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The Relationship between Gastric Acid Secretion and Gastric H^+,K^+-ATPase Activity*

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The H^+,K^+-ATPase has been postulated to be the enzyme responsible for H^+ secretion by the parietal cell. Omeprazole has been shown to be an inhibitor of acid secretion *in vivo*, but also in *in vitro* test models for acid secretion, including partly purified H^+,K^+-ATPase, the inhibitory action of omeprazole has been demonstrated (Wallmark, B., Jaresten, B. M., Larsson, H., Ryberg, B., Brändström, A., and Fellenius, E. (1983) *Am. J. Physiol.* 245, G64–G71). It was thus possible to use this compound to demonstrate a correlation between H^+,K^+-ATPase activity in rat oxyntic mucosa and *in vivo* H^+ secretion. Two results were found. (*a*) Increasing oral doses of omeprazole progressively inhibited acid secretion, H^+,K^+-ATPase activity, and phosphoenzyme formation of a microsomal fraction isolated from the inhibited rat mucosa. Furthermore, a Mg^{2+}-stimulated ATPase activity, associated with the H^+,K^+-ATPase membrane fraction, was not affected by the omeprazole treatment. (*b*) Recovery of H^+,K^+-ATPase activity following complete omeprazole inhibition was correlated with the appearance of acid secretion. The results indicate a strict relationship between the activity of the gastric H^+,K^+-ATPase in the microsomal fraction and gastric acid secretion.

Ever since the discovery of the presence of a K^+-stimulated ATPase in the gastric mucosa, research has been focused on its suggested role as the gastric proton pump (6). Several lines of evidence have favored this hypothesis.

The enzyme was found to be located in the tubulovesicular and secretory membranes of the acid-producing parietal cell by antibody techniques (11, 14). Gastric vesicles containing the enzyme can be isolated and purified either from resting or stimulated mucosae (22, 23). These vesicles exhibit both a K^+-stimulated ATPase- and an electroneutral H^+,K^+-antiport transport activity (13). Both the transport and ATPase activities were shown to depend on intravesicular K^+. Further evidence indicating that the H^+,K^+-ATPase[1] is the proton pump has been obtained in permeable gastric glands in which acid formation can be driven by the addition of exogenous ATP. This process was also K^+ dependent but did not require metabolic energy, since good responses were obtained in the presence of mitochondrial inhibitors or under anoxia (1, 10). However, so far no direct correlation has been proven between the H^+,K^+-ATPase activity and acid secretion. By the design and synthesis of substituted benzimidazoles, a new class of gastric acid secretion inhibitors became available.

Omeprazole, which belongs to this group of compounds, has been shown to inhibit gastric acid secretion in rats, dogs, and humans (8, 9). Based on studies in several different *in vitro* preparations, inhibition of acid formation by omeprazole and related substituted benzimidazoles has been proposed to be due to their ability to block the H^+,K^+-ATPase (4, 5, 15, 16). The selectivity of omeprazole for the H^+,K^+-ATPase can be attributed to several factors. Omeprazole is a permeable weak base ($pK_a = 4$) and will, accordingly, be charged and trapped within the acid compartments of the parietal cell. The protonated form of omeprazole is very labile and undergoes transformation into an inhibitor of the H^+,K^+-ATPase. By this mechanism the active inhibitor is generated in the acid compartments of the parietal cell close to its target receptor, the H^+,K^+-ATPase (17). The inhibitory action of omeprazole in isolated H^+,K^+-ATPase preparations has been shown to be due to modification of SH groups. The inhibition was found to be reversible by the addition of thiol-reducing agents, such as β-mercaptoethanol or dithiothreitol, suggesting a disulfide inhibitory bond between the inhibitor and the H^+,K^+-ATPase (17). In the present study, it was possible to use omeprazole to relate variations in acid secretion to different levels of H^+,K^+-ATPase activity in isolated microsomal fractions from treated rat mucosae, either during the phase of peak inhibition of secretion or during the recovery phase.

MATERIALS AND METHODS

Female Sprague-Dawley rats, with body weights between 200–250 g, were used. Omeprazole was suspended in Methocel®, while controls were given this vehicle only. Gastric acid secretion and H^+,K^+-ATPase activities were investigated in parallel groups of animals. For the secretory studies, the animals were provided with chronic fistulas. Two experimental series were conducted: 1) a dose-response study, where acid secretion and the mucosal H^+,K^+-ATPase activity were analyzed 3 h after administration of single oral doses (5–40 μmol/kg); 2) a time-course study for the recovery of the inhibitory effect following three consecutive doses of omeprazole at a level exceeding that required for maximal inhibition of gastric acid secretion. The effects were recorded 6, 12, 18, 24, 30, 36, 42, or 48 h after the third oral dose of vehicle or of omeprazole (suspended in 0.25% Methocel®) at 40 or 400 μmol/kg.

Measurements of Gastric Acid Secretion—Thirty rats were provided with plastic cannulas, chronically implanted into the rumen of the stomach (8). Before each secretory test, the animals were starved for 20 h. The stomach was then rinsed with tap water (+37 °C), and the animals were placed in Bollman cages. Each secretion test consisted of a 2-h maximal stimulation of acid secretion (carbachol (110 nmol·kg^{-1}·h^{-1}) + pentagastrin (20 nmol·kg^{-1}·h^{-1}), subcutaneous infusion). Gastric juice was collected by free flow from the cannula in 30-min samples. The samples were weighed, and their acid content was determined by titration to pH 7.0 with 0.1 M NaOH. Effects were

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[1] The abbreviations used are: H^+,K^+-ATPase, magnesium-dependent, hydrogen ion-transporting, and potassium-stimulated adenosine triphosphatase (EC 3.6.1.3); Na_2ATP, adenosine 5′-triphosphate disodium salt; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

recorded during the second 30-min sampling period of the secretory test, and during this period maximal acid secretion was recorded.

Preparation of Gastric Membranes Containing the Gastric H^+,K^+-ATPase—The membranes were prepared essentially as described in Ref. 21. Scrapings of the corpus mucosa were homogenized in a glass/Teflon homogenizer at 1500 rpm in a buffer containing: 250 mM sucrose, 2 mM $MgCl_2$, 1 mM EGTA, and 2 mM HEPES, pH 7.4. The tissue was diluted 10 times with buffer prior to homogenization. The crude homogenate was spun at 20,000 × *g* for 20 min. The resulting supernatant was subsequently spun at 100,000 × *g* for 1 h. The final 100,000 × *g* supernatant was discarded and the crude microsomal pellet was further separated on a discontinuous density gradient consisting of 30% sucrose at 100,000 × *g* for 60 min. The membranes sedimenting above the 30% sucrose interface were used for analysis of the ATPase activities.

Assays of H^+,K^+-ATPase Activity and Phosphoenzyme Formation—The ATPase activity was determined in a solution containing 180 mM sucrose, 40 mM Tris acetate, pH 7.4, 2 mM $MgCl_2$, 2 mM Na_2ATP, 5–20 μg of membrane protein, and 5 μg of nigericin/ml with and without 10 mM KCl. The incubation time was 10 min at 37 °C. The assay was stopped and inorganic phosphate analyzed as described in Ref. 24. The K^+-stimulated ATPase activity was taken as the difference between the Mg^{2+}-stimulated and the Mg^{2+},K^+-stimulated ATPase activity. The phosphoenzyme concentration was determined in the solution given above for the ATPase assay, with the following modifications: 5 μM [γ-^{32}P]ATP was used instead of 2 mM Na_2ATP, and 100 mM KCl instead of 10 mM KCl. The incubation time was 15 s at 22 °C. Determination of the amount of phosphoenzyme was as described in Ref. 18.

Protein Determination—Protein was determined according to the method of Bradford (2).

Polyacrylamide Gel Electrophoresis (PAGE)—SDS-PAGE was performed on a slab gel consisting of an 11% polyacrylamide running gel and a 4% polyacrylamide stacking gel. The samples, 5–20 μg of protein, were dissolved in 0.5% SDS. The gels were run in a two-buffer system with a separation buffer consisting of 0.42 mM Tris-HCl, pH 9.18, and an upper buffer consisting of 40 mM boric acid, 41 mM Tris, and 0.1% SDS, pH 8.64. The samples were run at 40 mA until they had entered the stacking gel, thereafter at 80 mA. After the run the gels were stained with Coomassie Brilliant Blue R-250 and after destaining, scanned in a laser densitometer.

Chemicals—Omeprazole (5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole) was synthesized by AB Hässle. The compound was suspended in 0.25% Methocel® before administration. Na_2ATP and [γ-^{32}P]ATP were obtained from Sigma and Amersham Corp., respectively. All other chemicals were obtained from commercial sources and were of the highest purity grade available.

RESULTS

Dose-Response Relationship for Inhibition of Gastric Acid Secretion and H^+,K^+-ATPase Activity in Rats Treated with Omeprazole—Omeprazole was found to dose dependently reduce the rate of maximally stimulated acid secretion when measured 3 h after administration of omeprazole (Fig. 1*A*). The ED_{50} value was about 10 μmol/kg, body weight. The K^+-stimulated ATPase activity, which was measured in the purified microsomal membrane fraction, was found to be inhibited in a dose-related manner. The ED_{50} value was about 12 μmol/kg (Fig. 1*B*). In the same membrane fraction, the formation of steady-state concentrations of the phosphoprotein was analyzed. This phosphoprotein was found to be acid stable, ouabain insensitive, and up to 95% K^+ sensitive (Table I). Since these three criteria are shared by the better characterized phosphoenzyme of the hog gastric H^+,K^+-ATPase preparation, the phosphoprotein of the rat preparation can most likely be identified as an enzyme intermediate of the catalytic cycle of the rat H^+,K^+-ATPase (19). Parallel to inhibition of the H^+,K^+-ATPase activity, following administration of increasing doses of omeprazole to the animals, the steady-state phosphoenzyme levels were also decreased (Fig. 1*C*). The ED_{50} value was about 10 μmol/kg. The K^+-stimulated ATPase activity, present in the microsomal density gradient fraction, was associated with an Mg^{2+}-stimulated ATPase activity. In contrast, this Mg^{2+}-ATPase was not affected by the omeprazole treatment (Fig. 2).

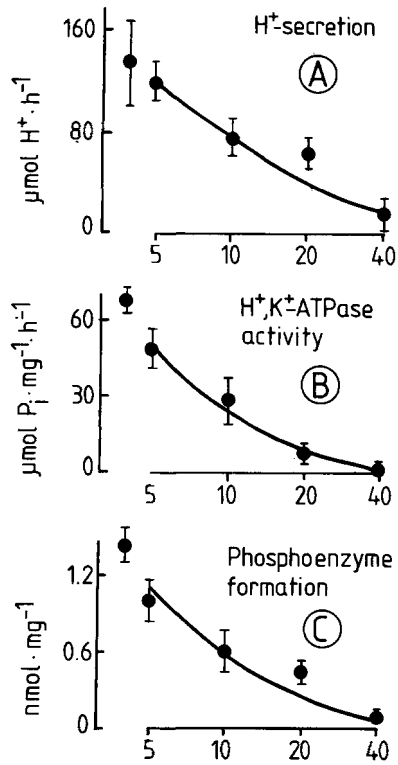

FIG. 1. **Dose-response relationship for inhibition of acid secretion, H^+,K^+-ATPase activity, and phosphoenzyme formation in rats treated with omeprazole.** Omeprazole was given as single oral doses. The anti-secretory effects were measured and the membranes prepared 3 h after administration. *A*, effect of omeprazole on supramaximal carbachol- and pentagastrin-stimulated acid secretion. *B*, effect on K^+-stimulated ATPase activity in the density-gradient fraction. *C*, effect of K^+-sensitive phosphoenzyme formation in the density-gradient fraction.

TABLE I

Effect of K^+ and ouabain on acid-stable phosphoprotein formation in the density-gradient fraction of rat gastric microsomes

Conditions	Phosphoprotein (mean ± S.E.)	
	nmol·(mg protein)$^{-1}$	
5 μM [γ-^{32}P]ATP, 2 mM $MgCl_2$	1.52 ± 0.14	$n = 21$
5 μM [γ-^{32}P]ATP, 2 mM $MgCl_2$, 100 mM KCl	0.074 ± 0.007	$n = 15$
5 μM [γ-^{32}P]ATP, 2 mM $MgCl_2$, 0.1 mM ouabain	1.41 ± 0.26	$n = 3$

Recovery of Acid Secretion and H^+,K^+-ATPase Activity following Omeprazole Treatment—Because of the long duration of the omeprazole action, the inhibitory degree of acid secretion is increased during the first few days of repeated treatment (9). Therefore, omeprazole was administered once daily for three consecutive days at dose levels of 40 and 400 μmol/kg in order to obtain steady-state inhibition of gastric acid secretion. After the last dose, acid secretory rates and H^+,K^+-ATPase activities were followed up to 48 h. For both dose levels acid secretory rates and H^+,K^+-ATPase activities were restored in a parallel manner (Fig. 3). For the lower dose group, both acid secretion and H^+,K^+-ATPase activity were back to control levels about 24–30 h after the last dose, while a lapse of almost 48 h was required for the higher dose. A linear relationship was obtained when the percentage of con-

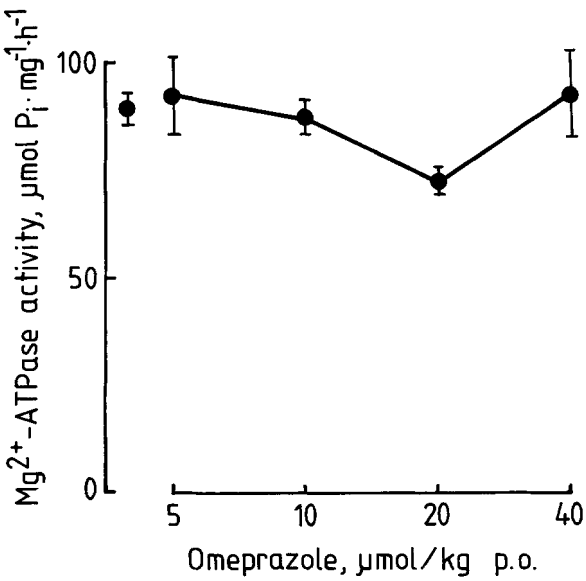

FIG. 2. **Effect of omeprazole treatment on Mg^{2+}-stimulated ATPase activities associated with the microsomal density-gradient fraction.**

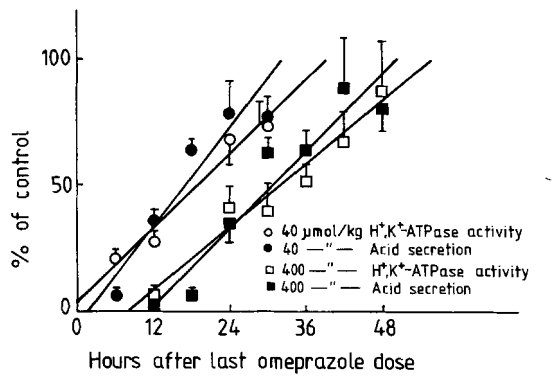

FIG. 3. **Recovery of acid secretion and H^+,K^+-ATPase activity following omeprazole treatment.** Omeprazole was given once daily for three consecutive days. The effects on excessively stimulated acid secretion and K^+-stimulated ATPase activity in the microsomal density-gradient fraction were measured at the times indicated. The results are expressed as percentages of the secretory rates and K^+-stimulated ATPase activities obtained in control rats.

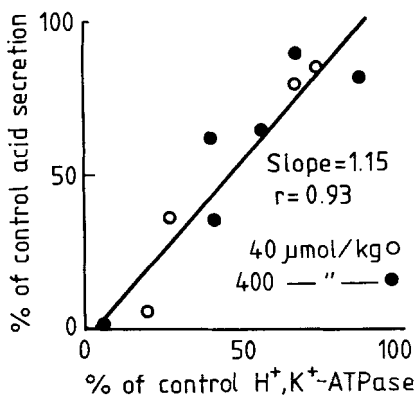

FIG. 4. **Correlation between inhibition of acid secretion and H^+,K^+-ATPase activity under the influence of omeprazole.** Data were taken from Fig. 3. The correlation line was drawn according to the method of least squares.

trol acid secretory rates was plotted against the percentage of control H^+,K^+-ATPase activity (Fig. 4). The slope obtained did not significantly deviate from unity, indicating a direct correlation between acid secretion and H^+,K^+-ATPase activity.

Determination of the Amount of H^+,K^+-ATPase Present in the Fraction Used for Assay of the K^+-stimulated ATPase Activities—Although the specific K^+-stimulated ATPase activity is the highest in the microsomal fraction of the rat gastric mucosa, it only represents about 20–40% of the total H^+,K^+-ATPase activity present in the initial homogenate (20). Moreover, since secretagogues and inhibitors of acid secretion have been shown to influence the distribution of the gastric H^+,K^+-ATPase (20, 23), it was of importance to quantitate the amount of enzyme present in the same fraction in which the K^+-ATPase activity and phosphoenzyme level were assayed. From Fig. 5*A* it follows that the total protein content in the fractions was not influenced at any of the times indicated after the last dose of omeprazole. It has been shown that the peptide comprising the catalytic subunit of the gastric H^+,K^+-ATPase has a molecular mass of about 95,000 daltons (12, 15). It is evident from the SDS-PAGE gel scan (Fig. 5*C*) that the peptides in this region of the gel only constitute a

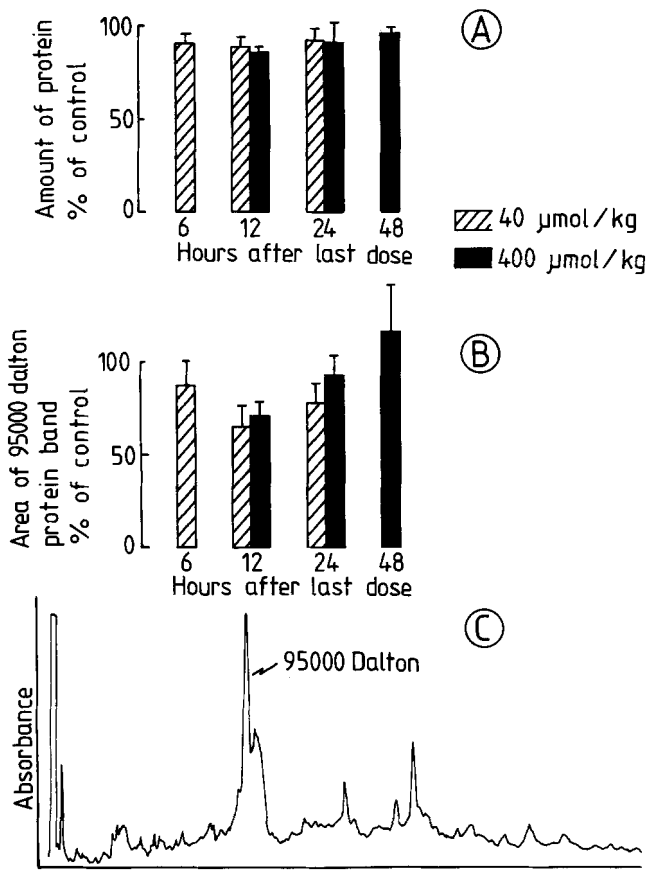

FIG. 5. **Determination of the total amount of protein and 95,000-dalton protein band present in the microsomal density-gradient fractions after omeprazole treatment.** The same fractions were used as for measurements of the K^+-stimulated ATPase activity shown in Fig. 3. *A*, total amount of protein present at different times following omeprazole administration. The amount of protein obtained in control fractions was set at 100%. *B*, total amount of 95,000-dalton peptides at different times following omeprazole treatment. The areas obtained under the 95,000-dalton peaks were determined. The areas obtained for control rats in the respective groups were set at 100%. *C*, a representative SDS-PAGE gel from the microsomal density-gradient fraction.

fraction of the total protein present. Therefore, the area under the 95,000-dalton peak was quantified and taken as a measurement of the amount of the H^+,K^+-ATPase which was present in the microsomal density gradient fraction after omeprazole treatment. The areas under the 95,000-dalton peak quantified at specific intervals after omeprazole treatment are shown in Fig. 5*B*. They are expressed as percentages of the mean area determined for control animals at each time. The areas did not significantly differ from those of controls at any of the measuring times, despite the fact that acid secretion and the H^+,K^+-ATPase activity were totally inhibited 6 h after the low dose and 12 h after the high dose of omeprazole.

DISCUSSION

The inhibitory action of omeprazole on acid secretion has been determined in intact mucosa *in vivo* and *in vitro*, in isolated gastric glands, and on purified H^+,K^+-ATPase. In brief, it has been shown that this compound is a potent and long-lasting inhibitor of acid secretion. Inhibition is maintained in spite of the disappearance of the compound from blood plasma (3, 8, 9). Autoradiographic studies in whole animal sections and in gastric mucosal sections have shown persistence of radioactivity in the stomach, especially in the tubulovesicles and secretory canaliculi of the parietal cell (7). Thus, the radioactivity is bound to the same parietal cell membranes that have been shown to be the binding sites of monoclonal antibodies directed against the gastric H^+,K^+-ATPase (17). In this study, we have shown that changes in the activity of the H^+,K^+-ATPase are directly related to the rate of acid secretion by the intact mucosa. This was the case when omeprazole was either administered in increasing submaximal doses and the inhibitory effect was measured at peak response or when the recovery period following the excessive doses of omeprazole was investigated.

The observed inhibition is most likely explained by binding and/or modification of the H^+,K^+-ATPase by the active inhibitor generated from omeprazole. This leads to deactivation of the enzyme activity rather than redistribution of the H^+,K^+-ATPase to different membrane fractions. Evidence for this hypothesis resides in the fact that the Mg^{2+}-stimulated ATPase, which was associated with the same membrane fraction holding the K^+-stimulated ATPase, was not affected by the omeprazole treatment. Furthermore, neither the amount of protein nor the concentration of the 95,000-dalton peptides containing the catalytic subunit of the H^+,K^+-ATPase were significantly altered in the microsomal density-gradient fractions, despite the near-complete inhibition of acid secretion 6 and 12 h after omeprazole administration, depending on the dose given.

The inhibition induced by omeprazole represents covalent binding of the inhibitor to the H^+,K^+-ATPase (25). Therefore, regeneration of active enzyme following omeprazole treatment could depend on *de novo* synthesis of the enzyme. In this case, the half-life of recovery of acid secretion and H^+,K^+-ATPase activity should be related to the half-life of the enzyme, which appeared to be about 14 h in the rat, assuming a first-order mechanism for the recovery phase. Alternatively, the rate of the recovery of the H^+,K^+-ATPase activity might depend on reactivation of essential SH groups by endogenous sulfhydryl compounds, *e.g.* glutathione. The fact that mercaptanes have been shown to reverse omeprazole-induced inhibition *in vitro* (17) supports this view.

The presented data not only strengthen the hypothesis that inhibition of acid secretion by omeprazole is mediated by inactivation of the H^+,K^+-ATPase but also draw attention to the important physiological role of the gastric H^+,K^+-ATPase in gastric acid secretion and encourage further exploration of H^+,K^+-ATPase inhibitors for use in ulcer therapy.

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