Ann. Rev. Pharmacol. Toxicol. 1988. 28:269–84

GASTRIC H,K-ATPASE AS THERAPEUTIC TARGET[1]

G. Sachs

CURE, Wadsworth Veterans Administration Hospital and the University of California, Los Angeles, California 90073

E. Carlsson, P. Lindberg, and B. Wallmark

Hässle Gastrointestinal Research Laboratories S-431 83 Mölndal, Sweden

INTRODUCTION

The upper gastrointestinal tract comprising the lower esophagus, the stomach, and the proximal duodenum are frequently exposed to extremely acidic pH. The large majority of the population tolerates the acidic environment, without major pathological consequences. However, in about 10% of the population, lesions of the epithelium occur that range from superficial destruction of the mucosa to ulcers penetrating the full thickness of the tissue. Although, with the exception of the massive hypergastrinemia of the Zollinger-Ellison syndrome, it is not possible to establish a cause-effect relationship between acid and peptic ulcers, the adage "no acid-no ulcer," first enunciated in 1910, has stood the test of time. Thus the vast majority of interventionist measures taken to promote the healing of peptic ulcers have focused on reduction of gastric acidity.

The traditional methods of achieving this goal involve the use of a variety of antacids, but their effectiveness is brief. In particular, the neutralization of nighttime acidity is too short-lived for effective maintenance treatment. Nevertheless, antacids are useful in therapy. Muscarinic antagonists, such as atropine or pirenzipine, are effective inhibitors of perhaps 50% of maximal meal-stimulated acid production, but they have the side effects expected of

compounds that interfere with cholinergic receptors distributed throughout the body. The histamine-H_2 receptor antagonists (1)—cimetidine, ranitidine, and famotidine—have several advantages, such as potent inhibition of secretion and relative selectivity for the stomach. Complete inhibition of parietal cell acid secretion by receptor antagonists may be difficult to accomplish for two reasons. One is the multiplicity of known receptors on the parietal cell, and the other is the variety of second messenger signalling systems, which involve at least cyclic adenosinemonophosphate (cAMP) in the histamine-H_2 receptor pathway and intercellular calcium ($[Ca]_i$) in the acetylcholine and gastrin pathways (2, 3, 4). The release of histamine, and hence the histamine-H_2 receptor system, apparently plays a role in the gastric effects of acetylcholine and gastrin that would account for the actions of histamine-H_2 receptor antagonists on meal-stimulated acid secretion.

As agents that significantly alter the clinical history of peptic ulcers but do not inhibit acid secretion, bismuth (5) and sucralfate (6) have produced good results in duodenal ulcer healing trials. The prostaglandins were thought to offer the advantage of mucosal protection but so far have shown effectiveness only in antisecretory doses (7).

The effectiveness of the drugs mentioned above in treatment of duodenal ulcers, gastric ulcers, the Zollinger-Ellison syndrome, and gastroesophageal reflux disease can be related to the degree of inhibition of acid necessary to restore the healthy physiological status of the diseased region (8). Since the duodenum has a greater neutralizing capacity than the antrum or fundus and usually has only transient exposure to low pH, the degree of acid inhibition required to heal ulcers in this region may be less than for lesions in the antrum or fundus, while the poor acid resistance of the terminal esophagus with lower esophageal incompetence would also increase the need for inhibition of acid secretion in comparison with that required for the healing of duodenal ulcers. Thus there are indications that more potent inhibition of acid secretion is needed than that achieved by receptor antagonists. The discovery of the gastric proton pump in 1973 (9) and the elucidation of its mechanism of action in the following years (10, 11, 12) made the H,K-ATPase an alternative to the above-mentioned receptors as a target for antisecretory agents. In this review, therefore, the emphasis is on the development of agents that interfere selectively with this enzyme and on their mechanisms of action. From this study one can expect not only interesting pharmacology and therapeutics, but also insight into structural and mechanistic aspects of the target enzyme.

H,K-ATPase

This enzyme is found in smooth membrane structures in the parietal cell called tubulo-vesicles as long as the cell is not secreting acid. As acid

secretion increases, it transfers progressively to the microvilli of the secretory canaliculus of this cell (11). This transformation is due to the change in levels of cAMP or $[Ca]_i$ in the cell. The secretory canaliculus can be thought of as an infolding of the apical surface of the parietal cell, having a restricted connection with the gastric gland lumen. Thus, in essence, the stimulated canalicular membrane encloses the product of the active H,K-ATPase system, namely HCl and a small quantity of KCl. This results in a highly acidic space lined by a membrane containing the H,K-ATPase, as is demonstrated by anti-ATPase monoclonal antibody staining (11) (Figure 1).

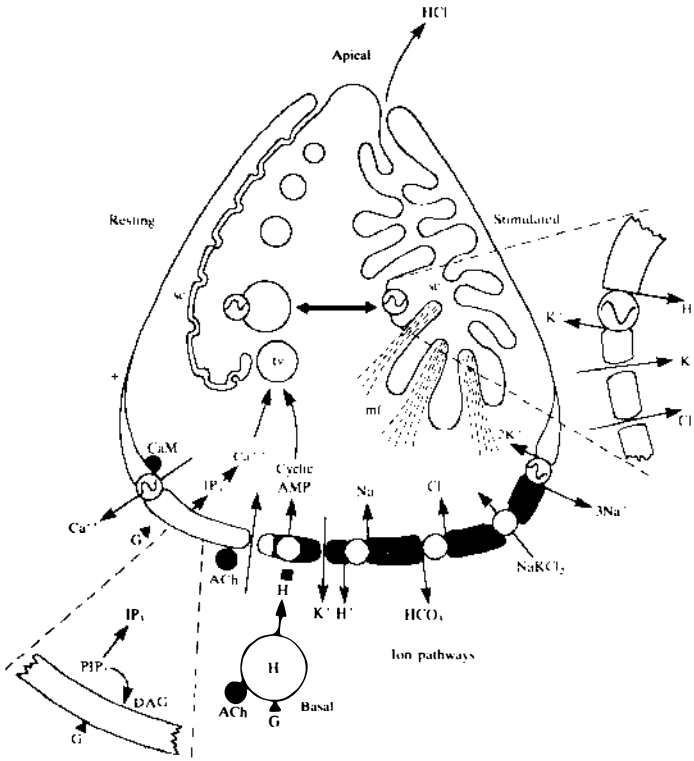

Figure 1 A composite model of the parietal cell. The receptor complex on the basal-lateral surface of the parietal cell includes the H_2-receptor, which is linked to the production of cyclic AMP; the ACh-receptor, which alters membrane permeability to Ca^{2+}; and the gastrin receptor, which releases inositol triphosphate and thence intracellular Ca^2. Ion pathways on the basal-lateral surface show the presence of a K^+-conductance, Na^+/H^+ exchange, HCO_3^-/Cl^- exchange, the Na^+-pump and the Ca^{2+}-pump. The $NaKCl_2$ porter has not been shown as yet. On activation, the tubulovesicles (tv), containing the H,K-ATPase, are transformed into the microvilli of the secretory canaliculus. The K^+ and Cl^- conductances are shown in the canalicular membrane. the drawing is taken from Ref. 65.

Visible accumulation of the metachromatic weak base, acridine orange (12), or by the uptake of the radioactive weak base, aminopyrine (13), in isolated gastric glands shows that the canaliculus of the parietal cell in the stimulated state is very acidic. In the latter case, given that 10% of cell volume is occupied by the canaliculus, and that the pKa of aminopyrine is 5, with an accumulation ratio of 1000 for this weak base, a calculated pH of 1 is obtained for the canalicular space. There does not seem to be any other place in the body where this level of acidity is obtained, and thus the parietal cell's caniculus provides a uniquely favorable environment for the luminal face of this enzyme.

With respect to its mechanism of action, this enzyme belongs to the class of transport ATPases forming in their reactions a covalent phosphoenzyme that is an aspartyl phosphate (14). It is also thought that at least two major conformations of the catalytic subunit (i.e. the subunit that is phosphorylated by ATP during the reaction cycle) determine the binding of the transported ion on the ATP side of the enzyme (the E_1 conformation) and release of the ion on the trans-side (the E_2 conformation). In the case of a countertransport pump, such as Na,K-ATPase and H,K-ATPase, the counterion binds to the E_2 form and is released from the E_1 form. Examples of these pumps include the Na,K-ATPase, the Ca-ATPase of the plasma membrane and the sarcoplasmatic reticulum, and the H,K-ATPase of the parietal cell (15).

The H,K-ATPase displays a basal Mg-ATPase activity that is enhanced up to 30-fold by K. Other monovalent cations can substitute for K, in the sequence $Tl > K > Rb > NH_4 > Cs > Na, Li$ (15, 16). In the absence of K, the enzyme forms a relatively stable phosphoenzyme (E-P), the slow turnover of which determines the Mg-ATPase activity. When K is added to the luminal, but not to the cytosolic side, the E-P is hydrolyzed, thus accounting for the K stimulation of ATPase activity (17, 18).

When the ATPase is prepared in inside-out, ion-tight vesicles, MgATP-dependent H transport into the vesicles is obtained only when K is presented to the internal face of the vesicles. This correlates with the catalytic properties of the enzyme.

During the transport reaction, as H is moved into the vesicle, K is transported outward with equal stoichiometry. Thus the H,K-ATPase catalyzes an electroneutral H for K exchange (10). The rate of transport and ATPase activity in vesicles isolated from a resting mucosa is limited by the entry of K, owing to the absence of both a K and Cl pathway. In vesicles isolated from stimulated tissue, a K and a Cl conductance are present (19) that remove the K restriction on enzyme activity and H transport. Thus stimulation of acid secretion involves translocation of the H,K-ATPase to the canalicular membrane and activation of K and Cl pathways.

In view of the enzyme's extremely low luminal pH, the continuing ability

of K to react with the activating site of the H,K-ATPase suggests a specialization of this region with regard to the Na,K-ATPase. A further notable difference in the handling of K by the two enzymes is the absence of K occlusion in the H,K-ATPase and the presence of K occlusion in the Na,K-ATPase (20, 21).

The phosporylated intermediate and the sidedness of the reactions can be illustrated with the following simplified kinetic scheme:

$$MgATP + E \rightarrow MgATP \cdot E_1$$

$$H_c + MgATP \cdot E_1 \rightarrow H_cE_1 - P$$

$$H_cE_1 - P \rightarrow H_LE_2 - P$$

$$K_L + H_LE_2 - P \rightarrow K_LE_2 - P + H_L$$

$$K_L \cdot E_2 - P \rightarrow K_LE_2 + P_i$$

$$K_LE_2 \rightarrow E_1 \cdot K_c$$

$$MgATP + E_1K_c \rightarrow MgATP \cdot E_1 + K_c,$$

where c denotes cytosol and L denotes lumen.

In addition to reacting with ATP in these ways, this enzyme is able to hydrolyze p-nitrophenylphosphate in a K-dependent manner by reaction with this substrate in the E_2 enzyme form.

The primary amino-acid sequence of the H,K-ATPase has been deduced by means of cloning techniques (22). There is 60% homology with Na^+,K^+-ATPase, the greatest in the region thought to be involved in ATP breakdown via transphosphorylation into the protein. As for the hydrophobic region of the enzyme, eight membrane spanning sequences are postulated to exist there, five of which exhibit considerable homology with the sodium pump sequences. However, the three sequences located towards the C terminal region show differences from them. Perhaps relevant to this review is the large number of cysteine residues located in the enzyme's hydrophobic region, as well as one cysteine that is calculated to be in its luminal sector. The enzyme has been sought in various tissue, but research has not provided convincing evidence for the presence of H,K-ATPase elsewhere than in the colon, where both the enzyme and transport activity have been found (23, 24).

Thus, although there is similarity between the gastric proton pump and the ubiquitous sodium pump with respect to their structures and reaction mechanisms, the differences that can be significant consist in (*a*) the acid environ-

ment of the luminal region of the active H,K-ATPase, (*b*) the nature of the K site and the absence of tight K occlusion, and (*c*) the presence of a large number of cysteine residues in the membrane sector, one or more of which might be accessed from the luminal side. These differences can be used to achieve selective inhibition of the proton pump.

APPROACHES TO SELECTIVE INHIBITION OF THE H,K-ATPase

From the above considerations, it is apparent that H,K-ATPase represents a unique target for antisecretory compounds, compared to the more generally distributed histamine-H_2 and cholinergic receptors. Novel strategies for the development of selective inhibitors of H,K,-ATPase have emerged.

Substituted Benzimidazoles

A new class of antisecretory compounds was made available through the development of substituted benzimidazoles and the recognition of those compounds as H,K-ATPase inhibitors (25). In this group of compounds, omeprazole represents the first example of a clinically useful drug.

INHIBITION OF GASTRIC ACID SECRETION BY OMEPRAZOLE Omeprazole is a potent inhibitor of gastric acid secretion in several species, including the human (26, 27). The drug is effective in the dog, regardless whether secretion is stimulated by histamine, pentagastrin, or urecholine (26, 28). The same high degree of potency was recorded against basal acid secretion in the human and against secretion induced by pentagastrin, histalog, betazole, and sham-feeding (27, 29). Also in isolated gastric glands or purified parietal cells, omeprazole effectively inhibited both histamine and dbc-AMP or K-stimulated secretion (30, 31). The inhibition of gastric acid secretion by omeprazole was of long duration in rats, dogs, and human beings (26, 27). Following a dose that initially gave complete blockade of acid secretion in dogs, normal secretory rates were not observed until the fourth day. Furthermore, increasing the dose to supramaximal levels only marginally increased the inhibitory effect. The same duration of the antisecretory effect was observed following two months of daily treatment (Figure 2). Since the antisecretory effect of omeprazole has a long duration, an increase of the drug's inhibitory effect is to be expected during the first days of its administration, when it is given once daily. This was indeed found to be the case, and steady-state levels of inhibition were obtained after 4–5 days of daily dosing, both in humans and in dogs (27, 32). In dogs, a dose of 0.5 μmol/kg results in about 20% inhibition of stimulated acid secretion, when measured 3 hr after the first dose, while the inhibitory effect during steady-state is 60%.

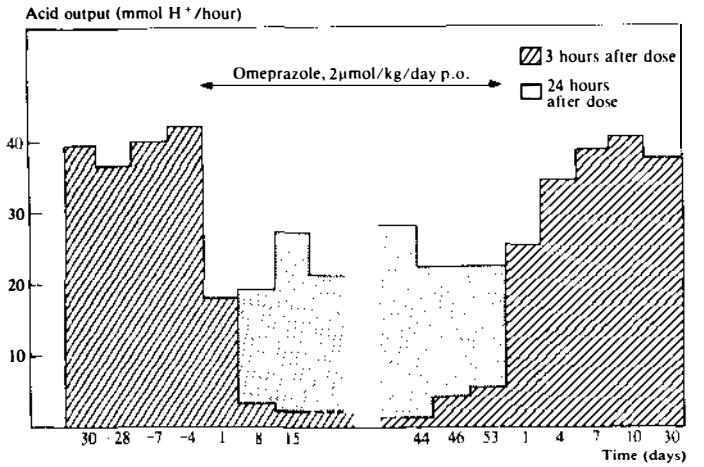

Figure 2 Histamine stimulated gastric acid secretion before, during, and after 2 months of daily oral treatment with omeprazole, 2 μmol/kg/day, in the gastric fistula of the dog. During the treatment period, acid secretory tests were performed either 3 or 24 hr after dosing. Values are means, n = 4. Data are taken from Ref. 32.

PHARMACOKINETICS In spite of the long duration of the antisecretory effect, omeprazole was found to have a plasma half-life of only 40–60 min (26, 27, 34). Approximately the same half-life was found in rats, dogs and humans. Thus, no correlation exists between the plasma concentration of omeprazole and the inhibitory effect. However, in both dogs and humans a good correlation was obtained between the area of the omeprazole plasma-concentration curve (AUC) and the inhibitory effect (26, 27). This indicates that the amount, rather than the concentration at any given time, of drug that reaches the blood determines the antisecretory effect (Figure 3).

MECHANISM OF ACTION OF OMEPRAZOLE Autoradiography was conducted following intravenous ^{3}H-omeprazole administration. After 1 min the drug was generally distributed within the animal. However, 16 hr after administration, the radioactivity was retained in the gastric epithelium (35). Further analysis of the distribution of radioactivity revealed that it was confined to the tubulovesicular and canalicular membranes of the parietal cell (35). These membranes have been shown to contain the gastric H,K-ATPase (see above). Thus, when administered in vivo, omeprazole was found to selectively label the gastric H,K-ATPase. Moreover, it was possible to show that in both gastric homogenates and gastric microsomes prepared from rabbits given ^{3}H-omeprazole, essentially the only peptide labelled was the catalytic subunit of the H,K-ATPase (Figure 4). That the inhibition of acid

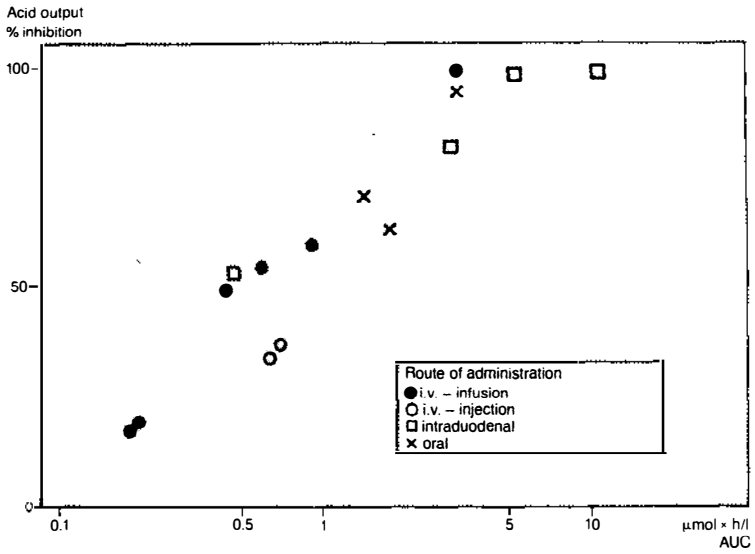

Figure 3 Gastric fistula of the dog. Relation between area under the plasma concentration curve (AUC) and antisecretory effect of omeprazole on histamine-stimulated acid secretion. The compound was given by various routes of administration. Data are from Ref. 33.

secretion by omeoprazole was due to blockade of the H,K-ATPase was shown by a series of experiments in which the inhibition of acid secretion was correlated with the inactivation of the gastric mucosal H,K-ATPase activity (36). Thus, following submaximal oral doses of omeprazole, both the maximal capacity to secrete acid and the H,K-ATPase activity were reduced to the same extent. Furthermore, when omeprazole was given in maximal dose levels, a parallel recovery of acid secretion and mucosal H,K-ATPase activity was obtained, indicating that H,K-ATPase plays a primary role in acid secretion (Figure 5).

Omeprazole's selectivity for inhibition of H,K-ATPase can be understood from consideration of the three important factors discussed above:

(*a*) H,K-ATPase is specifically located in the membranes lining the highly acidic (pH~1) canalicular system of the parietal cell and in luminally accessible cystein residues.

(*b*) Omeprazole is a weak base with a pKa value of 4. Accordingly, the acidic form of the drug, which is positively charged, will be concentrated within the canaliculus of the parietal cell.

(*c*) Omeprazole, which is inactive in its intact form, undergoes acid catalyzed conversion into an inhibitor of H,K-ATPase, which is a permanent cation, within the acid canaliculus of the parietal cell.

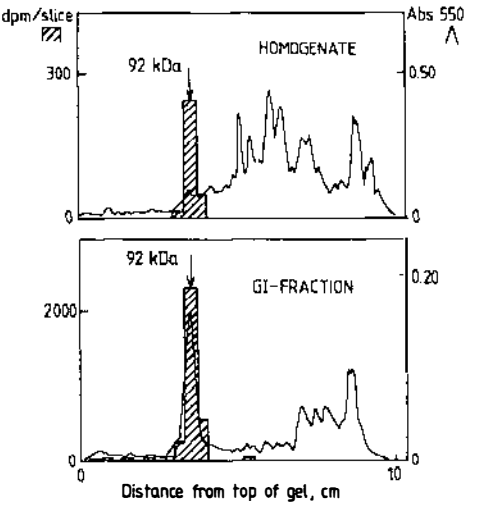

Figure 4 Distribution of radioactivity and protein, in a gastric homogenate and in a partly purified H,K-ATPase fraction (GI) following administration of ^{3}H-omeprazole to rabbits. The proteins in the homogenate and GI fractions were separated by SDS-polyacrylamide gelelectrophoresis. The hatched bars shows the distribution of radioactivity, while the continous line shows protein distribution.

This last point is schematically illustrated in Figure 6. Omeprazole reaches the parietal cell from the blood. The base form of the drug rapidly equilibrates into the canaliculi of the parietal cell. The acidic contents of these canaliculi lead to concentration of omeprazole in the protonated form and also to its conversion into an active inhibitor. This compound subsequently attacks H,K-ATPase in its luminal sector. Furthermore, both the acidic and converted form of omeprazole are positively charged and do not easily penetrate into the cell cytosol. This mechanism has been deduced by several investigators using a variety of in vitro and in vivo preparations.

Vesicles isolated from gastric mucosa were used to investigate the interactive omeprazole at the level of H,K-ATPase (37, 38). These vesicles contain H,K-ATPase in an asymmetric manner, so that the cytosolic sector to which ATP binds faces the external medium. The lumen of the vesicle is acidified by the vectorial transport effected by ATPase. These studies showed that omeprazole was without inhibitory effect unless the lumen of the vesicles was acidified by the proton transport activity of H,K-ATPase. This observation provides direct evidence that acid induced conversion of omeprazole is required for inhibition. In addition, the studies of isolated vesicles showed that the inhibitor generated from omeprazole interacted with the luminal sector of H,K-ATPase, since the impermeable mercaptan, gluthatione, did not prevent inhibition, in contrast to its effect in vesicles made permeable to

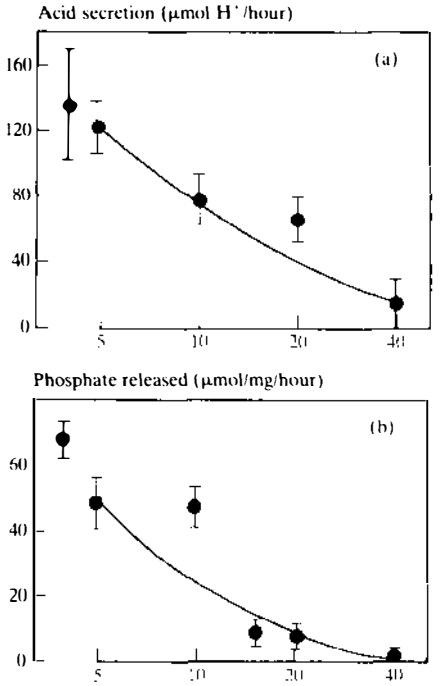

Figure 5 Correlation between (a) rates of acid secretion in the chronic fistula of the rat and (b) inhibition of H,K-ATPase activity, during omeprazole treatment. Results are from Ref. 36.

the molecule (see below) (38). A linear relationship was obtained between the inhibition of H,K-ATPase activity and binding of the inhibitor to the H,K-ATPase. At maximal inhibition, about 2 mol of inhibitor was bound per mol of phosphoenzyme (37, 38). The same stoichiometry for binding of omeprazole was obtained when omeprazole was given to rabbits before they were killed, and the relationship between the resultant ATPase activity and binding to the enzyme was subsequently analyzed. Studies conducted both in vivo and in vitro showed that the H,K-ATPase-inhibitor complex consists of a disulfide linkage. Thus, in vitro, mercaptans that were added prior to omeprazole effectively prevented inhibition. However, also when the enzyme-inhibitor complex had been formed, either in vivo or in vitro, the enzyme activity was restored and the inhibitor was displaced from the enzyme upon addition of mercaptans (39, 40, 41). Furthermore, in permeable vesicle preparations, a linear relationship was obtained between binding of the inhibitor and modification of sulfhydryl groups within the preparation (42, 43). The chemical reactions of omeprazole leading to inhibition of H,K-ATPase have been investigated in detail (44, 45, 46). The reaction mechanism is summarized in

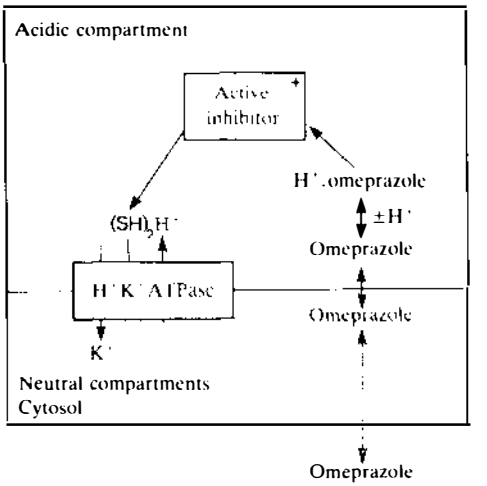

Figure 6 A scheme for acid induced transformation of omeprazole into an active inhibitor of acid secretion. From Ref. 64.

Figure 7. In acid, omeprazole is converted by a series of reversible steps and via a tetracyclic spiro intermediate into sulfenic acid and sulfenamide. These two compounds rapidly react with sulfhydryl groups in the luminal sector of H,K-ATPase. Both the sulfenic acid and the sulfenamide readily react with mercaptans, thereby forming a disulfide adduct. The structure of this adduct was verified by X-ray analysis and serves as a model for the H,K-ATPase inhibitor complex (44). When the disulfide complex is treated with excess mercaptan, the disulfide complex is split. This reaction can thus be compared to the displacement of bound inhibitor from H,K-ATPase in the presence of excess mercaptan.

TOXICOLOGY No significant drug-related toxic effects from omeprazole have been found in the species examined (47). However, since release of gastrin into the blood is regulated by the luminal pH of the stomach, among other factors, the plasma gastrin level will increase during pronounced inhibition of acid secretion (48). Gastrin is a general trophic hormone for gastric fundic mucosa. It is in accordance with this pronounced hypergastrinemia, that a uniform hyperplasia of the oxyntic mucosa has been observed in chronic toxicity studies following high doses of omeprazole (49).

In the rat stomach (in contrast to that of the mouse and dog), a selective hyperplasia of the enterochromaffinlike cells (ECL cells) occurred, which, after two years of treatment with doses of omeoprazole 50–500 times the recommended human dose, resulted in the development of ECL cell carcinoids (32, 47, 49). The ECL and mucosal response to omeprazole is pre-

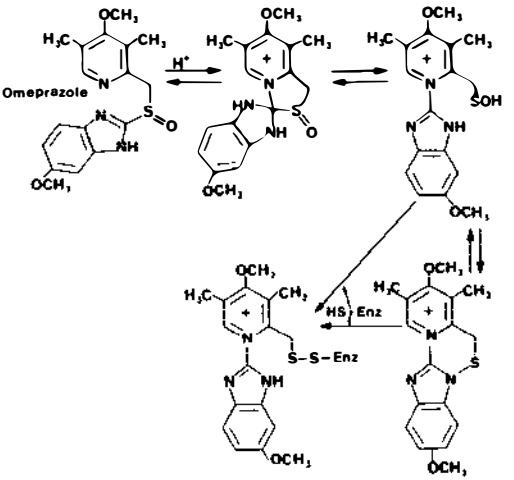

Figure 7 Mechanism of decomposition of omeprazole in acid. From Ref. 38.

vented by antrectomy. This finding is evidence that the development of the ECL cell hyperplasia and carcinoids is due to the secondary hypergastrinaemia and is not an effect of the drug itself (47, 49).

STUDIES IN HUMANS

Omeprazole provides long-lasting inhibition of acid secretion in humans (27) and has been shown to inhibit secretion in isolated human gastric glands and the human H,K-ATPase (50). When given in a single dose, it was found to have an ED_{50} value of about 27 mg for inhibition of acid secretion, irrespective of the stimulus used (27, 29). During the first days of repeated administration, an increasing antisecretory effect occurs that reaches a steady-state level after 4–5 days of treatment, as is to be expected from a drug with a long duration of action (27). Despite the long duration of the antisecretory effect, it was not possible, with 1 dose every 24 hr, to completely block acid secretion over the 24-hr period (27). Thus complete suppression of maximal acid output is of short duration, possibly owing to resynthesis of new pumps in the parietal cell. The degree of acid suppression is greater, and of longer duration, than in the case of other currently available agents.

Prior to the use of omeprazole, it was not clear that improved healing of duodenal ulcers would result from more pronounced inhibition of acid secretion. However, several double blind, randomized clinical trials have shown that pump inhibition in duodenal ulcers provides more rapid symptom relief and healing than do histamine H_2-receptor antagonists (51, 52, 53). Although there has been more confusion about the role of acid secretion in gastric

ulcers, the use of omeprazole has given good results for such ulcers when administered once a day (54). Erosive esophageal lesions also heal significantly better in response to omeprazole than to histamine H_2-receptor blockers (55). Finally, for the Zollinger-Ellison syndrome, in which the massive hypergastrinemia drives acid secretion by direct action on the parietal cell, omeprazole has proved itself to be the drug of choice (56).

The efficacy of this class of pump blocker in treating acid related disease in humans seems to be clearly established, and an increasing number of analogues are being synthesized and studied throughout the world.

Protonatable Amines

The K site of the H,K-ATPase, which is responsible for triggering the breakdown of E-P and hence for turnover and H transport, may have unique properties. It has been shown that various protonable amines, such as trifluperazine (57), are able to inhibit K activation of H,K-ATPase competitively, and thus that they reversibly inhibit H,K-ATPase. A substituted pyridyl 1,2a imidazole, SCH 28080, has been identified as an inhibitor of acid secretion with characteristics similar to those expected of a pump inhibitor (58, 59, 60). Analysis of its mechanism of action showed that SCH 28080 inhibited isolated gastric gland secretion and inhibited H,K-ATPase by K competition, although it was uncompetitive with ATP. Moreover, in gastric glands the drug inhibited secretion and the stimulated oxygen (O_2) consumption, irrespective of the stimulus used. Furthermore, inhibition of O_2 consumption was not blocked by buffering the acid space by means of imidazole, in contrast to the effects of omeprazole. The protonated form is probably the active species, since the $R\text{-}\overset{+}{N}\text{-}CH_3$ derivative shows activity only on the luminal surface (60). In light of the fact that SCH 28080 induces an E_2 conformation, as assessed by the quenching of the enzyme after it has been modified by fluoresceinisothiocyanate (60), SCH 28080 and perhaps other similar compounds appear to be luminally active cations that compete with K on this face of the enzyme. Based on a steady-state and transient kinetic analysis, the inhibited form of the enzyme is likely to be an $MgATP \cdot E_2$ inhibitor complex, with the inhibitor substituting for K. Since these compounds also are weak bases,

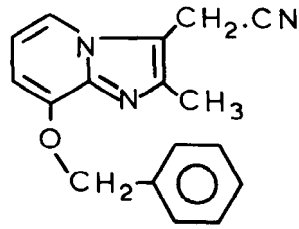

Figure 8 Formula of SCH 28080.

they will accumulate in the parietal cell at a concentration ratio dependent on their pKa and on the permeability of the protonated species. Remarkably, the structural requirements of SCH 28080 are rather narrow, suggesting some quite specific three dimensional structure in the luminally accessible E_2. K region of H,K-ATPase (61) (Figure 8).

The fact that SCH 28080 and the active species of omeprazole are both cationic and luminally active may render both compounds useful as probes of the ion transport sites and mechanism of H,K-ATPase.

Reversible H,K-ATPase inhibitors of this type have been shown to inhibit acid secretion in humans (60, 62), and in the future may provide another class of pump inhibitors that can be used in treatment of acid related disorders.

Literature Cited

1. Black, J. W., Duncan, W.A.M., Durant, C. J., Ganellin, C. R., Parsons, E. M. 1972. Definition and antagonism of histamine H_2-receptors. *Nature* 236:385–90
2. Chew, C. S., Sachs, G., Hersey, S. J., Berglindh, T. 1980. Histamine responsiveness of isolated gastric glands. *Am. J. Physiol.* 238:G312–20
3. Chew, C. S. 1986. Cholecystokinin, carbachol, gastrin, histamine, and forskolin increase $[Ca^{2+}]_i$ in gastric glands. *Am. J. Physiol.* 250:G814–23
4. Muallem, S., Sachs, G. 1984. Changes in cytosolic free Ca^{2+} in isolated parietal cells: differential effects of secretagogues. *Biochim. Biophys. Acta* 805:181–85
5. Barbara, L., Corinaldesi, R., Rea, E., Paternico, A., Stanghellini, V. 1986. The role of colloidal bismuth substrate in the short-term treatment of duodenal ulcer. *Scand. J. Gastroenterol.* 21(Suppl. 122):30–34
6. Lam, K. T., Lai, S. T., Kan, Y. S., Chan, A.X.T. 1985. Sucralfate compared with ranitidine in the short-term healing of duodenal ulcers. *J. Int. Med. Res.* 13:338–41
7. Nicholson, P. A. 1985. A multicenter international controlled comparison of two dosage regimes of misoprostol and cimetidine in the treatment of duodenal ulcer in out patients. *Dig. Dis. Sci.* 30:171S–77S
8. Hunt, R. M., Mowden, C. W., Jones, D. B., Burget, D. W., Kerr, G. D. 1986. The correlation between acid suppression and peptic ulcer healing. *Scand. J. Gastroenterol.* 21(Suppl.125):22–29
9. Ganser, A. L., Forte, J. G. 1973. K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochim. Biophys. Acta* 307:169–80
10. Sachs, G., Chang, H. H., Rabon, E., Schackmann, R., Lewin, M., Saccomani, G. 1974. A non-electrogenic H^+-pump in plasma membranes of hog stomach. *J. Biol. Chem.* 251:7690–98
11. Smolka, A., Helander, H. F., Sachs, G. 1983. Monoclonal antibodies against gastric H^+,K^+-ATPase. *Am. J. Physiol.* 245:G589–96
12. DiBona, D. R., Ito, S., Berglindh, T., Sachs, G. 1979. Cellular site of gastric acid secretion. *Proc. Natl. Acad. Sci. USA* 76:6689–93
13. Berglindh, T., Helander, H. F., Öbrink, K. J. 1976. Effects of secretagogues on oxyntic consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta Physiol. Scand.* 97:401–14
14. Kyte, J. 1981. Molecular considerations relevant to the mechanism of active transport. *Nature* 292:201–4
15. E. Carafoli, Scarpa, A., eds. 1982. Transport ATPases. *Ann. N.Y. Acad. Sci.* 402:
16. Saccomani, G., Stewart, M. B., Shaw, D., Lewin, M., Sachs, G. 1977. Characterization of gastric mucosal membranes. IX. Fractionation and purification of K^+-ATPase containing vesicles by zonal centrifugation and free-flow electrophoresis technique. *Biochim. Biophys. Acta* 465:311–30
17. Wallmark, B., Stewart, H. B., Rabon, E., Saccomani, G., Sachs, G. 1980. The catalytic cycle of gastric H^+,K^+-ATPase. *J. Biol. Chem.* 255:5313–19
18. Stewart, H. B., Wallmark, B., Sachs, G. 1981. The interaction of H^+ and K^+

with the partial reactions of gastric H^+,K^+-ATPase. *J. Biol. Chem.* 256: 2682–90
19. Wolosin, J. M., Forte, J. G. 1981. Changes in the membrane environment of the H,K-ATPase following stimulation of the gastric oxyntic cell. *J. Biol. Chem.* 256:3149–52
20. Rabon, E., Gunther, R. D., Soumarmon, A., Bassilian, S., Lewin, M., Sachs, G. 1985. Solubilization and reconstitution of the gastric H,K-ATPase. *J. Biol. Chem.* 260:10200–7
21. Karlish, S.J.D., Lieb, W. R., Stein, W. D. 1982. Combined effects of ATP and phosphate on rubidium exchange mediated by Na,K-ATPase reconstituted into phospholipid vesicles. *J. Physiol. London* 328:333–50
22. Shull, G. E., Lingrel, J. B. 1986. Molecular cloning of rat stomach H,K-ATPase. *J. Biol. Chem.* 261:16788–91
23. Kaunitz, J. D., Sachs, G. 1986. Identification of a vanadate-sensitive potassium-dependent proton pump from rabbit colon. *J. Biol. Chem.* 261:14005–10
24. Gustin, M. C., Goodman, D.B.P. 1982. Characterization of the phosphorylated intermediate of K-ATPase of rabbit distal colon. *J. Biol. Chem.* 257:9629–33
25. Fellenius, E., Berglindh, T., Sachs, G., Olbe, L., Elander, B., Sjöstrand, S. E., Wallmark, B. 1981. Substituted benzimidazoles inhibit gastric acid secretion by blocking H,K-ATPase. *Nature* 290:159–61
26. Larsson, H., Carlsson, E., Jungren, U., Olbe, L., Sjöstrand, S. E., Skånberg, I., Sundell, G. 1983. Inhibition of gastric acid secretion by omeprazole in the dog and rat. *Gastroenterology* 85:900–7
27. Lind, T., Cederberg, C., Ekenved, G., Haglund, U., Olbe, L. 1983. Effect of omeprazole—a gastric proton pump inhibitor—on pentagastrin-stimulated acid secretion in man. *Gut* 24:270–76
28. Konturek, S. J., Cieszkowski, M., Kwiecien, N., Konturek, J., Tasler, J., Bilski, J. 1984. Effects of omeprazole, a substituted benzimidazole, on gastrointestinal secretions, serum gastrin, and gastric mucosal blood flow in dogs. *Gastroenterology* 86:71–77
29. Lind, T., Cederberg, C., Ekenved, G., Olbe, L. 1986. Inhibition of basal and betazole- and sham-feeding-induced acid secretion by omeprazole in man. *Scand. J. Gastroenterol.* 21:1004–10
30. Wallmark, B., Jaresten, B.-M., Larsson, H., Ryberg, B., Brändström, A., Fellenius, E. 1983. Differentiation among the inhibitory actions of omeprazole, cimetidine, and SCN^- on gastric acid secretion. *Am. J. Physiol.* 245:G64–71
31. Sewing, K-Fr., Harms, P., Schultz, G., Hannemann, H. 1983. Effect of substituted benzimidazoles on acid secretion in isolated and enriched guinea pig parietal cells. *Gut,* 24:557–60
32. Carlsson, E., Larsson, H., Mattsson, H., Ryberg, B., Sundell, G. 1986. Pharmacology and toxicology of omeprazole—with special reference to the effects on the gastric mucosa. *Scand. J. Gastroenterol.* Suppl. 118:31–38
33. Larsson, H., Mattsson, H., Sundell, G., Carlson, E. 1985. Animal pharmacodynamics of omeprazole. A survey of its pharmacological properties in vivo. *Scand. J. Gastroenterol.* Suppl. 108:23–35
34. Regårdh, C.-G., Gabriclsson, M., Hoffman, K.-J., Löfberg, I., Skånberg, I. 1985. Pharmacokinetics and metabolism of omeprazole in animals and man—an overview. *Scad. J. Gastroenterol.* Suppl. 108:79–94
35. Helander, H. F., Ramsay, C.-H., Regårdh, C.-G. 1985. Localisation of omeprazole and metabolites in the mouse. *Scand. J. Gastroenterol.* Suppl. 108:95–104
36. Wallmark, B., Larsson, H., Humble, L. 1985. The relationship between gastric acid secretion and gastric H,K-ATPase activity. *J. Biol. Chem.* 260:13681–84
37. Keeling, D. J., Fallowfield, C., Underwood, A. H. 1987. The specificity of omeprazole as an H^+,K^+-ATPase inhibitor depends upon the means of its activation. *Biochem. Pharmacol.* 36:339–44
38. Lorentzon, P., Jackson, R., Wallmark, B., Sachs, G. 1987. Inhibition of H,K-ATPase by omeprazole in isolated vesicles requires proton transport. *Biochim. Biophys. Acta* 897:41–51
39. Im, W. B., Sih, J. C., Blakeman, D. P., McGrath, J. P. 1985. Omeprazole a specific inhibitor of gastric H,K-ATPase, is a H-activated oxidizing agent of sulfhydryl groups. *J. Biol. Chem.* 260:4591–97
40. Im, W. B., Blakeman, D. P., Sachs, G. 1985. Reversal of antisecretory activity of omeprazole by sulfhydryl compounds in isolated rabbit gastric glands. *Biochim. Biophys. Acta* 845:54–59
41. Wallmark, B., Brändström, A., Larsson, H. 1984. Evidence for acid-induced transformation of omeprazole into an active inhibitor of H,K-ATPase within the parietal cell. *Biochim. Biophys. Acta* 778:549–58
42. Lorentzon, P., Eklund, B., Brändström,

A., Wallmark, B. 1985. The mechanism for inhibition of gastric H^+,K^+-ATPase by omeprazole. *Biochim. Biophys. Acta* 817:25–32

43. Keeling, D. J., Fallowfield, C., Milliner, K. J., Tingley, S. K., Ife, R. J., Underwood, A. H. 1985. Studies on the mechanism of action of omeprazole. *Biochem. Pharmacol.* 34:2967–73
44. Lindberg, P., Nordberg, P., Alminger, T., Brändström, A., Wallmark, B. 1986. The mechanism of action of the gastric acid secretion inhibitor omeprazole. *J. Med. Chem.* 29:1327–29
45. Figala, V., Klemm, K., Kohl, B., Krüger, U., et al. 1986. Acid activation of H^+,K^+-ATPase inhibiting 2-(2-pyridylmethylsulfinyl) benzimidazoles: Isolation and characterization of the thiophilic 'active principle' and its reactions. *J. Chem. Soc. Chem. Commun.* 1986:125–29
46. Lindberg, P., Brändström, A., Wallmark, B. 1987. Structure-activity relationships of omeprazole analogues and their mechanism of action. In *Trends in Medical Chemistry*, ed. E. Mutscher, E. Winterfeldt. Weinheim, FRG: Verlagsgesellschaft
47. Ekman, L., Hansson, E., Havu, N., Carlsson, E., Lundberg, C. 1985. Toxicological studies on omeprazole. *Scand. J. Gastroenterol.* Suppl. 108:53–59
48. Håkanson, R., Oscarsson, J., Sundler, F. 1986. Gastrin and the trophic control of gastric mucosa. *Scand. J. Gastroenterol.* Suppl. 118:18–30
49. Larsson, H., Carlsson, E., Mattsson, H., Lundell, L., Sundler, F., Sundell, G., Wallmark, B., Watanabe, T., Håkanson, R. 1986. Plasma gastrin and gastric enterochromaffinlike cell activation and proliferation. *Gastroenterology* 90:391–99
50. Elander, B., Fellenius, E., Leth, R., Olbe, L., Wallmark, B. 1986. Inhibitory action of omeprazole on acid formation in gastric glands and on H^+,K^+-ATPase isolated from human gastric mucosa. *Scand. J. Gastroenterol.* 21:268–72
51. Lauritsen, K., Rune, S. J., Bytzer, P., et al. 1985. Effect of omeprazole and cimetidine on duodenal ulcer. *N. Engl. J. Med.* 312:958–61
52. Bardhan, D. K., Porro, G. B., Bose, K., et al. 1986. A comparison of two different doses of omeprazole versus ranitidine in treatment of duodenal ulcers. *J. Clin. Gastroenterol.* 8:408–13
53. Classen, M., Dammann, H. G., Domschke, W., et al. 1985. Kurzzeit-Therapie des Ulcus duodeni mit Omeprazol und Ranitidin. *Dtsch. Med. Wochenschr.* 110:210–15 and 628–33
54. Classen, M., Dammann, H. G., Domschke, W., et al. 1985. Abheilungsraten nach Omeprazol- und Randitidin-Behandlung des Ulcus Ventriculi. *Dtsch. Med. Wochenschr.* 110:627–33
55. Klinkenberg-Knol, E. C., Jansen, J.B.M.J., Festen, H.P.M., Menwissen, S.C.M., Lauers, C.B.M.W. 1987. Double-blind multicentre comparison of omeprazole and ranitidine in the treatment of reflux oesophagitis. *Lancet* 1:349–51
56. Lloyd-Davies, K. A., Rutgersson, K., Sölvell, L. 1986. Omeprazole in Zollinger-Ellison syndrome: Four-year international study. *Gastroenterology* 90:1523 (Abstr.)
57. Im, W. B., Blakeman, D. P., Mendlein, J., Sachs, G. 1984. Inhibition of H^+,K^+-ATPase and H^+ accumulation in hog gastric membranes by trifluoperazine, verapamil and 8-(N,N-diethylamino) octyl-3,4,5-trimethoxybenzoate. *Biochim. Biophys. Acta* 770:65–72
58. Beil, W., Hackbarth, I., Sewing, K.-Fr. 1986. Mechanism of gastric antisecretory effect of SCH 28080, *Br. J. Pharmacol.* 88:19–23
59. Scott, C. K., Sundell, E., Castrovilly, L. 1987. Studies on the mechanism of action of the gastric microsomal H^+,K^+-ATPase inhibitors SCH 32651 and SCH 28080. *Biochem. Pharmacol.* 36:97–104
60. Wallmark, B., Briving, C., Fryklund, J., Munson, K., et al. 1987. Inhibition of gastric H^+,K^+-ATPase and acid secretion by SCH 28080, a substituted pyridyl (1,2a) imidazole. *J. Biol. Chem.* 262:2077–84
61. Kaminski, J. J., Bristol, J. A., Puchalski, C., et al. 1985. Antiulcer agents. 1. Gastric antisecretory and cytoprotective properties of substituted imidazo[1,2-a] pyridines. *J. Med. Chem.* 28:876–92
62. Enc, M. D., Khan-Daneshmend, T., Roberts, C.J.C. 1982. A study of the inhibitory effects of SCH 28080 on gastric secretion in man. *Br. J. Pharmacol.* 76:389–91
63. Long, J. F., Chiu, J. S., Derelanko, M. J., Steinberg, M. 1983. Gastric antisecretory and cytoprotective activities of SCH 28080. *J. Pharmacol. Exp. Ther.* 266:114–20
64. Wallmark, B. 1986. Mechanism of action of omeprazole. *Scand. J. Gastroenterol.* Suppl. 118:11–17
65. Sachs, G. 1986. The parietal cell as a therapeutic target. *Scand. J. Gastroenterol.* Suppl. 118:1–10