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Omeprazole and bafilomycin, two proton pump inhibitors: differentiation of their effects on gastric, kidney and bone H^+-translocating ATPases

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The effects of omeprazole and bafilomycin on processes dependent on two different types of H^+-translocating ATPases were compared. A H^+-ATPase of the E_1E_2-type, the H^+,K^+-ATPase, was purified from gastric mucosa. Vacuolar type H^+-ATPases were prepared both from kidney medulla and from osteoclast-containing medullary bone. H^+,K^+-ATPase-mediated proton transport in gastric vesicles was selectively inhibited by omeprazole with a high potency (inhibitory concentrations ≥ 3 μM) and in a time- and pH-dependent manner. This result is consistent with the mechanism of action of omeprazole, which is dependent on acid-induced transformation of the drug into an active inhibitor reacting with luminally accessible sulfhydryl groups of the enzyme. Accordingly, the presence of the membrane-impermeable mercaptane glutathione did not affect the inhibitory action of omeprazole on the H^+, K^+-ATPase. Proton transport in kidney- and bone-derived membrane vesicles was also inhibited by omeprazole, but with a lower potency (inhibitory concentrations ≥ 100 μM). Furthermore, the presence of glutathione totally abolished this inhibition, indicating that cytosolic, rather than luminal, SH-groups of the respective vacuolar H^+-ATPase were interacting with omeprazole at high concentrations. In line with these results, it was found that omeprazole was much more potent in inhibiting acid production in isolated gastric glands ($IC_{50} \approx 0.25$ μM) than in inhibiting osteoclast-mediated ^{45}Ca-release in isolated mouse calvaria ($IC_{50} \approx 200$ μM). Bafilomycin, on the other hand, was much more effective in inhibiting proton transport mediated by the vacuolar H^+-ATPases in the kidney- and bone-derived membrane vesicles ($IC_{50} \approx 2$ nM) than in inhibiting H^+,K^+-ATPase-mediated proton transport in gastric membrane vesicles ($IC_{50} \approx 50$ μM). Thus, approximately 10^4 times higher concentrations of bafilomycin were needed to inhibit the H^+, K^+-ATPase to the same extent as the vacuolar H^+-ATPase. A similar difference in potency of bafilomycin was found when its inhibitory effect was determined in isolated mouse calvaria ($IC_{50} \approx 2.5$ nM) and in isolated gastric glands ($IC_{50} \approx 5$ μM). Hence, omeprazole was found to be a specific inhibitor of the H^+,K^+-ATPase under physiological conditions, i.e. in the presence of glutathione, while bafilomycin was found to be selective towards vacuolar H^+-ATPases.

Introduction

Gastric acid secretion, urinary acidification and osteoclast-mediated bone resorption are all processes directly dependent on H^+-translocating ATPases [1–5]. From a functional point of view, these proton pumps can be put in the same category since they, polarized to the apical membrane of the respective cell, translocate protons into the extracellular space. However, the H^+-ATPases are heterogeneous and exhibit differences in both structure and mechanism. The gastric H^+,K^+-ATPase, found in the parietal cell and responsible for acidification of the stomach, is an ion-translocating ATPase of the E_1E_2-type [6]. This family of ion-pumps, which includes the Na^+,K^+-ATPase [7] and the Ca^{2+}-ATPase [8], is characterized by its sensitivity to vanadate and the formation of a phosphorylated enzyme-intermediate in the catalytic cycle [6]. In contrast to acidification of the stomach, renal collecting duct acidification is accomplished by a proton pump exhibiting the same characteristics as the proton pumps of intracellular vacuoles, the vacuolar type (v-type) H^+-ATPases [2]. Unlike the E_1E_2-type, the v-type H^+-ATPases do not form phosphoenzyme intermediates and are re-

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sistant to vanadate [9]. The v-type H^+-ATPases also differ from the F_1F_0-ATPases of mitochondria, chloroplasts and bacteria in that they are resistant to oligomycin and are more sensitive to *N*-ethylmaleimide (NEM) [9,10]. Structurally, the vacuolar H^+-ATPases are, like the F_1F_0-ATPases, multisubunit complexes, whereas the E_1E_2-type consists of a single catalytic subunit and, in some enzymes, one or two additional subunits [6–10]. The cell responsible for bone resorption, the osteoclast, secretes acid into the compartment formed between its apical membrane and the bone surface, the so-called resorption lacuna [11]. This acidification was earlier suggested to be accomplished by a H^+-ATPase similar to the gastric H^+,K^+-ATPase [12,13]. However, recent data have demonstrated strong evidence that a v-type H^+-ATPase is responsible for osteoclast acidification [3–5]. Thus, v-type H^+-ATPases are responsible for renal collecting duct and osteoclast acidification, whilst gastric acid secretion is accomplished by a proton pump of the E_1E_2-type. This is manifested in the pH gradients generated by the respective type of proton pump. The collecting duct of the kidney and the resorption lacuna have a pH of 4–6, whereas the acid secreted by the parietal cell has a pH of 1 or less [1,11,14]

Enzyme-specific inhibitors are important biochemical tools. They can not only be used to identify and classify an enzyme but also to give structural and functional information about the enzyme. In these respects, omeprazole and bafilomycin have proved to be useful compounds in the studies of the gastric H^+,K^+-ATPase and vacuolar H^+-pumps, respectively.

Omeprazole, a substituted benzimidazole sulfoxide, is an inhibitor of the H^+,K^+-ATPase and is currently in clinical use in acid-related diseases of the gastrointestinal tract. The inhibitory effects of omeprazole on the H^+,K^+-ATPase and its mechanism of action have been extensively investigated [15]. The parent compound is inactive, but is transformed in the acidic compartment of the parietal cells into a cationic sulfenamide which inhibits the enzyme from the extracellular side. Its inhibitory action on the H^+,K^+-ATPase has been shown to be due to reaction with luminally accessible sulfhydryl groups in the enzyme [16]. The specificity of omeprazole thus depends on the acid catalyzed transformation into the active inhibitor and its subsequent reaction with luminally accessible, critical SH-groups of the enzyme.

The bafilomycins, a group of macrolide antibiotics, have recently been found useful as proton pump inhibitors. Bowman et al. [17] demonstrated that bafilomycin A_1 is a potent inhibitor of many v-type ATPases and suggested its use in distinguishing v-type pumps from the E_1E_2-type, which was much less sensitive, and the F_1F_0-type, which was insensitive to this compound. The sensitivity of v-type H^+-ATPases to bafilomycin A_1 has later been verified for v-type H^+-ATPases prepared from a variety of sources [18–21]. In contrast to omeprazole, the mechanism of action of bafilomycin is not yet known.

In the present study, we have investigated the effects of omeprazole and bafilomycin on ATP-dependent H^+-transport in gastric-, kidney medulla- and medullary bone-derived membrane vesicles. The proton pumps present in these preparations have previously been characterized as the H^+,K^+-ATPase (gastric) and the vacuolar H^+-ATPase (kidney and bone) [2,4,22]. In addition, the effect of the inhibitors on gastric acid secretion and bone resorption on a cellular level was studied in isolated gastric glands and isolated mouse calvaria, respectively.

Materials and Methods

Materials. All chemicals were purchased from Sigma, U.S.A, except acridine orange, which was obtained from Merck (F.R.G) and parathyroid hormone, which was obtained from Bachem (U.S.A.) Bafilomycin A_1, a gift from Dr. K. Altendorf (University of Osnabruck, Germany), was dissolved in dimethylsulfoxide and stored at −20°C. Omeprazole was synthesized by AB Hässle, Mölndal, Sweden.

Preparation of membrane vesicles. Gastric membrane vesicles containing the H^+,K^+-ATPase were isolated from hog stomachs according to Saccomani et al. [22]. The vesicles were stored in 35% sucrose at −70°C. Kidney medulla membrane vesicles were prepared from bovine kidneys as previously described [9], with some modifications. DTT was omitted from the homogenization buffer, and the sucrose step gradient was centrifuged for 4 h instead of overnight. Membrane vesicles containing NEM-sensitive H^+-ATPase proton transport activity were accumulated at the 25–40% sucrose (w/w) interface. Medullary bone membrane vesicles were prepared from the long bone of the hen according to [4], except that DTT and K_2CO_3 were excluded from the homogenization buffer. Kidney- and bone-derived membrane vesicles were stored in homogenization buffer at −70°C.

Proton transport measurements. Proton transport in the membrane vesicles was monitored by the fluorescence quenching of the weak base Acridine orange (AO), as previously described [23]. Measurements were made with a Schimadzu RF-5000 fluorimeter with excitation at 492 nm and emission at 528 nm. Effects of omeprazole were studied at 37°C using a temperature-controlled cell holder (Shimadzu). In some experiments, the initial rate of acidification, taken to be the maximum initial decrease in AO fluorescence, was determined from the slope of a tangent to the fluorescence trace after addition of ATP. In order to confirm that quenching of AO fluorescence was due to intravesicular acidification, the pH gradient was dissipated by the

addition of the protonophore nigericin (10 μg/ml, final concn.).

ATPase activity measurements. ATPase activity in gastric membrane vesicles was measured as inorganic phosphate (P_i) release from ATP. P_i released was analysed according to Yoda and Hokin [24]. The ATPase activity presented is the ΔValinomycin-stimulated activity, i.e., activity in the presence of valinomycin + K^+ minus activity in the presence of K^+ alone. The Δvalinomycin activity represents the fraction of the ATPase activity that correlates with acid accumulation [16].

Preparation of gastric glands and measurement of acid secretion. Gastric glands were prepared from rabbits as previously described by Berglindh et al. [25]. Acid formation in the glands was monitored as the uptake of the weak base amino[^{14}C]pyrine according to [26]. Briefly, the glands (approx. 3 mg, dry weight) were incubated in the presence or absence of omeprazole or bafilomycin for 60 min at 37°C in a 1.5 ml medium containing 0.20 μC_i; amino[^{14}C]pyrine and (in mM): NaCl, 132.4; KCl, 5.4; Na_2HPO_4, 5.0; NaH_2PO_4, 1.0; $MgSO_4$, 1.2; $CaCl_2$, 1.0; indomethacin, 0.01; glucose, 11; albumin, 1.0 mg/ml; and histamine, 0.1. Omeprazole and bafilomycin were dissolved in methanol and dimethylsulfoxide (DMSO) stock solutions, respectively. Aliquots of these were pipetted into the media, giving final concentrations of methanol and DMSO of less than 1%. After incubation the glands and medium were separated by centrifugation and their amino[^{14}C]pyrine content determined by liquid scintillation counting (LKB rackbeta). The aminopyrine distribution ratio was calculated as previously described [26]. The result is expressed as histamine-stimulated AP ratio.

Preparation of mouse calvaria and measurement of bone resorption. The bone organ culture technique used, modified from [27], has recently been described in detail [28]. Briefly, new-born mice were injected subcutaneously with ^{45}Ca (approx. 2 μCi per animal). After 4 days their calvaria were dissected, split into halves and cultured in CMRL-1066 medium (GIBCO, U.K.) which had been supplemented with bovine serum albumin and antibiotics (penicillin-streptomycin, GIBCO). The calvaria were pre-incubated at 37°C in the presence of indomethacin (1 μM, final concn.) and the bone resorption stimulator parathyroid hormone (PTH) (10 nM, final concn.). After 24 h the calvaria were placed in fresh medium of the same composition but containing omeprazole or bafilomycin, and incubation proceeded for another 24 h. Omeprazole and bafilomycin was added to the medium from DMSO stock solutions. Final concentrations of DMSO in the assay did not exceed 0.2%, which in itself had no effect in the assay, and control samples without inhibitor contained DMSO. The ^{45}Ca content of the bone and medium was determined by liquid scintillation counting. ^{45}Ca was extracted from the bones in 6 M HCl. The bone-resorbing activity is expressed as % ^{45}Ca released into the medium at 24 h of the initial total ^{45}Ca content in the calvarium.

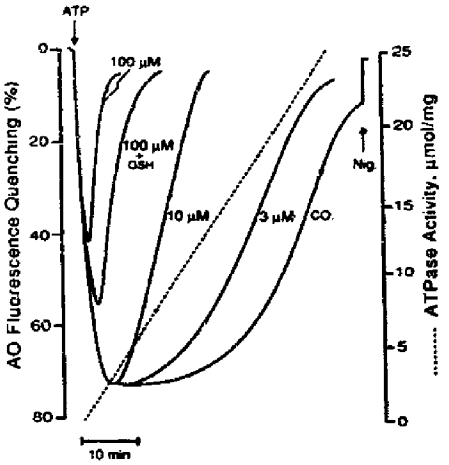

Fig. 1. Effect of omeprazole on ATP-dependent H^+-transport in gastric membrane vesicles and time-course of ATPase activity. Gastric membrane vesicles (approx. 5 μg of protein) were added to a medium containing 2 mM Hepes-NaOH (pH 7.4), 3 mM $MgCl_2$, 175 mM KCl, 5 μg/ml valinomycin, 1 μM Acridine orange and methanol (control) or the indicated concentrations of omeprazole in the presence or absence of 0.1 mM glutathione (GSH) in a final volume of 1 ml. At the time indicated nigericin (10 μg/ml, final concn.) was added to the media in order to dissipate the gradient. ATPase activity (- - - - - -) was measured under the same conditions as in the control proton transport assay (A). The ATPase activity presented is the ΔValinomycin activity, as described in Materials and Methods. Reactions were initiated by the addition of 2 mM Na_2ATP (pH 7.4), and both proton transport and ATPase activity were followed as a function of time at 37°C.

Protein determination. Protein was determined according to Bradford [31] using the Bio-Rad assay procedure and gammaglobulin as a standard.

Results

Effects of omeprazole

Addition of ATP to gastric membrane vesicles induced quenching of Acridine orange (AO) fluorescence, indicating proton transport into vesicles, leading to the formation of an acid-interior pH-gradient (Fig. 1). The maximal pH gradient obtained represents an equilibrium between transport of protons into the vesicles and passive leakage of protons outwards. Shortly after reaching its maximal value, the pH gradient slowly started to collapse and about forty minutes after the addition of ATP, the gradient was abolished, see control trace in Fig. 1. It is not likely that maintainance of the pH gradient was limited by reduced substrate concentration, since ATPase activity, measured under identical conditions, was linear over this period of time (Fig. 1). The pH collapse could possibly represent a time- and temperature-dependent decrease in membrane sta-

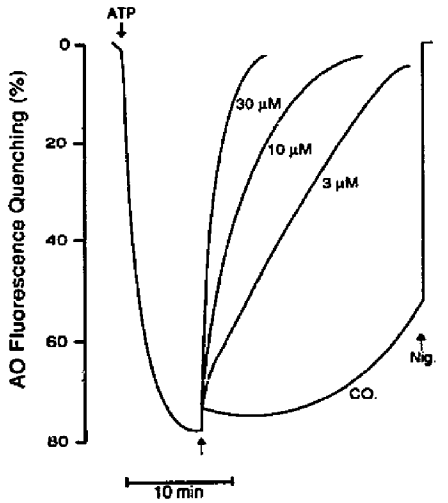

Fig. 2. Addition of omeprazole to the maximal pH-gradient in gastric membrane vesicles. Gastric membrane vesicles (approx. 5 μg of protein) were incubated in the medium described for the control in the legend to Fig. 1. Proton transport was initiated by the addition of 2 mM Na_2ATP. Methanol (control) or the indicated concentrations of omeprazole was added to the media (indicated with an arrow) when the pH gradient had reached its maximal value. Nigericin was added to a final concentration of 10 μg/ml at the point indicated.

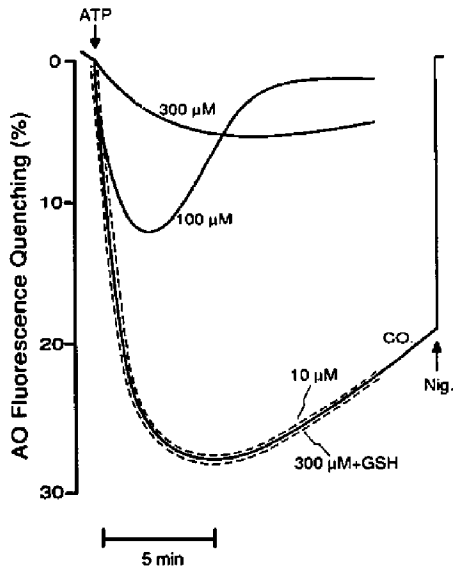

Fig. 3. Effect of omeprazole on ATP-dependent H^+-transport in medullary bone membrane vesicles in the presence or absence of glutathione. Bone membrane vesicles (approx. 30 μg of protein) were added to 1 ml medium containing 2 mM Hepes-NaOH (pH 7.4), 3 mM $MgCl_2$, 175 mM KCl, 0.6 μg/ml valinomycin, 1 μM acridine orange and methanol (control) or the indicated conctrations of omeprazole in the presence or absence of glutathione (GSH). Proton transport was initiated by the addition of 1 mM Na_2ATP (pH 7.4). At the time indicated, nigericin (10 μg/ml, final concn.) was added to the media.

bility and thereby ion-permeability. This slow dissipation of the pH-gradient was not specific to the gastric membrane vesicles; the same effect was observed in both bone- and kidney-derived membrane vesicles (Figs. 3 and 4).

Omeprazole, 3–100 μM, had no effect on the initial rate of acidification in gastric membrane vesicles (Fig. 1). However, as the vesicles were acidified, omeprazole induced a time- and concentration-dependent dissipation of the pH-gradient due to inhibition of the enzyme. This result is in agreement with a time- and pH-dependent transformation of omeprazole into the active inhibitor, the sulfenamide. That the time-lag for the response represents the time necessary for producing enough inhibitor is supported by the experiment shown in Fig. 2, where omeprazole was added after intravesicular acidification had reached the maximal level. Under these conditions, where an acid-interior pH gradient has been established, omeprazole immediately induced a concentration-dependent dissipation of the pH gradient, corresponding to inhibition of the proton pump and subsequent passive leakage of protons out of the vesicles.

Fig. 3 shows the effect of omeprazole on ATP-dependent proton transport in medullary bone-derived membrane vesicles. Concentrations of omeprazole (≤ 10 μM) that resulted in total collapse of the pH gradient in gastric membrane vesicles did not affect H^+-transport in bone-derived membrane vesicles. However, at high concentrations (≥ 100 μM), omeprazole inhibited both the rate and extent of development of the pH gradient. The active form of omeprazole, the sulfenamide, reacts readily with mercaptans such as β-mercaptoethanol and glutathione [15]. Earlier experiments [16] have shown that β-mercaptoethanol, which is lipid-permeable, can protect the H^+,K^+-ATPase in gastric membrane vesicles from inhibition by omeprazole, whereas glutathione, which is lipid-impermeable, is ineffective in protecting

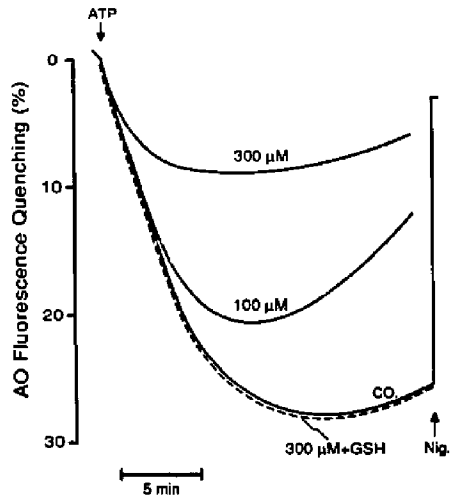

Fig. 4. Effect of omeprazole on ATP-dependent H^+-transport in kidney medulla membrane vesicles in the presence or absence of glutathione. ATP-dependent H^+-transport in kidney membrane vesicles (approx. 100 μg of protein) were measured under exactly the same conditions as for the bone membrane vesicles (legend to Fig. 3.).

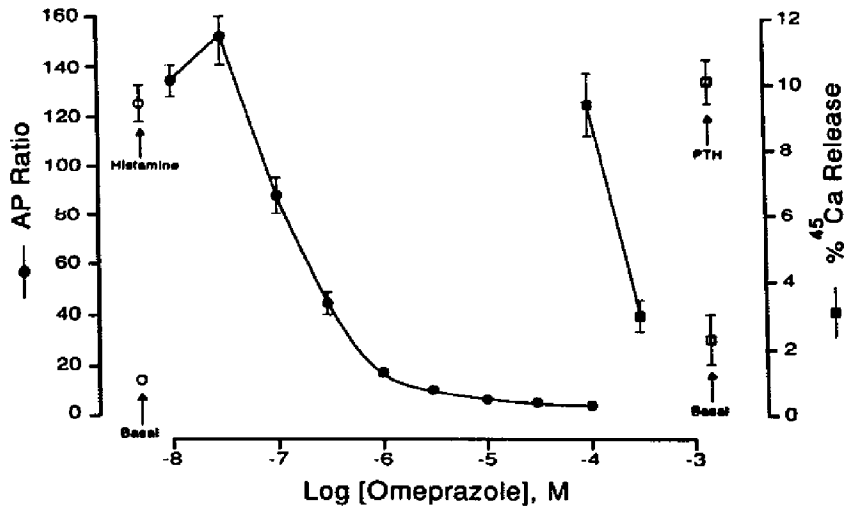

Fig. 5. Comparison of the effects of omeprazole on acid secretion and bone resorption. Histamine-stimulated AP accumulation in gastric glands and PTH-stimulated release of ^{45}Ca from mouse calvarias were measured in the presence of the indicated concentrations of omeprazole, as described in Materials and Methods. Values are (●) histamine-stimulated AP ratio (mean ± S.E., $n = 4$) and (■) % ^{45}Ca released into the medium at 24 h of the initial total ^{45}Ca content in the calvarias (mean ± S.E., $n = 13$). Stimulated and unstimulated (basal) values of (○) AP ratio (mean ± S.E., $n = 4$) and (□) % ^{45}Ca release (mean ± S.E.), $n = 13$) in the absence of omeprazole are indicated in the figure.

the enzyme. These results demonstrate that omeprazole is converted to the active inhibitor in the acidified gastric vesicle interior, and that inhibition is due to interaction with luminal SH-groups on the enzyme. In the present study, glutathione had no effect on the inhibitory action by low concentrations of omeprazole on H^+-transport in gastric membrane vesicles and had only a minor influence on the time-lag for the response by higher concentrations (Fig. 1). In contrast, the inhibitory effect of omeprazole on H^+-transport in the bone-derived membrane vesicles was totally abolished in the presence of glutathione (Fig. 3). Thus, these results indicate that the inhibition by omeprazole was due to interaction at extravesicular sites of the medullary bone H^+-ATPase.

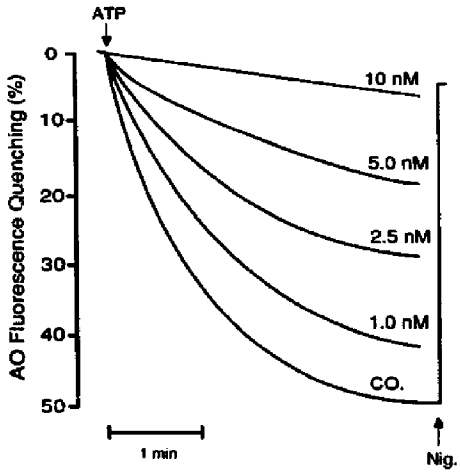

Fig. 6. Effect of bafilomycin A_1 on ATP-dependent H^+-transport in medullary bone membrane vesicles. Bone membrane vesicles (approx. 50 μg of protein) were incubated in 1 ml medium containing 5 mM Hepes-NaOH (pH 7.4), 3 mM $MgSO_4$, 150 mM KCl, 1 μM Acridine orange, 0.6 μg/ml valinomycin and the indicated concentrations of bafilomycin A_1. After a 10 min preincubation at room temperature, 1 mM Na_2ATP was added and the AO fluorescence quenching was followed as described in Materials and Methods. At the point indicated nigericin was added to a final concentration of 10 μg/ml.

The effects of omeprazole on ATP-dependent acidification in kidney medulla membrane vesicles in the presence and absence of glutathione are shown in Fig. 4. Similar results as those obtained in the bone-derived membrane vesicles were achieved, i.e., at concentrations above 100 μM, omeprazole was found to inhibit proton transport, but the presence of glutathione completely protected against this inhibition.

At the tissue level, gastric acid secretion and bone resorption can be studied in isolated gastric glands and isolated mouse calvaria, respectively. The effects of omeprazole on histamine-stimulated acid production (measured as aminopyrine distribution) in gastric glands and on PTH-stimulated bone resorption (measured as ^{45}Ca release) in mouse calvaria are compared in Fig. 5. Omeprazole was found to inhibit histamine-stimulated aminopyrine uptake with an $IC_{50} = 0.25 \pm 0.07$ μM (mean ± S.E., $n = 4$). By contrast, a concentration of approx. 200 μM omeprazole was needed to inhibit 50% of the ^{45}Ca released in PTH-stimulated mouse calvaria. These results correlate with the differentiated inhibitory effects of omeprazole on proton transport observed in the respective membrane vesicle preparations.

Effects of bafilomycin

Bafilomycin A_1 was found to be a potent inhibitor of ATP-dependent H^+-transport in both bone- and kid-

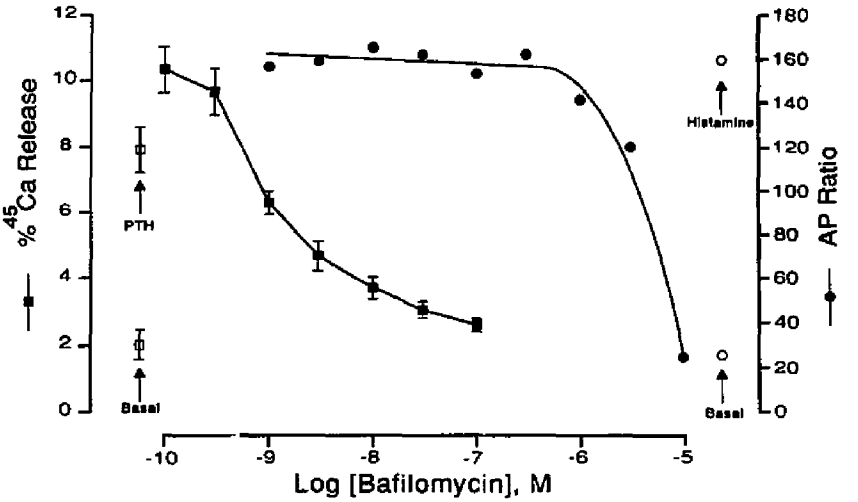

Fig. 7. Comparison of the effects of bafilomycin A_1 on acid secretion and bone resorption. Histamine-stimulated AP accumulation in gastric glands and PTH-stimulated release of ^{45}Ca from mouse calvarias were measured in the presence of the indicated concentrations of bafilomycin A_1, as described in Materials and Methods. Values are (●) histamine-stimulated AP ratio (mean, $n = 2$) and (■) % ^{45}Ca release of total ^{45}Ca content (mean ± S.E., $n = 15$), as described in the legend to Fig. 6.

ney-derived membrane vesicles (Fig. 6 and Table I). In both preparations, the initial rate of proton transport was completely inhibited by 10 nM bafilomycin. Half-maximal inhibition of the initial rate of acidification occurred at 210.5 nM (mean ± S.E., $n = 3$) in both the medullary bone membrane vesicles and the kidney medulla membrane vesicles (Table I). Similar concentrations of bafilomycin have previously been shown to inhibit H^+-transport activity in bone membrane vesicles [21] and vacuolar H^+-ATPases from other sources [17–20]. The inhibitory effect of bafilomycin has been reported to be dependent on the amount of protein in the assay [17]. However, since the amount of vacuolar H^+-ATPase protein present in the bone and kidney membrane vesicle preparations has not yet been determined, a meaningful calculation of the ratio of bafilomycin to protein needed for inhibition in these preparations can not be made.

TABLE I

Effect of bafilomycin on the initial rate of acidification in bone-, kidney- and gastric-membrane vesicles

ATP-dependent acidification in bone (50 μg)-, kidney (500 μg)- and gastric (10 μg)-membrane vesicles in the presence or absence of bafilomycin A_1 was measured as described in the legend to Fig. 7. except that the valinomycin concentration was 5 μg/ml in the assays of gastric membrane vesicles. Numbers in the parantheses refer to the amount of protein present in the assay volume of 1 ml. The initial rate of acidification was calculated as described in Materials and Methods and concentrations of bafilomycin A_1 which were required for 50% inhibition of the initial rate of acidification was determined. Values are mean ± S.E. of three determinations on three separate preparations.

Preparation	IC_{50} (M)
Bone membrane vesicles	$(2 \pm 0.5) \cdot 10^{-9}$
Kidney membrane vesicles	$(2 \pm 0.5) \cdot 10^{-9}$
Gastric membrane vesicles	$5 \cdot 10^{-5}$

ATP-dependent H^+-translocation in gastric membrane vesicles was not affected by bafilomycin at concentrations up to 1 μM. Concentrations of bafilomycin of approx. 50 μM were needed to achieve 50% inhibition of the H^+-transport (Table I). Thus, bafilomycin was approximately 10^4-times less potent in inhibiting the gastric H^+,K^+-ATPase than in inhibiting the H^+-ATPases of kidney and bone.

Bafilomycin inhibited PTH-stimulated ^{45}Ca release in mouse calvaria with similar potency, $IC_{50} \approx 2.5$ nM, as that found for inhibition of proton transport in bone-derived membrane vesicles (Fig. 7). Shown in the same figure is the effect of bafilomycin on histamine-stimulated aminopyrine-uptake in gastric glands. In line with the effects on H^+-transport in the vesicle preparations, bafilomycin was a much less potent inhibitor of acid secretion in gastric glands ($IC_{50} \approx 5$ μM) compared to its effectiveness in inhibiting bone resorption in the mouse calvaria.

Discussion

In line with its mechanism of action, the inhibitory effect of omeprazole on ATP-dependent acidification in gastric membrane vesicles was found to be time- and pH-dependent. The degree of inhibition of the H^+,K^+-ATPase is directly correlated to the amount of omeprazole converted to active inhibitor, the sulfenamide. At neutral pH, the conversion is slow, but as the pH is lowered, the rate of transformation is rapidly increased [15]. The amount of active inhibitor formed for a given concentration of omeprazole is therefore a function of the incubation time and the pH.

The sensitivity of vacuolar H^+-ATPases to sulfhydryl group reactive agents, such as *N*-ethylmaleimide (NEM), is thought to be due to interaction with critical cysteine residues in the vicinity of the putative catalytic site in the cytosolic domain of the enzyme [9,30,31]. The glutathione-protectable inhibitory effect of omeprazole on the vacuolar H^+-ATPases observed in the bone- and kidney-derived membrane vesicles may be the result of reaction with such cysteine residues. At high concentrations of omeprazole, sufficient amounts of the SH-group reactive sulfenamide to interact with the H^+-ATPase could be formed even at neutral pH ($t_{1/2}$ = 1400 min at pH = 7.4 [15]). Another possible reaction is a nucleophilic attack of the thiol groups on the electrophilic 2-carbon in the benzimidazole part of omeprazole, i.e., omeprazole acting as an alkylating agent. This type of reaction has been described in the metabolic pathway of omeprazole [32]. Thus, at the high concentrations ($\geq$ 100 μM) needed to affect the vacuolar H^+-ATPases, the selective action of omeprazole on luminal SH-groups is circumvented. In this context, it should be noted that the peak plasma concentration of omeprazole obtained during clinical use of omeprazole is approx. 1–2 μM. The presence of extravesicular glutathione in the assay mimics the physiological situation, where intracellular glutathione protects against the action of omeprazole on intracellular SH-groups. Omeprazole potently inhibited H^+-transport in gastric membrane vesicles despite the presence of glutathione, in accordance with a selective interaction with luminal, extracellular SH-groups. In contrast, the protective effect of glutathione found in the vacuolar H^+-ATPase preparations, indicates that omeprazole does not react selectively with luminal, but rather with cytosolic, SH-groups of this type of H^+-ATPase. These results suggest that vacuolar H^+-ATPases do not have critical SH-groups in the extracellular domain of the enzyme, or that existing SH-groups are inaccessible to reaction with omeprazole.

In bone tissue cultures and isolated osteoclast preparations, omeprazole has been shown to inhibit both bone resorption and the pH gradient formed by osteoclasts [13,33,34]. However, as found in the present study of bone resorption in isolated mouse calvaria, effects of omeprazole are only observed at high concentrations, IC_{50} > 100 μM. This is also the case for the effect of omeprazole on urinary acidification. High concentrations of omeprazole have been found to exert inhibitory effects on proton secretion in isolated renal tubular segments [35–37]. However, in one investigation of both renal proton secretion in vivo and proton secretion in isolated collecting duct segments, no effect of omeprazole was found despite the use of high concentrations, or even of pre-acidified omeprazole [38]. Thus, in these models effects could only be found at very high concentrations of omeprazole, if at all.

It seems resonable to conclude that the mechanism of action of omeprazole applicable in the parietal cell does not apply to urinary acidification or osteoclast-mediated bone resorption, since the H^+-ATPases responsible for these processes are, unlike the gastric H^+, K^+-ATPase, insensitive to inhibition by omeprazole at luminal sites.

The potency of bafilomycin to inhibit proton transport in bone- and kidney-derived membrane vesicles was similar to that found for other vacuolar H^+-ATPases [17–21], giving further support that H^+-translocation in these vesicles is dependent on this type of ATPase. However, bafilomycin is also an inhibitor of the E_1E_2-ATPases, in this study represented by the gastric H^+, K^+-ATPase, but approximately 10^4-times less potent. The selectivity of bafilomycin to vacuolar H^+-ATPases is thus dependent on the concentration used.

The potency difference of bafilomycin found on the isolated enzyme level was paralleled in the isolated tissue models. Acid secretion in gastric glands was inhibited by bafilomycin with an $IC_{50} \sim 5$ μM, while the corresponding IC_{50}-value for inhibition was found to be ~ 2.5 nM. Hence, the isolated mouse calvaria were inhibited in the same concentration range as the isolated vacuolar H^+-ATPase. Bone resorption by isolated osteoclasts has recently also been shown to be inhibited by bafilomycin in this concentration range [21]. These results thus substantiates further the view that a vacuolar-type H^+-ATPase plays a central role in osteoclast-mediated bone resorption.

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