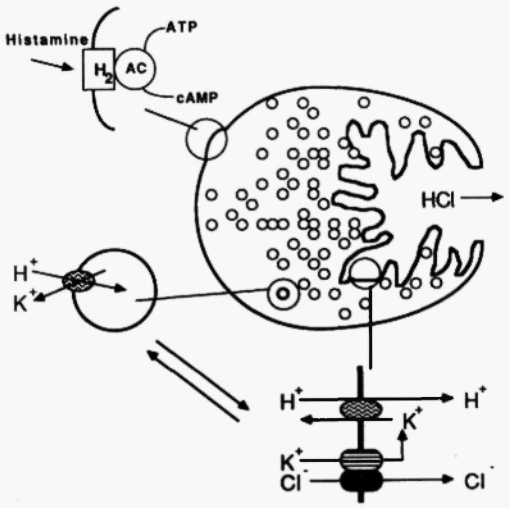


Fig. 4: Regulation of Acid Secretion by the Gastric Parietal Cell. The basolateral membrane of the parietal cell contains specific receptors for stimuli. The major stimulus is histamine acting at an H_2-type receptor. The stimulus-receptor complex activates adenylate cyclase (AC) resulting in the formation of intracellular cyclic AMP. The parietal cell contains numerous membrane elements known as tubulovesicles. These structures possess a functional H^+-K^+ ATPase but lack permeability to K^+. Because of the lack of K^+ access to the exchange site, the tubulovesicles do not transport protons. The secretory canaliculus possesses both the H^+-K^+ ATPase and permeability pathways for K^+ and Cl^-. This combination allows for proton transport in exchange for K^+ and thus a net secretion of HCl. The intracellular cyclic AMP generated during stimulation produces through an unknown mechanism, a conversion of tubulovesicle membrane to canalicular membrane.

As such, they are poorly suited for longterm usage and are prone to complications associated with abuse. Such complications include diarrhea (particularly with Mg^{2+} salts), disordered mineral metabolism (hypophosphatemia, hypernatremia, and hypercalcemia), and alkalosis /72-75/. The last symptom is due mainly to the fact that neutralized acid becomes unavailable for reabsorption or reaction with pancreatic bicarbonate which is necessary for maintenance of normal acid-base balance.

Interaction of antacids with the gastric proton pump are indirect primarily through incresing serum gastrin levels /76-79/. Following a meal, gastric acidity suppresses further stimulation by reducing gastrin release from the antrum of the stomach. With antacids this regulatory mechanism is disrupted and may result in hypergastrinemia /78, 79/.

In addition, the high calcium content of specific antacids can lead directly to enhanced release of gastrin /77/. Because gastrin acts both as a secretory stimulus and as a trophic factor /80/, sustained high levels of gastrin can lead to an increase in parietal cell mass and thus, to enhanced secretory capacity. While the effects of antacids on the proton pump are indirect, they should be taken into account when considering the overall action of these agents.

3.2 Receptor Antagonists

A second approach to reducing gastric acidity is aimed at inhibiting acid production by antagonism of endogenous stimulating agents. The major, known stimuli for acid secretion are gastrin, acetylcholine and histamine. Although each of these agonists are capable of stimulating acid secretion alone, a major part of the action of gastrin and acetylcholine is mediated by histamine /61-63/. Both gastrin and acetylcholine cause a local, or paracrine, release of histamine which accounts for much of the observed stimulation. In addition, the direct effects of gastrin and acetylcholine on the parietal cell are enhanced synergistically by histamine. Thus, of the total response to gastrin or acetylcholine, approximately 80% requires the simultaneous presence of histamine. The interaction among these stimuli serves as a basis for the observation that histamine antagonists are able to suppress acid secretion independent of the stimulus /64, 81-83/. It is not surprising, therefore, that the major effort in development of antisecretory agents has been devoted to histamine antagonists.

The potential use of anticholinergic agents in ulcer therapy has not been abandoned. The development of more selective anticholinergic agents, e.g. pirenzepine, has renewed interest in this area. Pirenzepine and related agents appear to be selective for the M_1-type muscarinic receptor, associated with acid secretion /84-86/. Moreover, because they are hydrophilic, these agents do not cross the blood-brain barrier easily /87/. Thus, most of the side-effects of previous anticholinergic

agents are avoided. Because of somewhat limited action of anti cholinergics, it is likely that their principal use will be in combination with histamine antagonists to promote more complete suppression of acid production /88/.

The identification of the H_2-histamine receptor subtype and development of specific antagonists by Black and associates /64, 89-92/ is a landmark in the medical management of ulcer disease. The results of those efforts led to the availability of potent and selective antisecretory agents, including cimetidine, ranitidine and famotidine. A key observation in the development of these agents was that the selectivity for the H_2-receptor depends upon the nature of the alkyl side chain rather than on the imidazole ring as for the H_1-receptor /89,92/. Based on this concept, current efforts are aimed at producing more potent and longer active H_2-receptor antagonists /91, 93, 94/.

The efficacy and duration of receptor antagonists depends, in part, on their circulating half-life and also on the levels of endogenous agonists. This follows directly from the fundamental nature of competitive inhibition. Thus, a dosage which is effective under some circumstances may become ineffective with changes that alter levels of stimuli, e.g., stress levels, dietary habits, etc. Additionally, if chronic usage leads to hypergastrinemia, the dosage must be adjusted to compensate for the increased level of stimulus. Finally, since receptor antagonists act at the initial event of stimulus-secretion coupling, inhibition of secretion will be delayed until intracellular mediators have recovered to their basal level. In the case of histamine stimulation, the H_2-receptor is coupled to activation of adenylate cyclase and elevation of intracellular cyclic AMP /59/. This second messenger activates a protein kinase which, through an unknown set of events, activates the proton pump, or H^+-K^+ ATPase. It is known that the activation of the H^+-K^+ ATPase occurs rapidly, within 1-2 min after addition of the stimulus /51, 68-70/. However, when the stimulus is withdrawn or the receptor blocked by an antagonist, acid secretion continues for 30-60 min before gradually returning to basal rates. This continued secretion is presumably due to persistent intracellular activation. Thus the biological effectiveness of histamine antagonists will depend upon the state of intracellular mediators at the time of administration.

It is certainly true that the H_2-receptor antagonists are effective in suppressing acid secretion and have been successful in treating ulcer

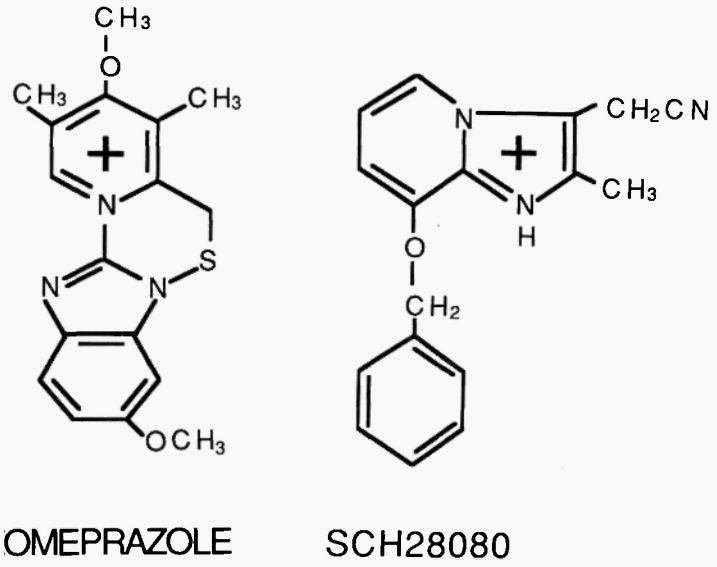
disease with few untoward side-effects. Cimetidine and other H_2-receptor antagonists tend to inhibit cytochrome P-450 mediated drug metabolism by the liver /95/. This delays metabolism of drugs which share this pathway and some adjustment of dosage is usually required. A few cases of bradycardia have been reported and likely are due to the presence of H_2-receptors in the atria. These few side-effects are generally well-tolerated and represent minor considerations /96/. The major drawback in the use of histamine antagonists is the poor predictabilily of biological effectiveness which largely is a consequence of the nature of receptor antagonism.

3.3 Proton Pump Inhibitors

Identification of the gastric H^+-K^+ ATPase as the proton pump of the stomach offered a potential target for antisecretory agents. Moreover, the unique biochemical and functional properties of this enzyme suggest that it can serve as a highly selective target. These unique properties have been exploited in the development of substituted benzimidazoles as a new class of antisecretory agents, including the first clinically useful compound in this group, omeprazole (Figure 5).

Omeprazole has been shown to be a potent inhibitor of acid secretion in animals /97-99/ and man /100, 101/, independent of the stimulus employed. In this regard, the compound resembles the action of histamine antagonists. However, in vitro studies demonstrated that omeprazole inhibits secretion induced by cyclic AMP analogues while histamine antagonists do not /97/. Subsequent studies have shown that omeprazole acts by a non-competitive inhibition of the H^+-K^+ ATPase /15, 102, 103/. In vivo studies in animals and man demonstrate that omeprazole inhibition is of long duration /98, 100/ with maximal inhibition requiring 3-4 days for complete recovery. This is despite a plasma half-life of only 60 min /98, 100, 104/. These findings suggest a relatively irreversible inhibition.

The rather unusual antisecretory properties of omeprazole have become understandable in light of the proposed mechanism of action Omeprazole is inactive in its native form but undergoes an acid-catalyzed conversion to the active inhibitor. The active inhibitor is a cationic sulfenamide which forms a covalent disulfide bond with available



OMEPRAZOLE SCH28080

Fig. 5: Structures of omeprazole and SCH28080. The strucure for omeprazole is the presumed active intermediate /106/. Note that both compounds are cationic weak bases.

sulfhydryl residues /105-114/. The selectivity of omeprazole for H^+-K^+ ATPase is primarily due to the generation of an acid environment by this enzyme. This functional property of the H^+-K^+ ATPase accomplishes two things. First, since the native omeprazole is a weak base, the acid environment leads to the accumulation of omeprazole in its protonated cationic form. Second, the high acidity converts the native molecule to its active form. Because the accumulation and activation occur only at pH values below about 4.0, omeprazole is selectively targeted to a highly acidic environment which, in man is restricted to the lumenal surface of the actively secreting parietal cell /109, 112/. Both the native protonated molecule and the active inhibitor are cationic and this restricts the membrane permeability, preventing the active inhibitor from entering the cell cytosol where it could result in non-specific inhibition /112/. Thus, omeprazole may be viewed as targeted to a

physiological environment which is uniquely provided by the activity of the H^+-K^+ ATPase.

Once it is acid-activated, the sulfenamide is short-lived but highly reactive and will form a covalent disulfide bond with exposed sulfhydryl groups /105-107/. However, studies in vivo and using isolated gastric membranes demonstrated that inhibition of acid secretion is associated with a highly selective binding to the H^+-K^+ ATPase /112, 113/. Thus, the lumenal sector of the H^+-K^+ ATPase appears to contain one or two sulfhydryl groups which are preferentially attacked by the active inhibitor /111/. This preferential binding adds another element of selectivity for the action of omeprazole. Based on recent observations /115/, it may be suggested that the selective binding of omeprazole to the H^+-K^+ ATPase is related to the cationic nature of the active inhibitor. Thus, a variety of organic cations are known to inhibit the H^+-K^+ ATPase by competing with the K^+ binding site (see below).

Given the proposed mechanism of action of omeprazole (Figure 6)

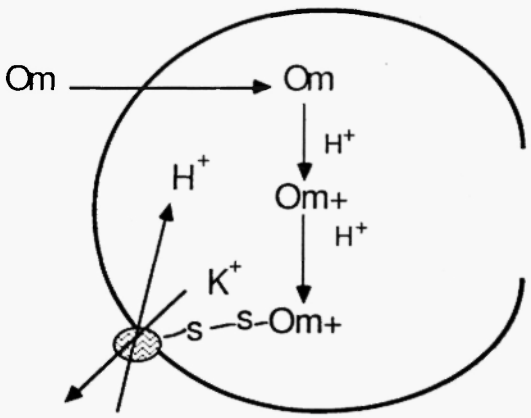


Fig. 6: Mechanism of Action of Omeprazole. Omeprazole is a membrane-permeant weak base which equilibrates in the secretory canaliculus. Under acid conditions, omeprazole becomes a cation and accumulates in the canaliculus. The low pH also allows omeprazole to convert to an active form which reacts with the H^+-K^+ ATPase to form a covalent disulfide bond.

the characteristics of its secretory inhibition are more understandable. Both the non-competitive inhibition and a duration of action which far exceeds the plasma half-life result from the irreversible covalent binding of omeprazole to the H^+-K^+ ATPase. It is likely that full recovery from inhibition requires replenishment of H^+-K^+ ATPase molecules and/or parietal cells. Direct inhibition of the H^+-K^+ ATPase by omeprazole allows for secretory inhibition which is essentially independent of both the nature and level of stimulus. Thus, omeprazole is effective even in the presence of the hypergastrinemia of Zollinger-Ellison syndrome /116/, whereas H_2-receptor antagonists are ineffective. Moreover, the rate of acid suppression by omeprazole is independent of the state of intracellular mediators, and therefore omeprazole may act more rapidly than receptor antagonists. Because omeprazole inhibits only active H^+-K^+ ATPase molecules or H^+-K^+ ATPase molecules that are present in a membrane which is actively acidifying, a cumulative inhibition would be expected with repetitive doses. This is found to be the case for both animals and humans /98, 100/ where the steady-state inhibition is reached after 4-5 days of daily dosage.

The cumulative irreversible inhibition of H^+-K^+ ATPase suggests that the timing, relative to secretory state, of omeprazole administration should not influence the steady-state level of inhibition. Although the timing would affect initial inhibition and the period required to achieve a steady-state. In man, where there is a finite basal secretion, this is likely to be true. The mechanism of action of omeprazole most likely contributes to the relative lack of toxicity. Administered as an inactive prodrug, circulating omeprazole would produce few, if any, direct side-effects. Because it is accumulated in acid spaces, the required dose of omeprazole can be kept low, thus avoiding potential interference with drug metabolism pathways. In vitro studies indicate that omeprazole inhibits P-450-mediated metabolism to about the same extent as cimetidine on a molar basis /104/. However, since the dose of omeprazole is likely to be 25-50 times lower than cimetidine, it would have a proportionately lower effect on the P-450 oxidase system. The only other reported untoward side-effect has been gastric hyperplasia which, most likely, was due to a hypergastrinemia associated with longterm, maximal suppression of acid secretion /117, 118/. With recommended doses (20 mg/day) designed to allow some acid secretion during a 24 hours period, hypergastrinemia should not be a significant problem even with longterm usage.

3.4 Reversible Pump Inhibitors

Development studies aimed at producing omeprazole analogues particularly more reversible analogues, led to the discovery (or rediscovery) of a different class of H^+-K^+ ATPase inhibitors. These are the so-called K^+-site inhibitors, of which SCH 28080, a substituted pyridyl 1,2 perazine (Figure 5), is the most thoroughly studied /119-122/. A number of organic cations have been shown to inhibit the H^+-K^+ ATPase by competing with K^+ for a lumenal binding site /123, 124/. Most of these compounds show rather poor selectivity for the H^+-K^+ ATPase. However, SCH 28080 displays a high degree of selectivity for the H^+-K^+ ATPase. This compound was shown to inhibit K^+ activation of the H^+-K^+ ATPase in competition with K^+ /119, 121/. The inhibition is uncompetitive with respect to ATP and occurs for both purified enzyme and enzyme associated with acidifying membranes /121/. Thus, SCH 28080 appears to act as a reversible competitive inhibitor of the lumenal K^+ exchange site on the H^+-K^+ ATPase. This K^+ exchange site may possess some unique properties since SCH 28080 does not interact with the K^+-site of the Na^+-K^+ ATPase /121/.

SCH 28080, and similar compounds, are protonatable amines and it is likely that the cationic form is the active species /121/. A methylated derivative of SCH 28080, which is a permanent cation, inhibits the H^+-K^+ ATPase but only when present at the lumenal surface /122/. The weak base properties of SCH 28080 allow this compound to permeate the parietal cell and reach the lumenal surface in the uncharged form The acid environment of the lumen will result in protonation and accumulation of the less permeable cationic form. To this extent, SCH 28080 behaves like omeprazole and can be selectively targeted to an acid environment. However, unlike omeprazole, SCH 28080 does not require an acid activation and thus can inhibit the H^+-K^+ ATPase even under non-acidifying conditions /121/. The effect of lumenal acidity, therefore, is on the apparent potency of SCH 28080 rather than the efficacy.

The structural requirements for H^+-K^+ ATPase inhibition by SCH 28080 analogues are quite narrow /122/. Assuming that the competitive nature of the inhibition demonstrates binding to the K^+-site rather than a conformational interaction, the specificity suggests a relatively well defined conformation of the lumenal K^+ binding site. The observation that both SCH 28080 and omeprazole have cationic active forms raises

the possibility that both agents act at the same site. Recent studies have shown that SCH 28080, which is reversible, can prevent irreversible inhibition by omeprazole /115/. This supports the notion that both compounds act selectively at a lumenal cation binding site, the K^+ exchange site, and that this site contains at least one exposed sulfhydryl group. Additionally, if omeprazole has a high affinity for a cation site, it would help to explain the selective binding of omeprazole to the H^+-K^+ ATPase as opposed to other lumenal membrane proteins.

As yet, there is insufficient data from in vivo and clinical studies to determine whether the reversible pump inhibitors represent the next generation of antisecretory agents. In principle, these agents should be like omeprazole in their selectivity and efficacy regardless of the nature or level of stimulus. The reversible nature of the inhibition would allow for better short-term control of secretion but would require more frequent administration with the inherent problem of patient compliance. Also, the timing of administration would become a significant variable since these agents will accumulate and thus be effective only if administered during periods of active acidification. This potential drawback is similar in nature but opposite to that encountered with receptor antagonists. Therefore, a combination of these approaches may be a more effective therapy than either one alone.

IV. FUTURE DIRECTIONS

Therapeutic approaches aimed at reducing gastric acidity have proven to be effective in the clinical management of peptic ulcer disease. Over the past decade, significant progress has been made in developing specific and effective pharmacological agents for inhibiting gastric acid production. The development of these agents has paralleled an improved understanding of the cellular and molecular basis of acid secretion. Thus, therapeutic agents have progressed from simple neutralization of acid to antagonism of stimuli for the parietal cell, and most recently towards inhibition of the gastric proton pump. The development of effective receptor antagonists depended upon the recognition of H_2-type histamine receptors as the primary mechanism for stimulation of the parietal cell. Similarly, identification of the H^+-K^+ ATPase as the gastric proton pump allowed development of omeprazole as a specific inhibitor.

As a logical extention of this sequence of developments, it may be anticipated that future studies will focus on production of more selective inhibitors of the proton pump. Possible sites of action for such inhibitors could be the lumenal cation binding site of the H^+-K^+ ATPase or the parallel ion pathways which provide K^+ to the lumenal exchange site. Studies aimed at defining the molecular nature of these sites will be critical in the development of site-specific inhibitors. Thus, advances in our knowledge of the molecular mechanism of acid production can provide guidance in the development of new agents as well as an improved system for testing their specificity of action.

An important concept which evolved from the development of omeprazole is that of environmental targeting. In the case of omeprazole, the acidic environment generated by the parietal cell serves both to accumulate the agent and to convert the prodrug to an active form. As a more general principle, unique physiological environments could be exploited as targets for the accumulation or activation of other types of therapeutic agents. Thus, unique conditions which may exist in various organs or intracellular organelles offer possible environmental targets. The combination of environmental targeting with site-specific compounds likely may thus result in classes of pharmacological agents with the highest selectivity and fewest side-effects.

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