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EVIDENCE FOR ACID-INDUCED TRANSFORMATION OF OMEPRAZOLE INTO AN ACTIVE INHIBITOR OF ($H^+ + K^+$)-ATPase WITHIN THE PARIETAL CELL

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The chemical reactions of omeprazole, leading to inhibition of gastric acid secretion, were investigated. In acid buffer solutions, omeprazole was found to be labile, whereas at physiological pH it was stable ($t_{1/2} > 17$ h at pH 7.4). The stability of omeprazole was also studied in isolated, acid producing, gastric glands under conditions where acid formation was either stimulated or inhibited. The rate of transformation of omeprazole was high ($t_{1/2} \approx 3$ min) under stimulation. Inhibition of acid formation in the gland greatly retarded the decomposition of omeprazole ($t_{1/2} \approx 73$ min). The time-course for inhibition of acid formation by omeprazole was parallel to that for decomposition. The major product formed from omeprazole was the reduced form, H 168/22. The inhibitory action of omeprazole was shown to depend on acid-induced transformation, since no inhibition was obtained when omeprazole was incubated under neutral conditions, both in the isolated gastric mucosal- and the ($H^+ + K^+$)-ATPase preparations. Despite the fact that H 168/22 was the major product formed in the glandular preparation, it was found to be virtually inactive in both the glandular- and ($H^+ + K^+$)-ATPase preparations. Therefore, a model is proposed in which the inhibition of acid formation by omeprazole is mediated by a compound formed during the reduction of omeprazole to H 168/22 within the acid compartments of the parietal cell. Furthermore, mercaptanes, such as β-mercaptoethanol, were found to prevent as well as reverse inhibition by omeprazole in both the glandular- and ($H^+ + K^+$)-ATPase preparations. This indicates that -SH groups are most likely involved in the chemical reactions leading to inhibition of acid secretion.

Introduction

The production of hydrochloric acid by the gastric mucosa is mediated by the ($H^+ + K^+$)-ATPase situated in the microvillar membrane lining the secretory canaliculus of the parietal cell [1–5]. This enzyme, although similar in many respects to the ($Na^+ + K^+$)-ATPase of plasma membranes [6], is rather sparsely distributed in the body. Thus, so far it has only been identified with certainty in the gastric mucosa. An enzyme with similar properties appears to be present in the colon [7]. In the latter case, however, no direct evidence linking the enzyme function to either H^+- or K^+-transport has been forthcoming.

Inhibition of gastric acid secretion plays an important part in ulcer healing therapy. Hitherto, this therapy has been dependent on H_2-antagonists or anticholinergic agents [8,9]. With the synthesis of substituted benzimidazoles, a novel group of gastric acid secretion inhibitors became available.

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Abbreviations: dibutyryl-cAMP, $N^6,O^{2'}$-dibutyryladenosine 3′:5′-cyclic monophosphate; DMSO, dimethylsulfoxide; Pipes, 1,4-piperazinediethanesulfonic acid.

In vivo, these agents have been shown to be effective in man, dog and rat [10–12]. In vitro, inhibition of acid formation by the benzimidazoles has been demonstrated in isolated mucosal preparations [13,14], in isolated gastric glands [15,16] and in isolated parietal cell fractions [17,18].

Based on their inhibitory action in these in vitro preparations and by virtue of their inhibition of the purified gastric ($H^+ + K^+$)-ATPase [19], a hypothesis has been put forward that blockade of this enzyme explains the inhibitory action of gastric acid secretion found in vivo [20].

Several factors might account for the selectivity of omeprazole as to its action in the parietal cell. Omeprazole is a lipid-permeable weak base ($pK_a = 4$). Protonation leads to an increased charge and, thereby, a lower tendency of the protonated species to penetrate the secretory membranes of the parietal cell. This probably explains the increase in uptake of omeprazole into the gastric glands under acidic conditions. Furthermore, protonation also induces transformation of omeprazole into an active inhibitor of the gastric ($H^+ + K^+$)-ATPase. The aim of the present study is to address the issues of these chemical reactions of omeprazole in acid, leading to inhibition of the ($H^+ + K^+$)-ATPase and thereby acid secretion.

Methods

Preparations

Preparation of gastric mucosa. Gastric mucosal preparations were obtained from male guinea pigs weighing 700–1100 g and experiments were performed according to previously described procedures [16], only modified by changing the end-point for titration of acid to pH 7.4 in the mucosal solution.

Preparation of gastric glands. Male or female albino rabbits, strain Swedish Lantras, were used. The preparation was carried out according to previously published methods [21].

Purification of the gastric ($H^+ + K^+$)-ATPase. This was done using hog stomachs in accordance with previously published methods [25].

Experimental procedures

Respiratory medium used for the glandular experiments. 130 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 1.0 mM $NaH_2PO_4 \cdot H_2O$, 5.0 mM Na_2HPO_4, 11 mM glucose, 10 μg/ml Phenol red and 1 mg/ml albumin.

Extraction of omeprazole and its transformation product. 0.5 or 1.0 ml samples of the supernates and glandular pellets were extracted at pH 7.0–7.4 with 0.5 and 1.0 ml, respectively, of dichloromethane by shaking for 1 min. After centrifugation for 5 min the aqueous phase was sucked off and 200 μl of the organic phase were injected into the chromatographic column.

Chromatography. Analyses were performed using a high-performance liquid chromatograph fitted with an absorbance detector operated at 280 nm. The length of the column was 150 mm.

Support: Lichrosorb SI 60 (5 μm). Mobile phase: methanol, containing 5% NH_4OH (v/v)/*n*-hexane/dichloromethane (2.5 : 10 : 87.5, v/v). The flow rate was 1 ml/min. The fractions, containing [^{14}C]omeprazole and [^{14}C]H 168/22, were collected for radioactivity measurements.

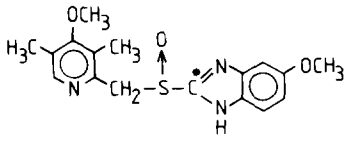
Determination of acid formation in the glands. Uptake of [^{14}C]aminopyrine was used as an index of acid formation within the glands, see Ref. 22. Samples of the incubates were taken and subsequently spun down for 15 s at approx. 1100 × *g*, in an Eppendorf centrifuge. The supernates were removed and the wet and dry weights of the pellets were determined. The pellets were digested for 60 min in 0.5 ml of 0.5 M NaOH at 50°C. The aminopyrine ratios were calculated as in Ref. 22.

Experiments with the isolated mucosal preparation. After an initial period of 30–40 min of recording basal acid secretion, the weak base, 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), was added (30 mM) to the serosal side. Half an hour later, omeprazole was also added to the serosal side to a final concentration of 5 μM and after an additional 30 min, acid secretion was stimulated by histamine (20 μM) for 1 h.

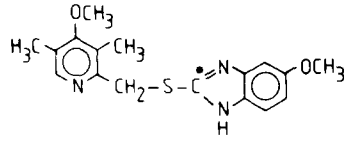
Protein determination. Protein was determined by the method of Lowry et al. [23].

Analysis of inorganic phosphate. This was done as described in Refs. 26 and 27.

Reagents. Collagenase (Type I) and dibutyryl-cAMP were purchased from Sigma, St Louis, MO, U.S.A. Aminopyrine was obtained from Amersham, U.K. Omeprazole (H 168/68), H



Omeprazole (H168/68)



Omeprazole Reduced form (H168/22)

Fig. 1. Chemical structure of omeprazole (H 168/68), 5-methoxy-2-(((4-methoxy-3,5-dimethyl-2-pyridinyl)methyl)sulfinyl)-1*H*-benzimidazole, and the reduced form of omeprazole (H 168/22), 5-Methoxy-2-(((4-methoxy-3,5-dimethyl-2-pyridinyl)methyl)-thio)-1*H*-benzimidazole. The asterisk indicates the position of the ^{14}C radiolabel.

168/22, [^{14}C]omeprazole and [^{14}C]H 168/22, shown in Fig. 1, were all synthesized by AB Hässle, Mölndal, Sweden. Na_2ATP was purchased from Sigma. All other reagents were of the highest purity available.

Determination of the rate constants for transformation of omeprazole in the glandular preparation. Omeprazole was found to decompose within the glands, since negligible decomposition occurred in the glandular medium. The rate of decomposition is dependent on the volume of the glands (V_g) and the volume of the medium (V_m). V_g was taken as the intraglandular water volume, see Ref. 22. The quantity (Q) of omeprazole decomposed is V_g [H 168/22]$_g$ + V_m [H 168/22] = Q. If first-order kinetics for this decomposition is assumed, the change in Q with time is:

$$dQ = k[\text{omeprazole}]_g \cdot V_g \, dt$$

or in integrated form

$$Q = k \cdot V_g \int_0^t [\text{omeprazole}]_g \, dt$$

A plot of Q/V_g against $\int_0^t [\text{omeprazole}]_g \, dt$ gives a straight line with the slope k, where k denotes the apparent decomposition rate constant.

Results

Transformation of omeprazole in buffer medium and in glands

When [^{14}C]omeprazole was suspended in the glandular medium (pH 7.4) at 1 μM, omeprazole was found to have a half-life of 1050 min. Likewise, when the glands were homogenized prior to incubation, omeprazole was found to have a similar half-life, Table I. Thus, during the 60-min time-course studied, less than 4% of the initial amount of omeprazole was degraded.

The medium and glandular concentrations of omeprazole were followed under different degrees of glandular stimulation of acid formation. Firstly, the glands were prestimulated to maximal acid formation, then [^{14}C]omeprazole was added, time = 0, Fig. 2, left panel, and Table I. Omeprazole almost instantaneously accumulated in the glands with a gland-to-medium concentration ratio of 28. The concentration of omeprazole was found to rapidly decrease both in the medium and in the glands. However, the gland-to-medium concentration ratio of omeprazole was constant, i.e., equilibrium between glands and medium was obtained. The degradation of omeprazole was accompanied by the appearance of its reduced form, H 168/22, both in the medium and the glands. The gland-to-

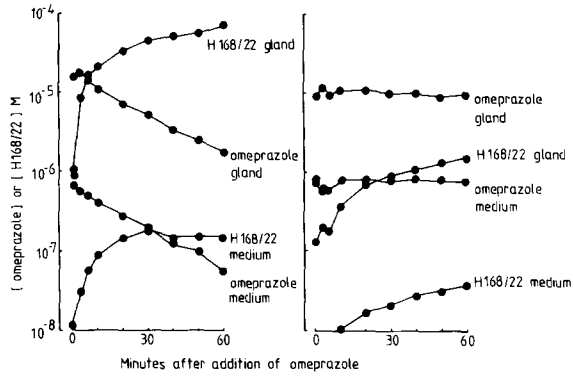


Fig. 2. Transformation of omeprazole in glands. Left panel, glands were stimulated with 1 mM dibutyryl-cAMP before the addition of [^{14}C]omeprazole to a final concentration of 1 μM, time = 0. The concentrations of omeprazole and H 168/22 were followed both in the glandular medium and the glands. Right panel, glands were treated with 20 mM NaSCN before the addition of [^{14}C]omeprazole.

TABLE I

TRANSFORMATION OF OMEPRAZOLE IN GLANDS

[^{14}C]Omeprazole was added to the glands (5 mg dry weight/ml). Samples of the incubation mixtures were subsequently withdrawn at time intervals up to 60 min for analysis of [^{14}C]omeprazole, its sulfide analogue (H 168/22) and total radioactivity. The glands were laid on an 8% Ficoll cushion and spun down in order to separate the glands from the medium. The gland pellets were resuspended by homogenisation, at pH 8.0, before analysis.

Conditions	Decomposition half-life (min)	Gland-to-medium concentration ratios	
		Omeprazole	H 168/22
1. No glands present. 1 μM [^{14}C]-omeprazole was incubated in the medium at pH 7.4	1050	–	–
2. Homogenised glands. Glands were homogenised in 0.2 M Tris-HCl buffer pH 8.0 45 min before addition of 1 μM [^{14}C]omeprazole	1050	–	
3. Stimulated. 1 mM dibutyryl-cAMP was added 45 min before 1 μM [^{14}C]omeprazole	3	28	322
4. Inhibited by omeprazole. 10 μM omeprazole was added 45 min before 1 μM [^{14}C]omeprazole	37	15	74
5. Inhibited by SCN^-. 20 mM NaSCN was added 45 min before 1 μM [^{14}C]omeprazole	73	13	39

medium concentration ratio of H 168/22 was about 10-times higher than for omeprazole. Summation of the total amount of omeprazole and H 168/22 accounted for more than 90% of the total radioactivity at any point in time, indicating that H 168/22 was almost quantitatively formed.

In a second experiment, the glands were inhibited with 20 mM SCN^- and, subsequently, omeprazole was added, Fig. 2, right panel. In contrast to stimulated conditions, omeprazole was now found to be more stable and the gland-to-medium concentration ratios both for omeprazole and H 168/22 were reduced (Fig. 2, right panel, and Table I). Similar results were obtained when the glands were preinhibited by 10 μM omeprazole (Table I).

Thus, during the 60-min time-course, omeprazole was found to be reduced to its sulfide derivative at a rate that varied with the degree of glandular stimulation.

Inhibition of aminopyrine-accumulation

Under conditions identical to those shown in Fig. 2, left panel, the time-course for inhibition of aminopyrine accumulation ratio by omeprazole was studied. After omeprazole addition to prestimulated glands, the aminopyrine ratio reached a new, lower level of about 20% of the control level within 30 min (Fig. 3). Thus, the inhibition of the aminopyrine accumulation occurred simultaneously with the decomposition of omeprazole.

Omeprazole was found to be a potent inhibitor of aminopyrine accumulation in the glandular preparation with an IC_{50} value of 0.63 ± 0.03 μM ($n = 6$). This was in contrast to its reduced form (H 168/22), which was found to be virtually inactive since the IC_{50} value was higher than 0.1 mM.

Uptake of H 168/22

Since one explanation for the low potency of H 168/22 might be a limited penetration into the

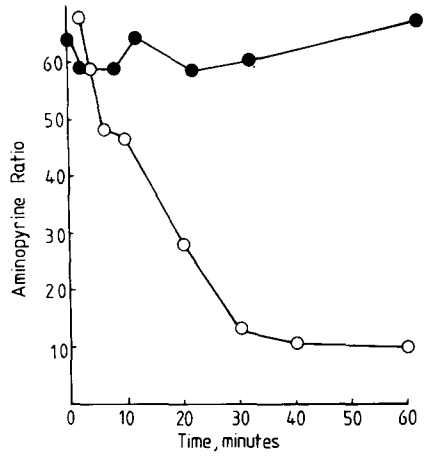


Fig. 3. Time-course for the inhibitory effect of omeprazole on [^{14}C]aminopyrine accumulation. The glands (5 mg dry weight/ml) were preincubated for 45 min in respiratory medium complemented with 1 μM aminopyrine and 1 mM dibutyryl-cAMP. After this time, indicated as time = 0 at the time axis, omeprazole was added to a final concentration of 1 μM (○——○). Control glands contained no omeprazole (●——●).

gastric gland, the uptake of H 168/22 into the glandular preparation was studied. Fig. 4 shows that H 168/22 was accumulated in the gland. Furthermore, the uptake was dependent on the degree of stimulation of the gland. Thus, under

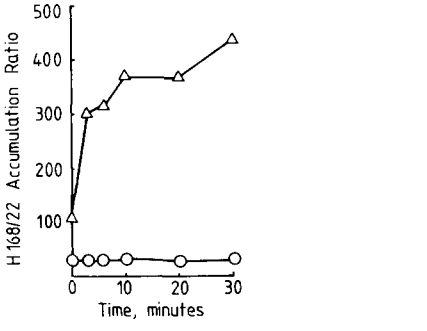


Fig. 4. Uptake of [^{14}C]H168/22 into the gastric glands. The glands (4 mg dry weight/ml) were incubated in the respiratory medium complemented with either 1 mM dibutyryl-cAMP (△——△) or 20 mM NaSCN (○——○). In addition, both conditions contained 10 μM [^{14}C]H168/22 which was added after 45 min of preincubation. Samples of the incubation mixtures were withdrawn at the indicated times. The glands were processed and the accumulation ratios determined as described in the Methods section.

stimulated conditions, high accumulation ratios were found, whereas, under SCN^--inhibited conditions, low accumulation ratios occurred (Fig. 4).

Effects of a weak base on the inhibitory activity of omeprazole in the isolated mucosal preparation

In the above experiments, it was shown that omeprazole was transformed in the glandular preparation at a rate dependent on the degree of stimulation/inhibition of acid formation. In order to test the possibility of acid-induced transformation of omeprazole being a prerequisite for its inhibitory activity, the isolated mucosal preparation was used as a test model. The advantage of this preparation is that acid secretory rates can be monitored in the presence of permeable buffers, such as 4-aminoantipyrine, and with the luminal solution kept at pH 7.4. Under these conditions, the acid compartments of the parietal cell will be expected to have a virtually neutral pH, since 4-aminoantipyrine will enter the acidic areas and act as a buffer [24]. Inclusion of 4-aminoantipyrine alone into the serosal medium had no significant effect on the acid secretory rates; 5.9 ± 0.87 and 6.4 ± 1.1 μequiv. $H^+ \cdot cm^{-2} \cdot h^{-1}$ ($n = 4$) for the control and 4-aminoantipyrine experiments, respectively. When omeprazole, at 5 μM, was added to the serosal side, a strong inhibition of the control secretory rate was obtained 1.3 ± 0.5 μequiv. $H^+ \cdot cm^{-2} \cdot h^{-1}$ ($n = 5$). However, inclusion of 4-aminoantipyrine before omeprazole in the serosal medium largely prevented the inhibition observed by omeprazole, the secretory rate being 5.1 ± 0.6 μequiv. $H^+ \cdot cm^{-2} \cdot h^{-1}$ ($n = 4$).

Effects of acid treatment of omeprazole on inhibition of the ($H^+ + K^+$)-ATPase activity

When omeprazole was degraded at pH 5.2, an apparent rate constant for the disappearance of omeprazole of 0.017 min^{-1} was obtained (Fig. 5A). The decomposition rate constant was not influenced when the concentration of omeprazole was increased 10-fold, indicating first-order kinetics for the degradation of omeprazole (data not shown). After various time intervals, aliquots of the breakdown mixture were tested for inhibition of ($H^+ + K^+$)-ATPase activity.

No inhibition was found without acid treatment ($t = 0$ in Fig. 5B). Treatment of omeprazole at pH

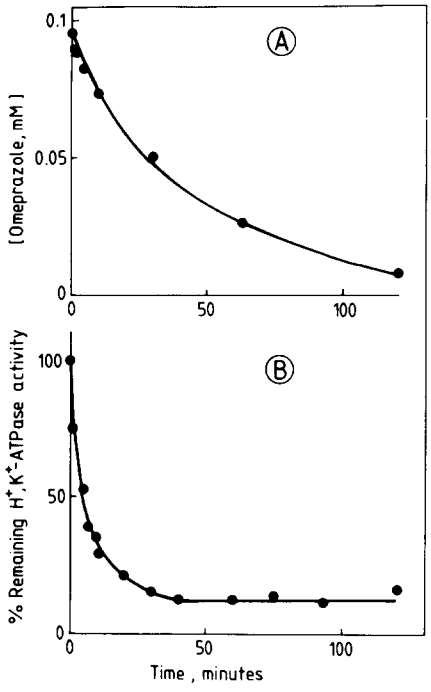


Fig. 5. A, decomposition of omeprazole at pH 5.2. [^{14}C]Omeprazole was suspended in 2 mM acetate/Tris buffer (pH 5.2), at 0.2 mM. At the time intervals indicated, samples were withdrawn and adjusted to pH 8.0 which stopped further degradation of omeprazole. These samples were used for analysis of the omeprazole concentrations. B, effect of acid-decomposed omeprazole on inhibition of the ($H^+ + K^+$)-ATPase activity. Samples were taken from the reaction mixture described in A. These samples, at 10 μM, were subsequently incubated in a solution containing ($H^+ + K^+$)-ATPase (10 μg protein) and 2 mM Pipes/Tris (pH 8.0) for 30 min. After this time, the enzyme activity was determined ($n = 3$). The control enzyme activity was 82 μmol P_i liberated/mg protein per h.

5.2 induced a rapid onset of the inhibitory activity of the ($H^+ + K^+$)-ATPase, being maximal after 40 min of exposure in the acid solution (Fig. 5B). Thus, in order to generate inhibition of the ($H^+ + K^+$)-ATPase, exposure of omeprazole to acid was required.

Potency relationship between omeprazole and H 168/22 for inhibition of isolated ($H^+ + K^+$)-ATPase

According to previous publications, omeprazole has been found to have an IC_{50} value of 2 μM at pH 6.1 [16]. Under identical conditions, H 168/22 did not have an inhibitory action in concentrations up to 0.1 mM.

Effects of mercapto compounds on inhibition induced by omeprazole in glands and ($H^+ + K^+$)-ATPase

In order to investigate the involvement of SH-groups in the inhibitory mechanism of omeprazole, the preparations were incubated in the presence of β-mercaptoethanol in addition to omeprazole.

Omeprazole, at 10 μM, (Fig. 6) in the absence of β-mercaptoethanol, induced near-maximal inhibition of aminopyrine accumulation in glands. This inhibition was counteracted by increasing concentrations of β-mercaptoethanol and, at a concentration of 10 mM of the mercaptane, no inhibition was present (Fig. 6). Inclusion of β-mercaptoethanol at up to about 10 mM in the incubation medium had on its own no effect on the aminopyrine uptake. However, higher concentrations reduced the aminopyrine accumulation.

In addition to β-mercaptoethanol, dithiothreitol and glutathione were also found to afford protection against inhibition by omeprazole (data not shown). The protective effects of the mercapto compounds appeared to be specific to inhibition induced by omeprazole, since inhibition of di-

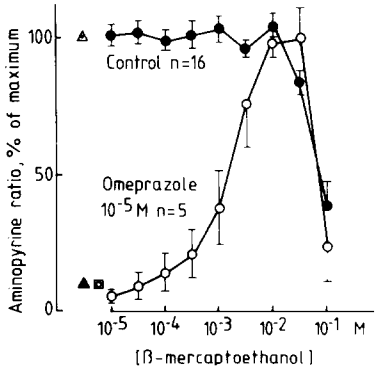


Fig. 6. Effect of β-mercaptoethanol on inhibition of aminopyrine uptake induced by omeprazole in glands. The glands (4 mg dry weight/ml) were incubated for 5 min with β-mercaptoethanol and 1 mM dibutyryl-cAMP. Subsequently, omeprazole was added and incubated for another 55 min (○ ——— ○). Control (● ——— ●) contained β-mercaptoethanol and 1 mM dibutyryl-cAMP but no inhibitor. △ denotes the response to 1 mM dibutyryl-cAMP alone, which was set to 100% and corresponded to an aminopyrine ratio of 178 ± 32 ($n = 16$); ▲ indicates basal aminopyrine uptake; □ denotes the response to 1 mM dibutyryl-cAMP and omeprazole.

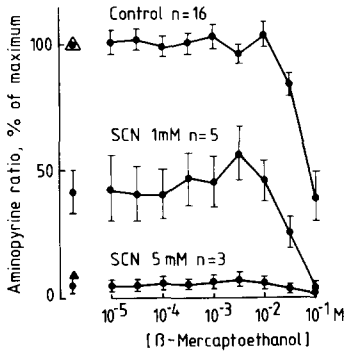


Fig. 7. Effect of β-mercaptoethanol on inhibition of aminopyrine uptake induced by SCN^- in glands. The same protocol was used as in Fig. 6. Control contained β-mercaptoethanol and 1 mM dibutyryl-cAMP but no inhibitor. The SCN^- samples also contained 1 mM dibutyryl-cAMP and β-mercaptoethanol. △ denotes the response to 1 mM dibutyryl-cAMP alone, which was set to 100%; ▲ indicates basal aminopyrine uptake.

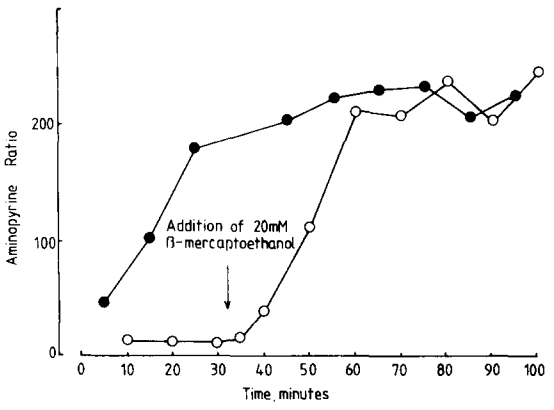


Fig. 8. Reversal of inhibition induced by omeprazole, by β-mercaptoethanol. Gastric glands were incubated with 10 μM omeprazole for 32 min after which time 20 mM β-mercaptoethanol (○——○) was added. The control curve (●——●) contained 20 mM β-mercaptoethanol added before 10 μM omeprazole. Both incubations contained 1 mM dibutyryl-cAMP.

butyryl-cAMP-stimulated aminopyrine uptake by SCN^-, which inhibits gastric acid secretion by a mechanism different from that of omeprazole [16] either at 1 mM or at 5 mM was not counteracted by β-mercaptoethanol (Fig. 7).

In order to study whether mercapto compounds could reverse inhibition already established by omeprazole, the glands were incubated with omeprazole before the addition of β-mercaptoethanol, Fig. 8. Addition of β-mercaptoethanol (20 mM final concentration) induced a reversal of the aminopyrine response. The time-course for the reversal of omeprazole inhibition by β-mercaptoethanol was similar to the control response. Moreover, the same maximal aminopyrine ratios were reached.

When purified gastric $(H^+ + K^+)$-ATPase was incubated in the presence of β-mercaptoethanol alone, no change in the ATPase activity was found (Fig. 9). However, when the concentrations of β-mercaptoethanol were increased in the presence of omeprazole at 10 μM, which by itself inhibits the enzyme activity to about 90%, the enzyme activity was protected. Half maximal protection was obtained at β-mercaptoethanol concentrations of between 1 and 10 μM. Similar protective properties were found for dithiothreitol (data not shown).

In order to investigate whether β-mercaptoethanol could reverse the inhibitory effect of omeprazole on the $(H^+ + K^+)$-ATPase, the en-

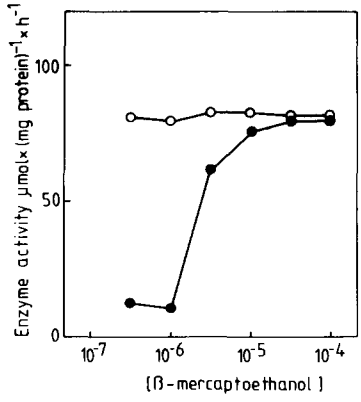


Fig. 9. Effect of β-mercaptoethanol on the inhibitory action of omeprazole on the $(H^+ + K^+)$-ATPase-activity. $(H^+ + K^+)$-ATPase (10 μg protein/ml) was incubated with increasing concentrations of β-mercaptoethanol in 2 mM Pipes/Tris buffer (pH 6.1). In the control experiment (○——○), no omeprazole was present, whereas in the compound experiment (●——●), the inhibitory reaction was started by the addition of 10 μM omeprazole and run for 30 min. After this time, the pH was changed to 7.4, in order to prevent further breakdown of omeprazole and the ATPase activity was determined. The results are means of two determinations. The control ATPase activity was 81 μmol P_i liberated/mg protein per h.

TABLE II

REACTIVATION OF INHIBITED ($H^+ + K^+$)-ATPase BY β-MERCAPTOETHANOL

($H^+ + K^+$)-ATPase (50 μg/ml) was incubated under the conditions listed below. After 20 min incubation, a pH 7.4 buffer, with or without 0.14 M β-mercaptoethanol, was added for 20 min. Thereafter, the preparation was spun down (200000 × g, 2 h) and washed with 2 mM Pipes/Tris (pH 7.4). The remaining enzyme activity and the protein content were determined.

Conditions	Reactivation conditions	Enzyme activity	
		μmol P_i/ mg per h	%
20 min, pH 6.1 Enzyme + 10 μM omeprazole	20 min, pH 7.4	17.2 ± 2.7	23.3
20 min, pH 6.1 Enzyme + 10 μM omeprazole	20 min, pH 7.4 + 0.14 M β-mercaptoethanol	45.3 ± 5.2	61.4
20 min, pH 6.1 Enzyme	20 min, pH 7.4 0.14 M β-mercaptoethanol	73.8 ± 12.6	100

zyme was inhibited for 20 min, which reduced the enzyme activity by about 23% of the control activity (Table II). Subsequently, a pH 7.4 buffer, with or without 0.14 M β-mercaptoethanol, was added and incubated with the inhibited enzyme for an additional 20 min. In both cases, the inhibitory reaction was effectively stopped. However, only after addition of the medium containing β-mercaptoethanol was the enzyme activity partially restored (Table II).

Thus, despite the fact that initial experiments showed that β-mercaptoethanol did not interact with either omeprazole or H 168/22 under the experimental conditions (data not shown), β-mercaptoethanol was able both to prevent the inhibition, when added prior to omeprazole, and to reverse the inhibition, when added subsequently to omeprazole.

Discussion

A model for the events leading to inhibition of acid secretion by omeprazole is shown in Fig. 10. Omeprazole enters the parietal cell acid compartments in its neutral form. Omeprazole has a pK_a value of 4 and, since the parietal cell contains acidic compartments with a pH below 4, omeprazole will be protonated and accumulated. Protonation also induces its decomposition. This hypothesis is based on the fact that the accumulation ratio of omeprazole was found to increase as a function of glandular stimulation. Furthermore, negligible decomposition of omeprazole occurred in the gland medium (pH 7.4) or in gland preparations homogenised in physiological buffers prior to addition of the inhibitor, while the compound was labile in glands stimulated to acid formation or in acid buffer solutions.

The time-courses for inhibition of the aminopyrine ratio and decomposition of omeprazole were found to be parallel. This leads to an inverted relationship between the omeprazole concentration and its pharmacological effect and this suggests that omeprazole itself is not the active inhibitor but rather a compound formed from

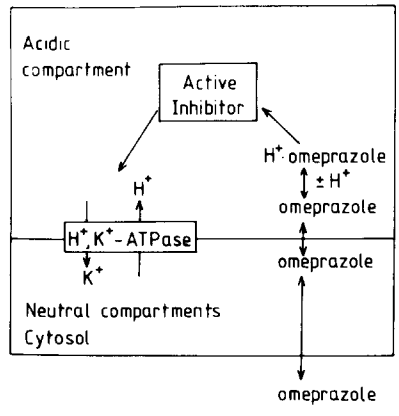


Fig. 10. Model for the events leading to inhibition of gastric acid secretion by omeprazole within the parietal cell.

omeprazole. In this way, acid-induced transformation of omeprazole would be obligatory for the events leading to inhibition of acid secretion (Fig. 10). This hypothesis is favoured by the finding from the isolated mucosal preparation, where elevation of the intramucosal pH by 4-aminoantipyrine substantially reduced the inhibitory activity of omeprazole and by the observation that treatment of omeprazole, under neutral or slightly alkaline conditions, prevented inhibition of the $(H^+ + K^+)$-ATPase. However, exposure of omeprazole to acid or unbuffered mucosal preparations induced its inhibitory activity.

In a previous publication, it has been shown that incubation of omeprazole and the $(H^+ + K^+)$-ATPase, at pH values below neutral, accelerated the inhibition of the enzyme [16]. However, back titration to pH 7.4 did not restore the enzyme activity. In the same publication, it was also shown that addition of antipyrine to the isolated mucosal preparation, after inhibition by omeprazole had been established, did not lead to a reversal of the inhibitory effect [16]. These results show that a low pH value is necessary to induce the inhibition by omeprazole. However, once inhibition is established, it is not reversed by elevation of the pH.

In the glandular preparation, one product, H 168/22, was predominantly formed. The fact that H 168/22 was virtually inactive in both the glandular and purified $(H^+ + K^+)$-ATPase preparations, at a concentration as high as 0.1 mM, supports the argument that H 168/22 is not the true inhibitor. This indicates that a product on the reaction pathway from omeprazole to H 168/22 acts as the inhibitor.

This conclusion was further supported by the results from experiments with β-mercaptoethanol. Despite the fact that β-mercaptoethanol did not significantly interact with omeprazole or H 168/22, it was able to abolish the inhibitory action of omeprazole in both the glandular- and $(H^+ + K^+)$-ATPase preparations. This would suggest that β-mercaptoethanol might either react with, and deactivate, an inhibitory compound formed from omeprazole, or that the inhibitory bond between the active compound and the $(H^+ + K^+)$-ATPase is sensitive to cleavage by β-mercaptoethanol. Moreover, since β-mercaptoethanol prevented inhibition in both the glandular- and ATPase preparations, the inhibitory mechanism most likely proceeds through the same reaction pathway in the two preparations.

Thus, the active inhibitor has two fates. Firstly, it can react with the $(H^+ + K^+)$-ATPase, which is the inhibitory reaction; secondly, it can be transformed, by a yet unknown pathway, to the non-inhibitory H 168/22. In this mechanism, two events are of prime importance in connection with the action of omeprazole: its accumulation in the acid compartment and the subsequent transformation to the inhibitor.

Accordingly, the fact that the parietal cell contains one of the most acidic compartments known to biology and that the target for the active compound, the $(H^+ + K^+)$-ATPase, is situated in the membranes separating the cell cytosol from the acid luminal space are two important factors for the specific inhibition of gastric acid secretion by omeprazole.

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