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INHIBITION OF GASTRIC ($H^+ + K^+$)-ATPase BY THE SUBSTITUTED BENZIMIDAZOLE, PICOPRAZOLE

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The substituted benzimidazole, picoprazole, inhibited the gastric ($H^+ + K^+$)-ATPase in a concentration- and time-dependent manner. Half-maximal inhibition of the ($H^+ + K^+$)-ATPase activity was obtained at about $2 \cdot 10^{-6}$ M under standard conditions. In addition to the inhibition of ATPase activity, parallel inhibition of phosphoenzyme formation and the proton transport activity were achieved. Radiolabelled picoprazole was found to bind to 100 kDa peptide; this peptide was shown by phosphorylation experiments to contain the catalytic centre of the ($H^+ + K^+$)-ATPase. Studies on the ($Na^+ + K^+$)-ATPase indicated that this enzyme was unaffected by picoprazole. From the data presented and from other pharmacological studies, it is proposed that this compound inhibits acid secretion at the level of the parietal cell by its ability to inhibit the gastric proton pump, the ($H^+ + K^+$)-ATPase.

Introduction

The substituted benzimidazoles inhibit gastric acid secretion both in vivo and in vitro [1–3]. Furthermore, in isolated gastric gland preparations [4] these inhibitors have been shown to inhibit acid production monitored as accumulation of a weak base, aminopyrine [5,6]. In this glandular system and in isolated guinea pig mucosa, the substituted benzimidazoles inhibited not only basal secretion, but also secretion induced by the secretatogues histamine and dibutyryl-cAMP. Inhibition was found to be noncompetitive [1,6]. Furthermore, aminopyrine accumulation stimulated by high K^+ concentration was also antagonized by the compounds [6]. Based on these data, a mechanism of action peripheral to cell receptor sites and probably located within the parietal cell seemed likely [5,6]. During recent years, an ($H^+ + K^+$)-ATPase has been purified from the gastric mucosa of various species [7–9] and has been localized to the secretory surface of the parietal cell [10]. The addition of ATP to isolated vesicles containing the enzyme results in H^+ uptake into the intravesicular space in exchange for intravesicular K^+ [11,12]. The transport process is apparently electroneutral [13]. Entry of KCl into the vesicle is rate-limiting for proton translocation. The ($H^+ + K^+$)-ATPase thus plays an important role in H^+ secretion across the membranes of the secretory canaliculus of the

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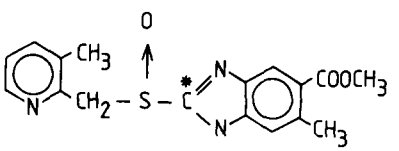
Abbreviations: ($H^+ + K^+$)-ATPase, Proton plus potassium-stimulated ATP phosphohydrolase; ($Na^+ K^+$)-ATPase, Sodium plus potassium ion transport ATP phosphohydrolase (EC 3.6.1.3); Pipes, piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid); Tris, tris(hydroxymethyl)aminomethane; dibutyryl-cAMP, N^6,O^{21}-dibutyryladenosine 3′:5′-cyclic monophosphate.

parietal cell [13,14]. It seemed possible, therefore, that the substituted benzimidazoles affect acid secretion by acting directly on the $(H^+ + K^+)$-ATPase. This paper presents data for one substituted benzimidazole, picoprazole, and describes its inhibitory action on the $(H^+ + K^+)$-ATPase in more detail.



Picoprazole (H 149/94)

Fig. 1. Structural formula of picoprazole. * indicates the position of the ^{14}C label.

Material and Methods

Materials. Gastric $(H^+ + K^+)$-ATPase was prepared from hog stomachs as described by Saccomani et al. [15]. In brief, membrane vesicles containing the $(H^+ + K^+)$-ATPase were prepared by differential and zonal density gradient centrifugation. The microsomal fraction was centrifuged through a 0.25 M sucrose/0.25 M sucrose-8% (w/w) Ficoll ® step gradient in a Kontron TZT 48 zonal rotor (Basel, Switzerland) for 4 h at 47000 rpm. The material retained at the interface was used for the experiments.

For transport experiments, the vesicles were equilibrated in a mixture of 150 mM KCl, 2 mM $MgCl_2$ and 2 mM Pipes adjusted to pH 6.7 with Tris base. After equilibration, the vesicles were stored at 4°C until use on the same day. All subsequent mixing of the sample solutions was done near isotonicity of the equilibrated sample. For experiments in which free access of ions to either face of the enzyme was required, the vesicle preparation was lyophilized and stored at −80°C until resuspension. The $(Na^+ + K^+)$-ATPase preparation was obtained as described by Jørgensen [16].

Reagents. [γ-^{32}P]ATP was obtained from the Radiochemical Centre, Amersham, U.K. ATP was obtained from Sigma, U.S.A. Acridine orange was obtained from Merck, Darmstadt, F.R.G. Picoprazole, (H 149/94), methyl 6-methyl-2-[[(3-methyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-5-carboxylate and [^{14}C]picoprazole were synthetized by the Chemical Department, AB Hässle, Mölndal, Sweden (Fig. 1). Picoprazole was dissolved in methanol. The final methanol concentration did not exceed 1% which alone had no effect on any of the activities measured.

Enzyme assays. K^+-stimulated ATPase activity of the $(H^+ + K^+)$-ATPase is designated K^+-ATPase activity. $(Na^+ + K^+)$-stimulated ATPase activity of the $(Na^+ + K^+)$-ATPase is designated $(Na^+ + K^+)$-ATPase activity. K^+-stimulated *p*-nitrophenylphosphatase activity of the $(H^+ + K^+)$-ATPase and $(Na^+ + K^+)$-ATPase are designated *p*-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase) and *p*-nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase), respectively. It should be noted that *p*-nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase) was assayed in the absence of Na^+.

ATPase activity. This was measured by the release of inorganic phosphate, which was assayed according to the method of Fiske and SubbaRow [17].

K^+-ATPase activity. The assay medium consisted of 2 mM $MgCl_2$, 75 mM Pipes-Tris buffer (pH 7.4), 2 mM Na_2ATP, with or without 10 mM KCl in a total volume of 2 ml. The assay was incubated for 15 min at 37°C. The reaction was then stopped by the addition of 1 ml 10% trichloroacetic acid. A maximum of 10% of the substrate was hydrolyzed over the incubation period, giving linear reaction rates. In lyophilized material and in the absence of K^+, reaction rates were about 10 μmol P_i/mg protein per h. In the presence of both Mg^{2+} and K^+, reaction rates were about 100 μmol P_i/mg protein per h. For all inhibition experiments, reaction rates in the presence of Mg^{2+} and K^+ but in the absence of inhibitor were set to 100%.

$(Na^+ + K^+)$-ATPase activity was assayed in a medium consisting of 5 mM Pipes-Tris buffer (pH 7.4), 2 mM $MgCl_2$, 2 mM Na_2ATP with or without 10 mM KCl plus 100 mM NaCl in a total volume of 2 ml, and incubated for 3 min at 37°C. The reaction was stopped by the addition of 1 ml 10% trichloroacetic acid. In the absence of K^+ and Na^+, reaction rates were about 20 μmol P_i/mg protein per h. In the presence of Na^+ and K^+,

reaction rates were about 400 μmol P_i/mg protein per h. For the inhibition experiments reaction rates in the presence of Mg^{2+}, Na^+ and K^+ but in the absence of inhibitor were set to 100%.

p-Nitrophenylphosphatase activity. This was measured by the release of *p*-nitrophenol from *p*-nitrophenyl phosphate [18].

p-Nitrophenylphosphatase ($(H^+ + K^+)$-ATPase) activity was assayed in a medium consisting of 20 mM imidazole-acetic acid buffer (pH 7.5), 3 mM $MgCl_2$, 3 mM Tris-*p*-nitrophenyl phosphate with or without 10 mM KCl in a total volume of 0.5 ml. The assay was incubated for 3 min at 37°C. The reaction was stopped by the addition of 25 μl 50% trichloroacetic acid. In the absence of K^+, reaction rates were about 4 μmol *p*-nitrophenol/mg protein per h. In the presence of both Mg^+ and K^+, reaction rates were about 75 μmol *p*-nitrophenol/mg protein per h. This reaction rate was given a value of 100%.

p-Nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase) activity. This was assayed as described for *p*-nitrophenylphosphatase ($H^+ + K^+$-ATPase). In the absence of K^+, reaction rates were about 7 μmol *p*-nitrophenol/mg protein per h. In the presence of both Mg^+ and K^+, reaction rates were about 160 μmol *p*-nitrophenol/mg protein per h, which was taken as the 100% value.

Phosphorylation of the gastric membranes. ($H^+ + K^+$)-ATPase was preincubated for 30 min at 37°C. The medium contained 10^{-6}, 10^{-5} and 10^{-4} M picoprazole, 40 mM Tris-HCl (pH 7.4) and 20 μg of membrane protein. The phosphorylation reaction was then measured by the addition of 2 mM $MgCl_2$ and 5 μM [γ-^{32}P]ATP for 15 s, at 22°C. The reaction was stopped by the addition of 1 ml ice-cold 10% $HClO_4$ containing 5 mM ATP and 40 mM Na_2PO_4. The precipitated protein was collected on a 4 μm Millipore® type SSWP filter. The filter was washed with 70 ml of 5% $HClO_4$ containing 10 mM Na_2PO_4. The Cerenkov radiation of the filter was measured in a scintillation counter.

SDS-polyacrylamide gel-electrophoresis. Membranes undergoing SDS-polyacrylamide gel electrophoresis were either first phosphorylated by means of [γ-^{32}P]ATP or incubated with [^{14}C]picoprazole. The phosphorylation procedure was carried out in a medium consisting of 200 μg/ml gastric membranes, 2 mM $MgCl_2$ and 5 μM [γ-^{32}P]ATP in the presence or absence of 0.1 M KCl. The final volume was 200 μl. The phosphorylation reaction was stopped with 1% SDS to solubilize the membranes. About 40 μg protein was applied to a density gradient polyacrylamide gel with a starting density of 4% and a final density of 30%. Gels were run in a 0.1 M Tris-borate buffer (pH 6.0) in 0.1% SDS for 2.5 h. Protein was stained with Coomassie blue and scanned in a gel densitometer at 550 nm. Gels run with ^{32}P-labelled membranes were sectioned manually immediately after electrophoresis and each slice was counted.

For binding studies with ^{14}C-picoprazole, gastric membranes (10 μg/ml) were incubated at 37°C in 2 mM Pipes-Tris (pH 7.4) with 10^{-4} M [^{14}C]picoprazole in a total volume of 20 ml for 2 h and then centrifuged for 1 h at 100000 × *g*. The resulting pellet was resuspended in 1% SDS and about 40 μg protein applied to each gel. Gels were then treated as described for the phosphorylation experiments.

Proton transport experiments. H^+ transport rates and the extent of pH gradient formation were monitored in an Aminco DW2 spectrophotometer (Americal Instrument Company, U.S.A.) in the dual beam model set at 490 and 535 nm [19]. Vesicles were preequilibrated at a concentration of 40 μg/ml in 2 mM Pipes-Tris, 150 mM KCl and 2 mM $MgCl_2$ at pH 6.7 for 3 h at 22°C. The equilibrated sample was mixed with 10^{-4} M picoprazole and after various incubation times, a sample was withdrawn and placed in a cuvette. 20 μM Acridine orange was added and a baseline recorded, after which 0.6 mM Na_2ATP was added to the incubation and the proton transport rate assayed.

Protein determination. Protein was measured by the method of Lowry et al. [20].

Results

Kinetics of the inactivation of K^+-ATPase activity

The time course for the inhibition of K^+-ATPase activity is shown in Fig. 2A. The inhibition was found to be both time- and concentration-dependent, since the reduction in enzyme activity increased progressively with increased

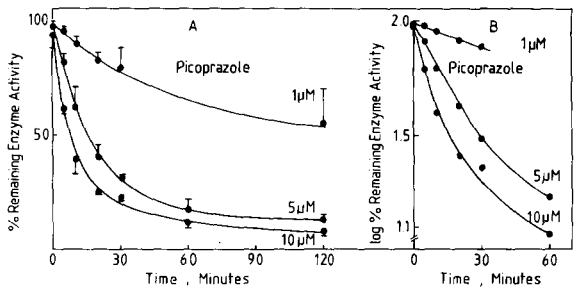


Fig. 2. A. Time course for inactivation of $(H^+ + K^+)$-ATPase. Membrane suspensions (10 μg of protein/ml) were incubated in a medium consisting of 5 mM Pipes-Tris (pH 7.4) at concentrations of picoprazole as indicated. After incubation times, indicated at the abscissa, samples were assayed for K^+-ATPase activity. The K^+-stimulated ATPase activity in the absence of picoprazole was given a value of 100%. Each time point is the mean of four separate determinations. The bars indicate the standard error of the mean (S.E.) for each time point. B. Semilogarithmic point of the data in Fig. 2A.

incubation time and inhibitor concentrations. When the data from Fig. 2A were replotted semilogarithmically (Fig. 2B), the reaction of picoprazole with the enzyme was linear over the first 75% of the total reaction. However, for the remaining 25% a deviation from a straight-line relationship between the logarithm of the remaining enzyme activity and time was obtained. From the linear portions of the curves in Fig. 2B, half-lives,

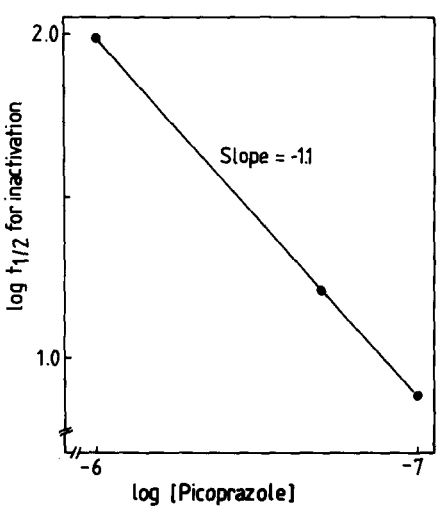


Fig. 3. Double logarithmic plot of the $t_{1/2}$ of inactivation against picoprazole concentrations. The $t_{1/2}$ was calculated from the linear part of Fig. 2B.

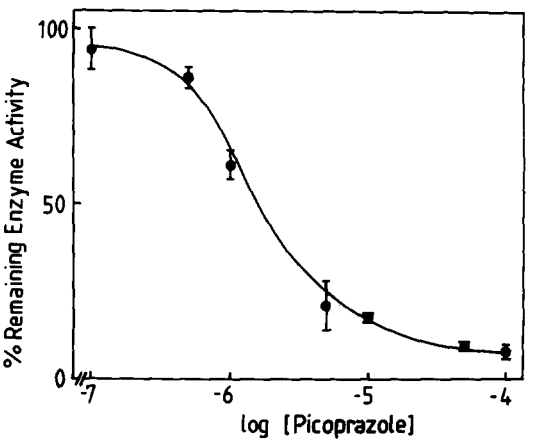


Fig. 4. Concentration dependence for inhibition of $(H^+ + K^+)$-ATPase by picoprazole. Gastric membranes (10 μg of protein/ml) were incubated for 30 min in 5 mM Pipes-Tris (pH 7.4) with picoprazole at concentrations indicated. After incubation, samples were assayed for K^+-ATPase activity. The enzyme activity in the absence of picoprazole was given a value of 100%. Each time point is the mean of two separate determinations.

$t_{1/2}$, were calculated for the inhibition reaction at each inhibitor concentration. These values were then plotted in a double logarithmic plot against the picoprazole concentrations (Fig. 3). This plot was linear with a slope close to one. This allows the conclusion that inhibition follows pseudo-first-order kinetics [21], at least over the first 75%. The concentration-effect relationship is shown in Fig. 4. Increasing concentrations of picoprazole resulted in progressive inhibition of the K^+-ATPase activity. Half-maximal inhibition occurred at about $2 \cdot 10^{-6}$ M.

pH dependence of inhibition

From the results in Fig. 5, it was found that enzyme inhibition increased progressively as the proton concentration was increased. Despite the greater inhibition at lower pH, however, a pH of 7.4 was taken as standard for most subsequent experiments, since this was the optimal pH for the enzyme activity [22].

Binding of picoprazole to $(H^+ + K^+)$-ATPase

The binding of picoprazole to $(H^+ + K^+)$-ATPase was analyzed by SDS-polyacrylamide gel electrophoresis. A major peak was evident at about

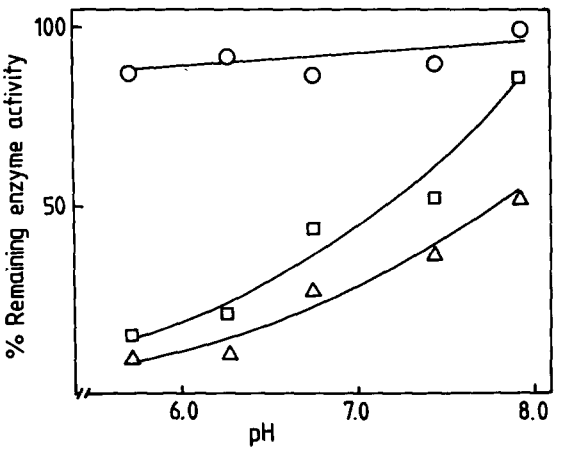


Fig. 5. Effects of pH on the inactivation of $(H^+ + K^+)$-ATPase. Membrane suspension (10 μg of protein/ml) was incubated in 2 mM Pipes-Tris buffer at pH 5.7, 6.3, 6.8, 7.4 and 7.8 for 30 min, with picoprazole at concentrations of 1 μM (○———○); 5 μM (□———□) and 10 μM (△———△). After incubation, samples were adjusted to pH 7.4 by the addition of 75 mM Pipes-Tris buffer and K^+-ATPase activity was assayed. K^+-ATPase activity in the absence of picoprazole was given a value of 100%.

100 kDa (Fig. 6B). This peak was also found to contain large amounts of radioactivity. However, large quantities of radioactivity that did not correspond to any protein peak were retained at the end of the gel. This was probably due to binding of [^{14}C]picoprazole to lipid components, as the low molecular weight region of the gel stained with Sudan black, a marker for lipids (data not shown). In order to identify the protein containing the catalytic peptide of the $(H^+ + K^+)$-ATPase, we studied the incorporation of ^{32}P from AT^{33}P [23]. The distribution of ^{32}P after phosphorylation of the membrane preparation is shown in Fig. 6A. The 100 kDa peptide was found to contain the ^{32}P-label (open bars) and, furthermore, when the experiment was performed in the presence of K^+, the label of the 100 kDa peptide was lost (shaded bars). It can therefore be concluded that both the ^{14}C- and ^{32}P-labels are confined to the same peptide.

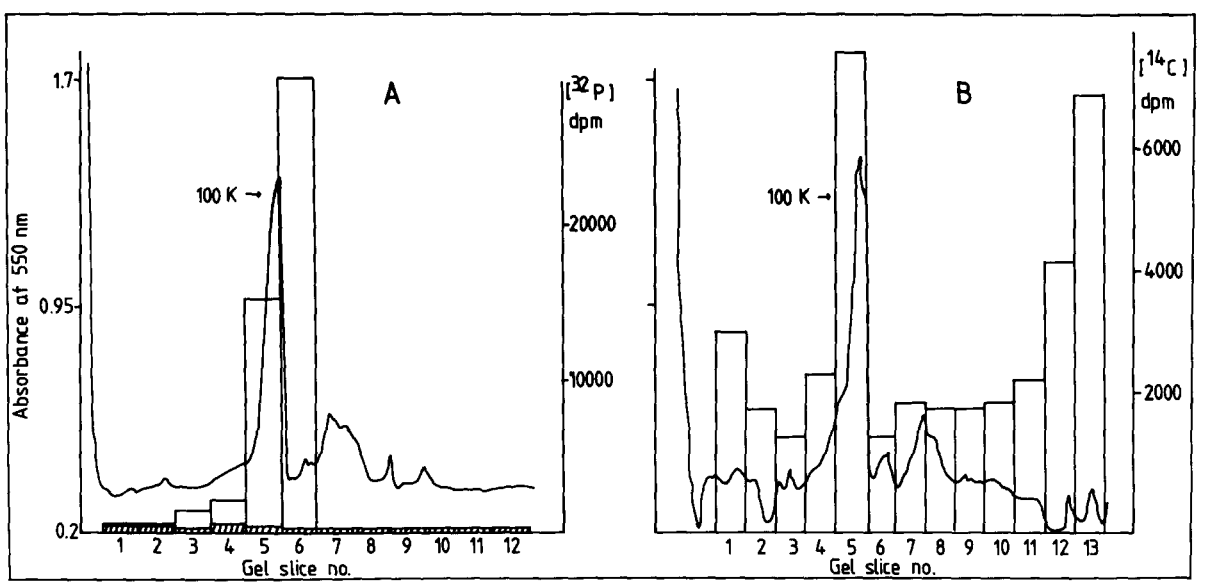


Fig. 6. A. Binding of [γ-^{32}P]ATP to $(H^+ + K^+)$-ATPase. Gastric membranes (200 μg of protein/ml) were incubated and phosphorylated as described in Methods. Open bars show the distribution of the ^{32}P-label in the presence of MgAT^{32}P and shaded bars in the presence of MgAT^{32}P plus 100 mM KCl. Gels were prepared and run as detailed in the Methods section. 100 K indicates the position of the 100 kDa peptide. B. Binding of [^{14}C]picoprazole to $(H^+ + K^+)$-ATPase. Gastric membranes (10 μg of protein/ml) were incubated with 10^{-4} M [^{14}C]picoprazole, after which the incubate was centrifuged at 100000 × g for 60 min and solubilized in 1% sodium dodecyl sulphate. Open bars show the distribution of [^{14}C]picoprazole. 100 K indicates the position of the 100 kDa peptide.

Effect of picoprazole on phosphoenzyme formation

During the catalytic cycle, the $(H^+ + K^+)$-ATPase forms a phosphoenzyme intermediate [24–26]. Since picoprazole was found to bind to the 100 kDa peptide and also inhibited the ATPase activity (Figs. 4 and 6), its effect on steady-state phosphoenzyme concentration was studied. Picoprazole inhibited the phosphoenzyme levels in a concentration-related manner. At 10^{-6} M, 95% of the control level was obtained; at 10^{-5} M and 10^{-4} M 61% and 35%, respectively, of the control level was reached. The control level was 720 pmol of phosphoenzyme per milligram of membrane protein. Thus, inhibition of K^+-ATPase activity and phosphoenzyme formation were found to occur in parallel.

Inhibition of proton transport by picoprazole

When the $(H^+ + K^+)$-ATPase is isolated in vesicular form, proton transport can be measured [12,19]. Addition of ATP to vesicles preequilibrated in a medium containing KCl results in vesicles energization, and proton transport rates can be monitored. In this experiment, gastric vesicles were incubated with picoprazole. After the times indicated under each curve in Fig. 7A, a sample was withdrawn and the transport signal measured. From Fig. 7A, three parameters were calculated for each curve: the inital rate of proton transport, taken as the initial slope; the magnitude of the signal, and the time at which the maximal magnitude was reached. These parameters are shown in Fig. 7B, from which it can be seen that both the intial rate and maximal magnitude of the transport signal were inhibited in parallel. In addition, the time required to reach the maximal amplitude of the transport curve increased progressively with incubation time.

Effects on p-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase)

As stated earlier, proton transport in the isolated vesicle system required extravesicular MgATP and intravesicular K^+. However, the $(H^+ + K^+)$-ATPase can hydrolyze substrates other than MgATP. In the presence of *p*-nitrophenyl phosphate and Mg^{2+}, K^+ stimulates the splitting of *p*-nitrophenyl phosphate into *p*-nitrophenol and inorganic phosphate. Two differences are evident

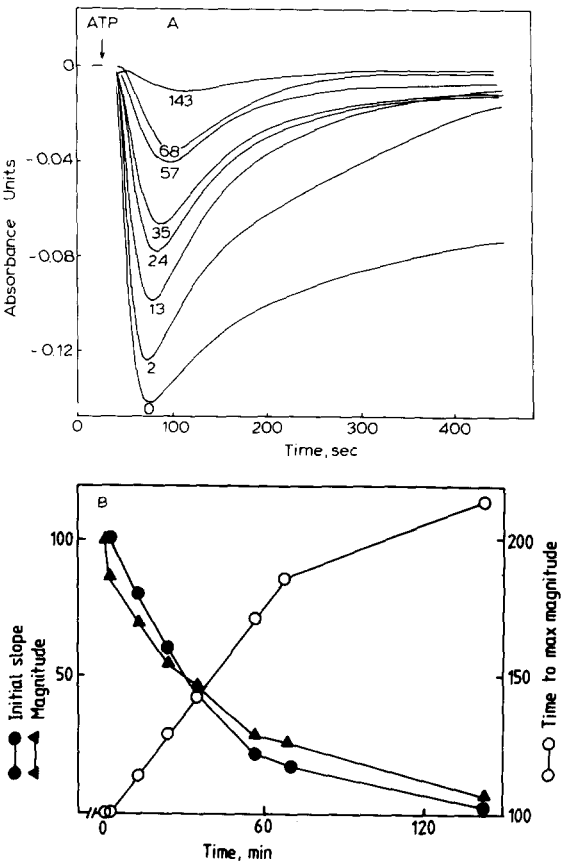


Fig. 7. A. Inhibition of proton by picoprazole. Gastric membrane vesicles (40 μg of protein/ml were equilibrated in 150 mM KCl, 2 mM $MgCl_2$ and 2 mM Pipes-Tris (pH 6.7) at room temperature for 3 h, after which 10^{-4} M picoprazole was added to the equilibration mixture. At the times indicated under the curves (0, 2, 13, 24, 35, 57, 68 and 143 min), a sample was withdrawn and the proton transport signal assayed. At time zero, indicated on the time axis in the figure, the sample and 20 μM Acridine orange were added to the cuvette (volume = 1 ml) and a base-line was recorded for about 25 s after which 0.6 mM ATP was added to initiate proton transport. B. Three parameters were calculated from the date in Fig. 7A: Initial slope (●———●); maximal magnitude taken as the lowest value of each curve (▲———▲) and the time from the addition of ATP at which the signal reached a maximum (○———○). All data are given as percent of the control signal, which was taken as the transport signal in the absence of inhibitor (lowest curve in Fig. 7A).

when *p*-nitrophenyl phosphate is used as substrate instead of MgATP. First, in order to stimulate hydrolysis of *p*-nitrophenyl phosphate, K^+ is required on the outside of the vesicle whereas intravesicular K^+ is required for the hydrolysis of

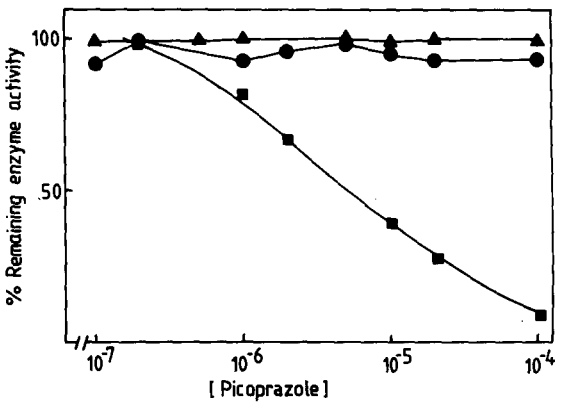


Fig. 8. Effects of picoprazole on *p*-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase), $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase) activity. Gastric membranes (10 μg of protein/ml) were incubated with picoprazole for 30 min in 5 mM Pipes-Tris buffer, pH 7.4 (■———■). Samples were assayed as detailed in Methods. K^+-*p*-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase) activity in the absence of inhibitor was taken as 100%. Kidney membranes (10 μg of protein/ml) were incubated with picoprazole in 5 mM Pipes-Tris buffer (pH 7.4) for 30 min. Samples were assayed either for $(Na^+ + K^+)$-ATPase activity (●———●) or for *p*-nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase) activity (▲——— ▲), $(Na^+ + K^+)$-ATPase activity and *p*-nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase) activity in the absence of picoprazole were taken as 100%.

MgATP. Secondly, hydrolysis of *p*-nitrophenyl phosphate cannot promote proton transport [23]. From the experiment presented in Fig. 8, it is evident that picoprazole inhibited the *p*-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase) activity in a concentration-dependent manner. Half-maximal inhibition was obtained at a concentration of $5 \cdot 10^{-6}$ M, which is in agreement with the value found for the inhibition of K^+-ATPase.

Effects on $(Na^+ + K^+)$-ATPase

Picoprazole was not able to discriminate between K^+-ATPase, *p*-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase) phosphoenzyme formation or proton transport. This finding led us to examine whether this lack of discrimination was only true of the various $(H^+ + K^+)$-ATPase reactions. We therefore also studied the effect of picoprazole on a related enzyme system, namely the $(Na^+ + K^+)$-ATPase which was chosen because of its close similarities to the $(H^+ + K^+)$-ATPase [27]. We found that even at concentrations as high as 10^{-4} M, the $(Na^+ + K^+)$-ATPase activity was unaffected by the inhibitor (Fig. 8). Furthermore, the *p*-nitrophenylphosphatase ($(Na^+ + K^+)$-ATPase) activity was not inhibited, even at high picoprazole concentrations (Fig. 8).

Discussion

The ability to inhibit acid secretion in mammalian gastric mucosa has hitherto depended on the availability of H_2-receptor antagonists, such as cimetidine, or of anticholinergic agents [28,29]. The substituted benzimidazoles represent a novel group of gastric acid secretion inhibitors. Their mechanism of action appears to be distinct from those of the H_2-blockers or anticholinergics, since pharmacological data for the benzimidazole derivative, picoprazole, indicate that its antisecretory activity is exerted at a site distal to the production of cAMP in the events leading to acid formation [1,6].

It has been established that the gastric $(H^+ + K^+)$-ATPase plays an essential role in the secretion of acid by the parietal cells [13]. The data presented here show that picoprazole, a known inhibitor of gastric acid secretion [1–3], inhibits the $(H^+ + K^+)$-ATPase. In addition, the phosphorylation and K^+-dependent phosphatase reactions were also inhibited. Moreover, SDS-polyacrylamide gel electrophoresis showed that the inhibitor was bound to the ATPase peptide. The inhibition of the proton transport reaction by picoprazole can thus be attributed to inhibition of the ATPase activity.

Since all partial reactions of the $(H^+ + K^+)$-ATPase were inhibited by picoprazole it was not possible to define the exact point at which the reaction pathway was inhibited. A puzzling feature of the inhibition of enzyme activity and of H^+ transport by the ATPase is the long preincubation requirement. Picoprazole is a hydrophobic compound, which in the unprotonated form, distributes as a weak base into acidic compartments [6]. Accordingly, limited penetration of picoprazole is unlikely to account for the time dependence of inhibition.

Acidic pH facilitated the inhibition, but it was not determined whether this was due to a change

in reactivity of the inhibitor or to the protein. However, both the enhanced reactivity at low pH and the trapping of picoprazole in the secretory canaliculus [6] would be expected to enhance the activity of the compound in the intact cell.

From the data presented, it can be concluded that picoprazole cannot discriminate between K^+-ATPase, *p*-nitrophenylphosphatase ($(H^+ + K^+)$-ATPase), phosphoenzyme levels or transport reactions of the enzyme. However, a closely related enzyme, $(Na^+ + K^+)$-ATPase, was not affected by picoprazole. It therefore appears that local acidic compartments within the gastric gland are not the only factor governing the selectivity of picoprazole; when tested on the isolated enzymes, picoprazole inhibited the $(H^+ + K^+)$-ATPase more readily than the $(Na^+ + K^+)$-ATPase. This compound thus appears to act selectively on the parietal cell, by virtue of its inhibition of the gastric proton pump.

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