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INTERACTION OF THE ANTI-INFLAMMATORY SELENO-ORGANIC COMPOUND EBSELEN WITH ACID SECRETION IN ISOLATED PARIETAL CELLS AND GASTRIC H^+/K^+-ATPase

WINFRIED BEIL, UTE STAAR and KARL-FR. SEWING
Abteilung Allgemeine Pharmakologie, Medizinische Hochschule, Hannover, D-3000 Hannover 61, Federal Republic of Germany

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Abstract—The effects of the anti-inflammatory seleno-organic compound ebselen on gastric H^+/K^+-ATPase, H^+/K^+-ATPase-mediated proton transport and on parietal cell HCl production was studied. Ebselen inhibited K^+-stimulated ATPase activity in leaky gastric membranes (IC_{50}: 0.15 μM) and H^+/K^+-ATPase-mediated proton transport in intact gastric membrane vesicles (IC_{50}: 0.7 μM). Histamine- and dibutyryl-cAMP-stimulated HCl production in isolated and enriched guinea-pig parietal cells was inhibited with an IC_{50} value of 12 μM. The mercaptan dithioerythritol and the nucleotide ATP prevents the H^+/K^+-ATPase against inactivation and dithioerythritol was found to restore already inhibited enzyme activity and ATPase mediated H^+ transport. Furthermore, dithioerythritol could prevent ebselen-induced inhibition of HCl production in the parietal cell preparation. It is concluded that ebselen inhibits acid secretion in the parietal cell by interference with SH groups of the gastric proton pump, the H^+/K^+-ATPase. Therefore ebselen can be regarded as an anti-inflammatory drug for which *in vitro* anti-secretory properties can be demonstrated.

The final step of gastric acid secretion is mediated by the H^+/K^+-ATPase localized in the secretory membrane of the parietal cell [1]. Sulfhydryl reagents, such as *p*-chloromercuribenzenesulfonate [1], *N*-ethylmaleimide [2] and 5′,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) [3], inhibit the H^+/K^+-ATPase, demonstrating that SH residues are crucial for enzyme activity as found for other ion-translocating ATPases, i.e. Na^+/K^+-ATPase [4]. It is well known that some selenium compounds oxidize or bind to SH groups [5]. Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a selenium compound with low toxicity which exhibits anti-inflammatory activity in various animal models [6]. *In vitro*, the compound catalysed a glutathione peroxidase-like reaction, i.e. the reduction of H_2O_2 and organic hydroperoxides by GSH [7]. Ebselen has been shown to protect the gastric mucosa against injury by necrotizing agents (0.6 N HCl and acidified ethanol) and it was postulated that the anti-oxidative properties of the drug may be, at least in part, responsible for this gastroprotective effect [8].

The present study was undertaken to investigate the anti-secretory activity of ebselen. We have studied the effect of ebselen on histamine and dibutyryl-cAMP-induced acid secretion in guinea-pig parietal cells and the interaction of the drug with pig gastric H^+/K^+-ATPase.

MATERIALS AND METHODS

Preparation of gastric membranes enriched in H^+/K^+-ATPase

Gastric H^+/K^+-ATPase was purified from pig stomachs as described previously [9]. Briefly, scraped fundic mucosa was minced in 20 mM Tris–HCl buffer (pH 7.0) containing 250 mM sucrose and 1 mM EDTA with a tissue chopper. The minced material was filtered through a nylon mesh and was then homogenized with a teflon–glass homogenizer. The 20,000 *g* supernatant of the homogenate was centrifuged for 60 min at 100,000 *g*. The resulting pellet was resuspended in sucrose buffer and used for the proton transport studies. The ATPase activity in this microsomal fraction was 16.4 μmol P_i/mg protein/hr in the presence of $MgCl_2$ alone, 34.8, 51.3 and 74.2 μmol P_i/mg protein/hr in the presence of KCl (50 mM) and KCl plus the K^+-ionophores valinomycin and gramicidin (25 μg/mL), respectively (values: means from three different enzyme preparations).

To render the membranes permeable for cations the 100,000 *g* pellet was resuspended in 20 mM Tris–HCl buffer (pH 7.3) containing 2 mM $MgCl_2$ and 0.1 mM EGTA and was then lyophilized. ATPase activity in the lyophilized material was 16.6 μmol P_i/mg protein/hr in the presence of $MgCl_2$ alone and 63.3 μmol P_i/mg protein/hr in the presence of $MgCl_2$ and 10 mM KCl (values: means from three different enzyme preparations). K^+-stimulated ATPase activity in the lyophilized material was not further enhanced by the K^+-ionophores valinomycin and gramicidin.

Assay of ATPase activities

Hydrolysis of ATP was measured by determination of the release of inorganic phosphate (P_i) from ATP according to the method described by Carter and Karl [10].

Inhibition studies on the H^+/K^+-ATPase. Leaky (lyophilized) gastric membranes were used for studying the effect of ebselen on Mg^{2+}-dependent

and K^+-stimulated ATPase activities of the H^+/K^+-ATPase. For concentration response studies the enzyme (15 μg protein) was preincubated with four concentrations (0.01–1 μM) of ebselen in 1 mL samples containing 20 mM Tris–HCl buffer (pH 7.3), 250 mM sucrose, 2 mM $MgCl_2$, 0.1 mM EGTA with or without 10 mM KCl. Ebselen was dissolved in dimethylsulfoxide (DMSO) such that after addition of the solution to the medium the final DMSO concentration was 1%, which on its own had no effect on enzyme activity. After preincubation for 20 min at 22° the ATPase was started by addition of ATP (final concentration: 2 mM). After 15 min incubation at 37° the reaction was stopped by addition of 1 mL 1 N HCl.

Time course for inhibition of K^+-stimulated ATPase activity at different pH conditions. The enzyme (150 μg protein) was incubated with or without 0.5 μM ebselen in 10 mL medium consisting of 2 mM $MgCl_2$, 0.1 mM EGTA, 250 mM sucrose and 10 mM PIPES/Tris buffer (pH 6.0) or 10 mM Tris–HCl (pH 8.0). After indicated times, 0.5 mL aliquots were taken and mixed with 0.5 mL medium containing 2 mM $MgCl_2$, 4 mM ATP and 100 mM Tris–HCl buffer (pH 7.3), with or without 20 mM KCl. The remaining enzyme activity was then determined for 5 min at 37°.

Protection experiments with dithioerythritol (DTE) and ATP. The enzyme (900 μg protein) was treated with 1 μM ebselen at 0–4° in 30 mL 20 mM Tris–HCl buffer (pH 7.3) containing 2 mM $MgCl_2$, 0.1 mM EGTA and 250 mM sucrose in the absence and presence of 0.1 mM DTE or 2 mM ATP. DTE or ATP was added to the samples prior to ebselen. After 30 min the samples were diluted three-fold with ice-cold 250 mM sucrose, 20 mM Tris–HCl (pH 7.3), 2 mM $MgCl_2$ and 0.1 mM EGTA, and the membranes were harvested by centrifugation at 100,000 *g* for 60 min. The pellets were resuspended in 1 mL ebselen-free buffer medium and the remaining ATPase activity in the presence of $MgCl_2$ and $MgCl_2$ plus KCl (10 mM) was determined. Control membranes were run in parallel under identical conditions without ebselen.

Inhibition studies on dog kidney Na^+/K^+-ATPase. Na^+/K^+-ATPase (15 μg protein) was incubated at 22° with four concentrations (0.01–1 μM) of ebselen in 1 mL samples containing 20 mM Tris–HCl buffer (pH 7.4) and 2 mM $MgCl_2$ in the absence and presence of 20 mM KCl and 100 mM NaCl. After 20 min ATPase was started by addition of Tris–ATP (final concentration: 2 mM) and the reaction was stopped after 15 min incubation at 37° by addition of 1 mL 1 N HCl. In the absence of K^+ and Na^+ control reaction rate was 11.0 μmol P_i/mg protein/hr. In the presence of Na^+ and K^+ reaction rate was 55 μmol P_i/mg protein/hr (values: means from three determinations).

Proton transport studies

Uptake of H^+ in intact gastric membrane vesicles was monitored by fluorescence quenching of the pH-sensitive dye Acridine Orange according to the method described by Lee and Forte [11]. Fluorescence was measured with an SPF-500 Ratio Spectrofluorometer. The excitation and emission wavelengths were 493 and 530 nm, respectively.

Vesicles (120 μg protein) were mixed in 2 mL medium containing 10 mM PIPES/Tris buffer (pH 7.0), 2 mM $MgCl_2$, 150 mM KCl and 5 μM Acridine Orange. After addition of ebselen, Na_2-ATP was added to the medium and proton uptake was initiated with the K^+-ionophore valinomycin (50 μg). When the protective effect of mercaptans or ATP was studied these compounds were added to the medium before ebselen.

Parietal cell isolation and enrichment

Guinea-pig parietal cells were isolated by collagenase and pronase digestion and enriched to about 80% purity by the elutriation technique as described previously [12].

Measurement of acid production in isolated and enriched parietal cells

Uptake of the weak base aminopyrine was used as index for acid formation within the parietal cell, as described previously [12]. 10^6 parietal cells were preincubated in 1 mL buffer medium (pH 7.4) containing 8.3 μM [^{14}C]aminopyrine and the ebselen concentration to be tested (0.1–30 μM). The buffer composition was (mM): NaCl 70, $NaHCO_3$ 20, NaH_2PO_4 0.5, Na_2HPO_4 1.0, HEPES 50, $CaCl_2$ 1.0, $MgCl_2$ 1.5 and glucose 11. After 20 min histamine or dibutyryl-cAMP (final concentration: 1 mM) were added and after 30 min incubation at 37° the process was stopped by separating the cells from the medium by centrifugation through silicone oil and the radioactivity of intracellular trapped aminopyrine was counted.

Protein determination

Protein was determined according to the Lowry method [13].

Drugs

The drugs used were: collagenase, Na_2-ATP, Tris–ATP, ionophores, Acridine Orange, Na^+/K^+-ATPase, histamine (Sigma, Munich); dibutyryl-cAMP (Boehringer, Mannheim); pronase E (Merck, Darmstadt); [^{14}C]aminopyrine (sp. act. 60–120 mCi/mmol) (New England Nuclear Corp., Dreieich); ebselen was kindly donated by Dr. Parnham, Rhone-Poulenc/Nattermann, Köln.

RESULTS

Interaction of ebselen with gastric H^+/K^+-ATPase

Ebselen inhibited H^+/K^+-ATPase activity in leaky pig gastric membranes. After treatment of the enzyme (15 μg protein/mL) for 20 min at pH 7.3 in the presence of 2 mM $MgCl_2$ and 10 mM KCl K^+-stimulated ATPase activity was inhibited with an IC_{50} value of 0.15 ± 0.02 μM (value: mean ± SE from three different enzyme preparations). The unstimulated, Mg^{2+}-dependent component of the H^+/K^+-ATPase was more resistant against inactivation. In the presence of 0.3 μM ebselen enzyme activity remained unaltered, whilst at 1 μM ebselen the enzyme activity was reduced to 60% of control activity. The protective effects of the mercaptan

Table 1. Protection of H^+/K^+-ATPase against inactivation by ebselen

Treatment	Enzyme activity (μmol P_i/mg protein/hr)	
	Mg^{2+}	Mg^{2+} + KCl (10 mM)
Control	15.4 ± 0.7	44.0 ± 1.0
Ebselen (1 μM)	9.3 ± 1.6	12.2 ± 0.6
ATP + ebselen	9.4 ± 1.5	34.5 ± 4.8
DTE + ebselen	13.6 ± 0.9	41.1 ± 9.5

Leaky gastric membranes were treated with 1 μM ebselen in the presence and absence of 2 mM ATP or 0.1 mM DTE at 0–4° in a medium containing 2 mM $MgCl_2$, 0.1 mM EGTA, 250 mM sucrose and 20 mM Tris–HCl (pH 7.3). After 30 min the membranes were diluted three-fold with ice-cold inhibitor-free buffer medium and harvested by centrifugation at 100,000 *g* for 60 min. The pellets were resuspended in 1 mL inhibitor-free buffer medium and the remaining enzyme activities were determined. Values are means ± SE from three enzyme preparations.

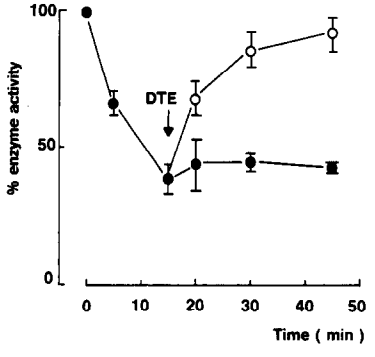

Fig. 1. Reactivation of ebselen-induced inhibition of K^+-stimulated ATPase by dithioerythritol (DTE). The enzyme (225 μg protein) was incubated with 0.5 μM ebselen in 15 mL medium containing 20 mM Tris–HCl buffer (pH 7.3), 250 mM sucrose, 2 mM $MgCl_2$ and 0.1 mM EGTA with or without 10 mM KCl. At the times indicated at the abscissa 1 mL aliquots were taken and the remaining enzyme activity was determined. DTE (final concentration: 2 mM) was added after 15 min. K^+-stimulated ATPase activity (enzyme activity measured in the presence of 2 mM $MgCl_2$ and 10 mM KCl minus the basal $MgCl_2$-dependent ATPase activity) in the absence of ebselen was set to 100%. Values are means ± SE from three different enzyme preparations.

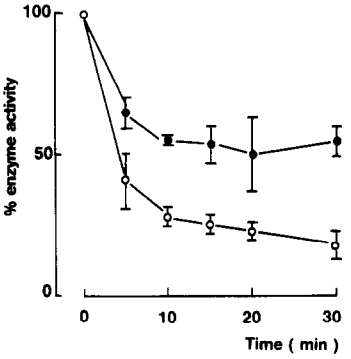

Fig. 2. Time course for inactivation of K^+-stimulated ATPase. The enzyme (150 μg protein) was incubated in 10 mL medium containing 2 mM $MgCl_2$, 0.1 mM EGTA, 250 mM sucrose and 10 mM PIPES/Tris (pH 6.0) (●) or 10 mM Tris–HCl (pH 8.0) (○) buffer and 0.5 μM ebselen. After the times indicated, 0.5 mL aliquots were taken and mixed with 0.5 mL inhibitor-free buffer medium containing 2 mM $MgCl_2$, 4 mM ATP and 100 mM Tris–HCl (pH 7.3) with or without 20 mM KCl. The remaining enzyme activity was then determined for 5 min at 37°. K^+-stimulated enzyme activity in the absence of ebselen was set to 100%. Values are means ± SE from three different enzyme preparations.

dithioerythritol and the substrate ATP on the inactivation of K^+-stimulated ATPase activity by ebselen is shown in Table 1. The presence of 0.1 mM DTE during treatment with 1 μM ebselen almost completely protects the enzyme from inactivation; in the presence of 2 mM ATP the enzyme activity could be protected to the extent of about 80%. Addition of DTE (final concentration: 2 mM) after 50% inactivation of the K^+-stimulated ATPase activity by 0.5 μM ebselen restored the enzyme activity (Fig. 1).

In the concentration response studies 1 μM ebselen completely abolished K^+-stimulated ATPase activity. This inhibition level persists after centrifugation of the membranes and subsequent dilution in inhibitor-free buffer medium (see Table 1). These findings demonstrate that inhibition of K^+-stimulated ATPase activity by ebselen was not reversible by washing.

The time-course for inhibition of K^+-stimulated ATPase activity was studied at slightly acidic (pH 6.0) and alkaline (pH 8.0) preincubation conditions. Inhibition was found to be more pronounced in more alkaline media and showed a rapid onset of action at both pH values tested (Fig. 2).

Interaction of ebselen with dog kidney Na^+/K^+-*ATPase*

The specificity of ebselen was examined by studying its effects on a related enzyme system, namely Na^+/K^+-ATPase. Ebselen inhibited Na^+/K^+-ATPase after treatment of the enzyme for 20 min

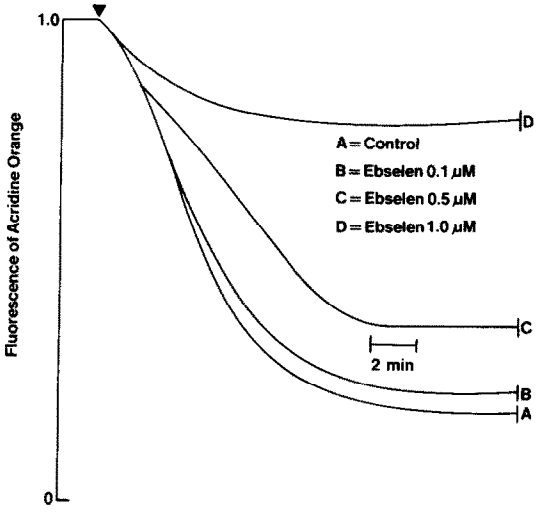

Fig. 3. Effect of increasing concentrations of ebselen on H^+/K^+-ATPase-mediated H^+ uptake in intact gastric membrane vesicles. The vesicle preparation (120 μg protein) was mixed in 2 mL medium containing 10 mM PIPES/Tris (pH 7.0), 150 mM KCl, 2 mM $MgCl_2$, 5 μM acridine orange and the ebselen concentration indicated. Subsequently, ATP (final concentration: 2 mM) was added and the pump reaction was started with 50 μg valinomycin (▼).

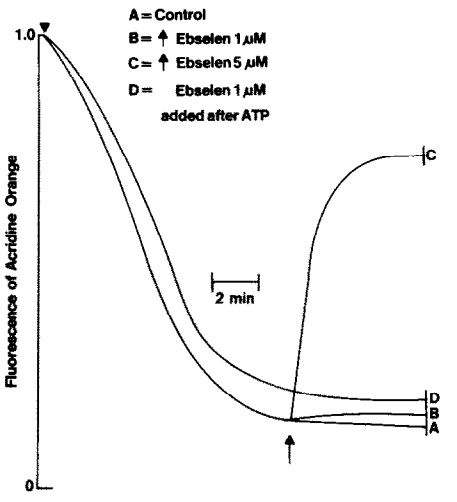

Fig. 4. Effect of ebselen on H^+/K^+-ATPase-mediated H^+ uptake when added after ATP to the medium or when added at the steady-state level of H^+ uptake to the vesicles. Experimental conditions were as described in Fig. 3 with the modification that ebselen was added after ATP to the vesicle preparation or was added at the steady-state level of ATP and valinomycin-induced H^+ uptake as indicated by arrows.

at pH 7.4 in the presence of 2 mM $MgCl_2$, 20 mM KCl and 100 mM NaCl, with an IC_{50} value of 0.46 ± 0.02 μM (values: mean ± SE from three determinations).

Interaction of ebselen with H^+/K^+-ATPase-mediated proton uptake in intact gastric membrane vesicles

When gastric membrane vesicles were incubated in a $MgCl_2$, KCl and ATP containing buffer medium and the K^+-ionophore valinomycin was added, which allows access of K^+ to the K^+ site of the H^+/K^+-ATPase, the fluorescence of the pH-sensitive dye Acridine Orange was progressively quenched during the first 6 min, demonstrating uptake of protons inside the vesicles. This pre-steady-state phase of H^+ uptake was followed by a steady-state phase with a stable extra/intravesicular pH gradient lasting at least 40 min.

When ebselen (0.1, 0.5 and 1.0 μM) was added to the vesicles before the H^+/K^+-ATPase reaction was started by subsequent addition of ATP and the K^+-ionophore valinomycin, the rate of proton uptake and the steady-state H^+ concentration was reduced in a concentration-dependent manner (Fig. 3). The IC_{50} value for inhibition of H^+ uptake was 0.7 μM. When ebselen (1 μM) was added after ATP to the vesicles or added at the steady-state level of ATP and valinomycin-induced H^+ uptake to the vesicles, this drug concentration had only a marginal inhibitory effect (Fig. 4). When 1 mM reduced glutathione (GSH) or DTE were present in the incubation mixture and afterwards ebselen (1 μM) was added, the inhibitory action of the drug was prevented (Fig. 5). DTE was more effective than GSH. DTE (2 and 10 mM) could restore the H^+ uptake rate when added after ebselen (Fig. 5) but GSH (2 and 10 mM) failed to restore the pump activity (data not shown).

Addition of ebselen up to 10 μM to vesicles in which the H^+/K^+-ATPase reaction was stopped by EDTA (final concentration: 10 mM) at the steady-state level of proton concentration did not alter the rate of dissipation of the H^+ gradient, demonstrating that the drug did not act as a protonophore (data not shown).

Interaction of ebselen with HCl production in isolated and enriched parietal cells

Acid secretion in isolated and enriched parietal cells, as measured by [^{14}C]aminopyrine uptake, was induced by 1 mM histamine or dibutyryl-cAMP. Ebselen inhibited aminopyrine accumulation in response to histamine and dibutyryl-cAMP in a concentration-dependent manner with IC_{50} values of 12.8 ± 1.3 and 11.8 ± 0.9 μM (values: means ± SE from three different cell preparations).

In order to study the involvement of SH groups in the inhibitory mechanism of ebselen in the cell preparation, parietal cells were incubated with ebselen in the presence of DTE. Ebselen, at a concentration of 20 μM, inhibited histamine-stimulated aminopyrine uptake to about 70%. DTE prevented the inhibitory action of ebselen (Fig. 6). In order to study whether mercaptans could reverse established inhibition in the cell preparation, the cells were incubated for 20 min with 20 μM ebselen before addition of DTE. In contrast to the findings with the purified enzyme preparation, DTE in a concentration up to 2 mM failed to restore histamine-stimulated aminopyrine uptake. Higher DTE concentrations could not be tested since they alone reduced the aminopyrine uptake.

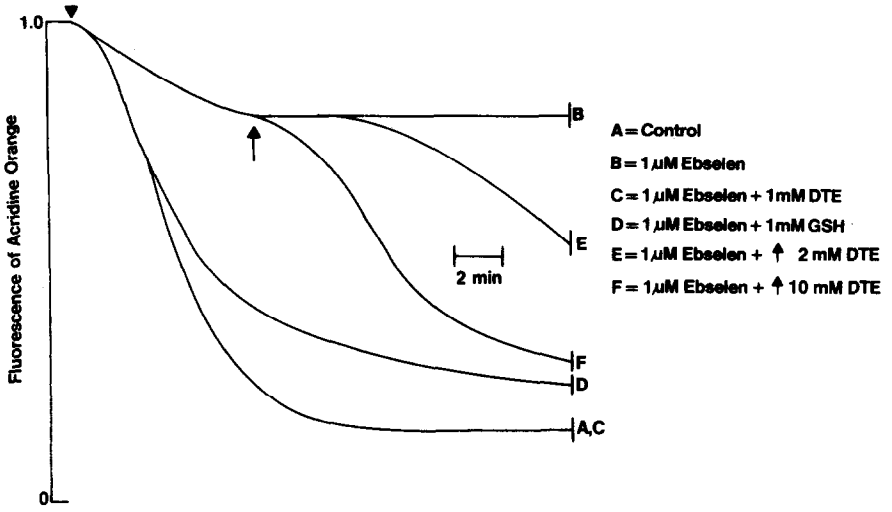

Fig. 5. Effect of the mercaptans dithioerythritol and glutathione in preventing and reversing the inhibitory action of ebselen on H^+/K^+-ATPase-mediated H^+ uptake in intact gastric membrane vesicles. Experimental conditions were as described in Fig. 3 with the modification that GSH or DTE (final concentration: 1 mM) were added to the incubation mixture before ebselen and afterwards the pump reaction was started with ATP and valinomycin. Reactivation of H^+ uptake was studied by addition of DTE (final concentrations: 2 and 10 mM) after pump inhibition was established.

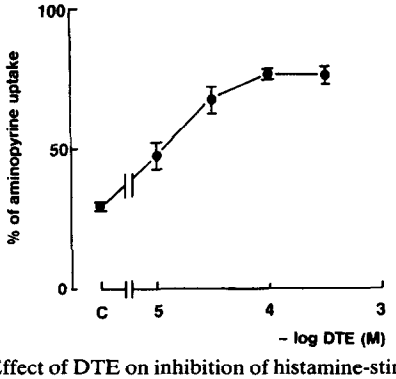

Fig. 6. Effect of DTE on inhibition of histamine-stimulated [^{14}C]aminopyrine uptake induced by ebselen in parietal cells. The cells were treated at 22° with 20 μM ebselen and the indicated concentrations of DTE. DTE was added before ebselen to the cell suspension. After 20 min, histamine (final concentration: 1 mM) was added and aminopyrine uptake was determined after 30 min incubation at 37°. The aminopyrine uptake in the absence of ebselen was set to 100%. DTE alone, up to a concentration of 0.3 mM, did not affect histamine-stimulated aminopyrine uptake. The values are means ± SE from three different cell preparations.

DISCUSSION

It has been established that gastric H^+/K^+-ATPase is the key enzyme for proton secretion by the parietal cell. The importance of thiol groups for H^+/K^+-ATPase function has been shown in several publications [1–3]. A clinically useful thiol reactive inhibitor of the H^+/K^+-ATPase is omeprazole [14, 15].

The experiments reported in this paper indicate that the seleno-organic compound ebselen inactivates the H^+/K^+-ATPase. Inhibition of enzyme activity and ATPase-mediated proton transport is protected by the mercaptan dithioerythritol; inhibited enzyme and proton transport were restored by subsequent treatment with dithioerythritol. From these findings we suggest that ebselen inactivates H^+/K^+-ATPase by an interaction with the sulfhydryl groups of the enzyme. The inhibition reaction was dependent on the pH in the preincubation medium. From the observation that inhibition increased at alkaline pH we suggest that the ionized thiolate anion (RS^-) is more reactive towards ebselen than the non-ionized SH group of the enzyme. However, the increased reactivity of ebselen against H^+/K^+-ATPase at alkaline pH could reflect a pH-dependent conformational change within the enzyme. Proton transport in intact membrane vesicles was protected by reduced glutathione and dithioerythritol. Dithioerythritol was more protective than glutathione and only the hydrophobic SH-reducing agent dithioerythritol was able to reactivate already inhibited H^+ uptake. The failure of reduced glutathione to reactivate proton uptake suggests that ebselen mainly reacts with thiol groups of the ATPase localized in the deeper regions of the membrane or the intravesicular face of the enzyme.

The presence of ATP during treatment of leaky (lyophilized) membranes with ebselen partially protects the enzyme from inactivation. Similar to the K^+-stimulated ATPase, H^+ uptake in intact membrane vesicles was partially protected when ebselen was added to the vesicles after ATP. These findings demonstrate that the thiol groups protected by ATP are involved in the transport function of the enzyme. A protective effect of ATP against enzyme inactivation induced by SH reagents has been described by other investigators [3, 16]. In their

detailed experiments Schrijen *et al.* [3] have shown that ATP and the SH reagent DTNB do not compete for the same binding site. The authors have postulated that the protective effect of ATP is due to a conformational change, which coincides with nucleotide binding and which leads to a decreased exposure of the inhibitor to the sulfhydryl groups of the enzyme.

There was a big difference, however, in the potencies of ebselen to inhibit the K^+-stimulated ATPase activity (IC_{50}: 0.15 μM) in leaky membranes, H^+ uptake in intact membrane vesicles (IC_{50}: 0.7 μM) and the ability of the drug to inhibit histamine and dibutyryl-cAMP-stimulated HCl production in the isolated perietal cell preparation (IC_{50}: 12 μM). The weaker potency in the cell preparation can be explained as follows: (1) the drug interacts with other enzymatic (non-H^+/K^+-ATPase) and non-enzymatic SH groups present in the cell compartments, which are not involved in the acid secretory process; (2) the cytosolic site of the H^+/K^+-ATPase in the cell is protected by endogenous ATP and higher drug concentrations are necessary to block the H^+/K^+-ATPase.

Since the mercaptan dithioerythritol prevented inhibition in both the cell and H^+/K^+-ATPase preparation we suggest that the inhibitory mechanism proceeds through the same reaction pathway in both systems. Furthermore, it seems likely that inhibition of HCl production in the cell preparation is due to inhibition of the H^+/K^+-ATPase, but we cannot exclude that inhibition of other SH enzymes (i.e. Na^+/K^+-ATPase) is responsible, at least in part, for the anti-secretory activity of the drug in the cell preparation.

Dithioerythritol failed to reverse the inhibitory action of ebselen in the cell preparation, whereas it restored K^+-stimulated ATPase and proton uptake in gastric membranes. This difference may be due to the different morphologies of both test systems and may be caused by the inability of DTE to reach the sulfhydryl groups involved in the inhibitory reaction mechanism in the cell system.

Ebselen was developed as an anti-inflammatory drug. *In vitro*, the drug catalyses the reduction of oxygen metabolites [7, 17]. Furthermore, ebselen inhibits the synthesis of the 5-lipoxygenase metabolite leukotriene B_4 [18], which is a potent inducer for superoxide production. Ebselen has only a weak inhibitory action on prostaglandin E_2 production [19].

In conclusion, the anti-inflammatory mechanism of action is different from that of classical non-steroidal anti-inflammatory drugs. Non-steroidal anti-inflammatory drugs induce as a most serious side effect mucosal damage and ulceration. This results from different mechanisms such as breaking the gastric mucosal barrier with resulting back diffusion of H^+ into the mucosa and inhibition of the biosynthesis of gastroprotective mucosal prostaglandins. In contrast, ebselen exhibits gastroprotective activity [8]. Gastro-protective agents can be divided into three classes: (i) anti-secretory and acid neutralizing drugs; (ii) drugs which protect the gastric mucosa against damaging agents via other mechanisms; (iii) drugs which possess both activities [20]. It seems likely that ebselen belongs to the third class of compounds, since the drug exerts anti-secretory activity *in vitro* (by blocking the H^+/K^+-ATPase) and has in addition a directly protective effect in the stomach, presumably via capturing oxygen free radicals [8]. Therefore this drug offers a promising approach for therapy of inflammatory diseases without ulcerogenic effects.

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