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Mechanism of gastric antisecretory effect of SCH 28080

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1 The mechanism of the gastric antisecretory action of SCH 28080 has been studied utilizing two different *in vitro* test systems, isolated and enriched parietal cells from the guinea-pig and guinea-pig gastric membranes purified and enriched with K^+/H^+-ATPase.

2 In guinea-pig isolated and enriched parietal cells SCH 28080 inhibited the acid response to histamine and high K^+ concentrations with IC_{50} values not significantly different from each other.

3 SCH 28080 inhibited the purified K^+/H^+-ATPase measured in the presence of 5 mM KCl with an IC_{50} value of 1.3 μM. Kinetic studies indicated a competitive inhibition of ATPase by SCH 28080 with respect to K^+. Studies on Na^+/K^+-ATPase showed that this enzyme was only slightly depressed by SCH 28080.

4 It is concluded that SCH 28080 acts with high selectivity on the parietal cell K^+/H^+-ATPase, establishing its antisecretory effect by a competitive interaction with the high affinity K^+-site of the gastric ATPase.

Introduction

Inhibitors of gastric acid secretion can be classified into two general categories based on their site of action: inhibitors working at the basolateral membrane of the parietal cell, such as histamine H_2-receptor antagonists (Black *et al.*, 1972) or anticholinergic agents (Albinus & Winne, 1983) and those working at the secretory membrane, such as inhibitors of the K^+/H^+-ATPase, the enzyme which can be regarded as the proton pump of the parietal cell (Sachs *et al.*, 1976). In recent years, agents belonging to the second category, such as the substituted benzimidazoles (Wallmark *et al.*, 1983; Beil & Sewing, 1984) have received considerable attention.

SCH 28080 (2-methyl-8-(phenylmethoxy) imidazo [1,2-a] pyridine-3-acetonitrile) (Figure 1) is a compound with gastric antisecretory and cytoprotective properties (Long *et al.*, 1983). The chemical structure of SCH 28080 is not related to known histamine H_2-receptor antagonists, anticholinergic agents or prostaglandins. *In vitro* studies in guinea-pig isolated gastric mucosa have shown that SCH 28080 inhibits acid secretion induced by histamine, methacholine and dibutyryl adenosine 3':5'-cyclic monophosphate (dibutyryl-cyclic AMP) (Chiu *et al.*, 1983) suggesting that the site of action of SCH 28080 is intracellular and distal from cyclic AMP. The present study was undertaken to define the mechanism of how

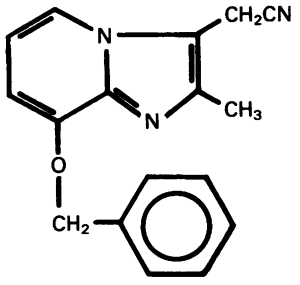

Figure 1 Structural formula of SCH 28080.

SCH 28080 interacts with parietal cell function, by use of two different *in vitro* test systems, the isolated and enriched parietal cell preparation from the guinea-pig and purified K^+/H^+-ATPase.

Methods

Studies with guinea-pig isolated and enriched parietal cells

Isolated and enriched parietal cells from the guinea-pig were prepared as described by Sewing *et al.* (1983). Acid secretion in parietal cells was determined by the [^{14}C]-aminopyrine (AP) accumulation technique. For

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inhibition studies, parietal cells were preincubated with SCH 28080 for 30 min at 22°C. Thereafter, 1 mM histamine was added and the secretory response was determined after 20 min incubation at 37°C. When KCl was used as stimulus, it was added at the same time as SCH 28080 and the concentration of NaCl in the incubation medium was reduced to maintain adequate osmolarity. AP accumulation rates in the absence of the inhibitor were 11.8 ± 1.8, 42.2 ± 8.0 and 68 ± 19.0 pmol [^{14}C]-AP per 10^5 cells in unstimulated, histamine- and KCl-treated cells respectively (mean ± s.e.mean from 6 different cell preparations). For inhibition experiments the control values for histamine- and KCl-treated cells were set as 100%. Control values in unstimulated cells were expressed as % of maximal AP-uptake measured in cells stimulated with 1 mM histamine.

Studies on purified K^+/H^+-ATPase

Gastric K^+/H^+-ATPase was purified by differential and density gradient centrifugation from a guinea-pig parietal cell homogenate essentially according to Beil & Sewing (1984). The method was modified in that the cells were isolated in the presence of 100 μM cimetidine and the cell separation process by the elutriation system was omitted.

Measurement of K^+/H^+-ATPase activity

The assay medium contained 2 mM $MgCl_2$, 50 mM Tris/HCl buffer (pH 7.5), 5–10 μg membrane protein with or without 5 mM KCl in a final volume of 1 ml. The reaction was started by addition of Na_2-ATP (final concentration 2 mM) and stopped after 15 min incubation at 37°C with 1 ml 20% trichloroacetic acid. Liberated inorganic phosphate (P_i) from ATP was measured according to the method of Sanui (1974). Reaction rates were 16.5 ± 0.3 μmol P_i mg^{-1} protein h^{-1} in the absence and 68.7 ± 4.5 μmol P_i mg^{-1} protein h^{-1} in the presence of 5 mM K^+ (mean ± s.e.mean from 4 different enzyme preparations). This K^+-stimulated ATPase activity was not enhanced by addition of the K^+ ionophore valinomycin (10 μM), indicating that the membrane vesicles used were freely permeable to K^+. For inhibition experiments the K^+-stimulated reaction rates in the absence of the inhibitor were set to 100%.

For kinetic analysis the ATP hydrolysis rate in the absence or presence of the inhibitor was determined with varying KCl (0.05–5 mM) concentrations.

Phosphorylation of K^+/H^+-ATPase

Samples, 20–30 μg, of K^+/H^+-ATPase-containing membranes were preincubated with the indicated concentrations of SCH 28080 for 30 min at 22°C in 0.2 ml 50 mM Tris/HCl buffer pH 7.5. Phosphorylation was started by addition of [γ-^{32}P]-ATP and $MgCl_2$ (final concentrations 10 μM and 2 mM) and 15 s later stopped by the addition of 0.2 ml 10% trichloroacetic acid containing 5 mM Tris-ATP and 5 mM K_2HPO_4. The protein precipitate was collected on Millipore membrane filters (0.45 μm pore size) and washed with 30 ml 5% trichloroacetic acid containing 5 mM K_2PO_4. Under control conditions, 718 ± 144 pmol phosphoenzyme per mg membrane protein was found.

p-Nitrophenylphosphatase assay

p-Nitrophenylphosphatase (*p*-NPPase) was assayed in a medium containing 6 mM $MgCl_2$, 6 mM *p*-nitrophenylphosphate, 50 mM Tris/HCl buffer (pH 7.5) and 10–20 μg membrane protein with or without 10 mM KCl in a total volume of 1 ml for 20 min at 37°C. The reaction was stopped by addition of 1 ml 1 N NaOH and liberated *p*-nitrophenol was measured at 410 nm. The reaction rate in the absence of an inhibitor was 35.5 ± 4.3 μmol *p*-nitrophenol mg^{-1} protein h^{-1} in the presence of Mg^{2+} and 10 mM K^+. For kinetic analysis the *p*-nitrophenylphosphate hydrolysis rate was determined in the absence or presence of the inhibitor with KCl concentration varying from 0.5–50 mM.

Studies on dog kidney Na^+/K^+-ATPase

SCH 28080 was preincubated for 30 min at 22°C with Na^+/K^+-ATPase (10 μg protein) in 1 ml medium consisting of 50 mM Tris/HCl buffer pH 7.5, 2 mM $MgCl_2$ with or without 20 mM KCl and 100 mM NaCl. Reaction was initiated by addition of Tris-ATP (final concentration 2 mM) and was, 15 min later, stopped by addition of 1 ml 20% trichloroacetic acid. In the presence of Na^+ and K^+ the uninhibited reaction rates were about 60 μmol P_i mg^{-1} protein h^{-1}.

Protein determination

Protein content was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Drugs and radiochemicals

The drugs used and their sources were: collagenase, 125–230 u mg^{-1}; Na_2-ATP; Tris-ATP; histamine dihydrochloride; Na^+/K^+-ATPase (Sigma, Munich); Pronase E (70,000 PUK g^{-1}) (Merck, Darmstadt); bovine serum albumin (Serva, Heidelberg); [^{14}C]-aminopyrine (specific activity 60–120 mCi mmol^{-1}; New England Nuclear Corp., Dreieich); [γ^{32}P]-adenosine triphosphate (specific activity 10 Ci mmol^{-1}; Amersham, Braunschweig). SCH 28080 was kindly donated

by Prof. Klemm, Byk Gulden, Konstanz. All other reagents were of the highest purity available.

Results

Effect of SCH 28080 on H^+ accumulation in intact parietal cells

Acid secretion, as measured by [^{14}C]-aminopyrine accumulation in guinea-pig isolated and enriched parietal cells, was stimulated in two ways: with 1 mM histamine as an H_2-receptor agonist and with 60 mM KCl which is likely to interact with one of the final steps in the process of acid formation. SCH 28080 inhibited H^+ secretion no matter what the agonist was, in a concentration-dependent manner with an IC_{50} value of approximately 6×10^{-8} M (Figure 2). In unstimulated parietal cells aminopyrine accumulation was reduced by 1 μM SCH 28080 to 53 ± 5.8% of the control value.

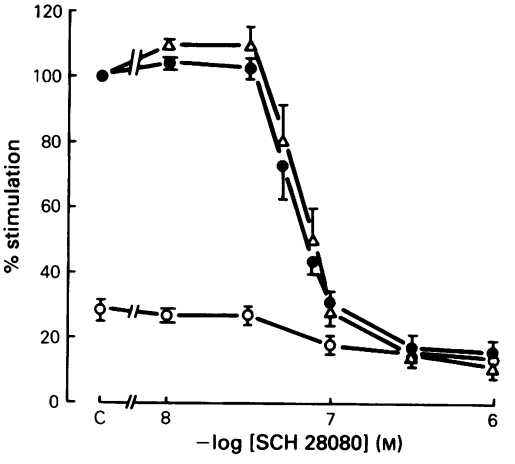

Figure 2 Effect of SCH 28080 on basal (○), histamine (●)- and K^+ (△)-stimulated uptake and accumulation of [^{14}C]-aminopyrine in guinea-pig parietal cells. Values are mean, with vertical lines indicating s.e.mean, from 6 different cell preparations.

Effect of SCH 28080 on isolated K^+/H^+-ATPase

SCH 28080 inhibited K^+/H^+-ATPase activity in guinea-pig purified gastric membranes in a concentration-dependent manner with an IC_{50} value of 1.3 ± 0.19 μM in the presence of 5 μM KCl (Figure 3). Lineweaver-Burk plots of the K^+-stimulated ATP hydrolysis rate in the presence and in the absence of

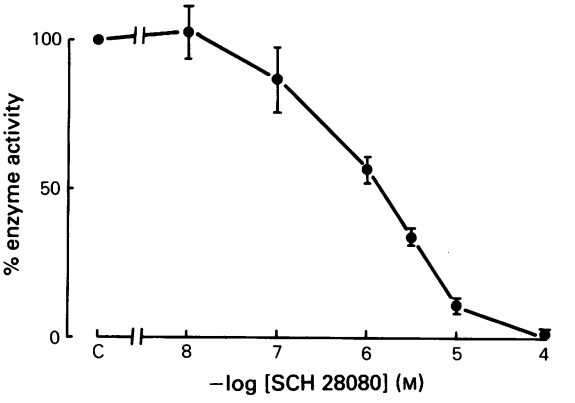

Figure 3 Concentration-response curve for inhibition of guinea-pig K^+/H^+-ATPase by SCH 28080. SCH 28080 was preincubated with the enzyme (5–10 μg protein) at pH 7.5 for 30 min at 22°C. The enzyme activity was then determined after incubation for 15 min at 37°C in the presence of 5 mM KCl. The uninhibited K^+-stimulated ATPase activity was set to 100%. Values are mean, with vertical lines indicating s.e.mean, from 3 different enzyme preparations.

SCH 28080 showed that the K_m of K^+, 0.18 mM, was increased to 1.4 and 8.3 mM in the presence of 0.5 and 3 μM SCH 28080 respectively, whereas V_{max} was not affected (Figure 4). These data indicated a competitive inhibition of the ATPase by SCH 28080 with respect to K^+. Calculated inhibitor K_i value for the ATPase reaction was 0.02 μM.

The basal, Mg^{2+}-dependent part of the K^+/H^+-ATPase was decreased to 65 ± 8.9% of the control activity by 100 μM SCH 28080 (data not shown).

Effect of SCH 28080 on phosphoenzyme formation

SCH 28080 was found to inhibit steady-state phos-

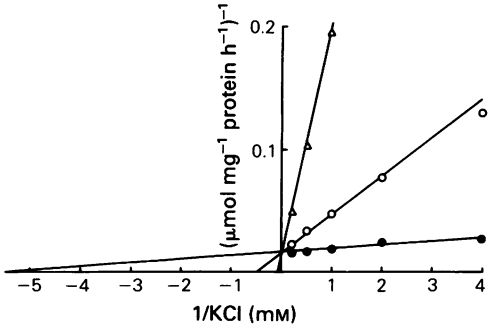

Figure 4 Lineweaver-Burk plots of the rate of K^+-stimulated ATP hydrolysis vs concentration of KCl (0.25 to 5 mM KCl) in the absence (●) or presence of 0.5 (○) and 3 (△) μM SCH 28080.

phoenzyme formation in the presence of 2 mM $MgCl_2$ in a concentration-dependent manner with an IC_{50} value of 5.3 ± 2.7 μM (Figure 5).

Effect of SCH 28080 on p-*nitrophenylphosphatase*

p-NPPase activity, measured in the presence of 10 mM KCl was inhibited by SCH 28080 in a concentration-dependent manner with an IC_{50} value of 7.3 ± 1.3 μM (values: mean ± s.e.mean from 3 different enzyme preparations). The Lineweaver-Burk plot of the K^+-dependent *p*-nitrophenylphosphate hydrolysis rate in the presence of SCH 28080 showed that the drug increased the K_m value for K^+ without affecting V_{max}. The K_m values were 2.0 mM without SCH 28080 and 5.2 and 11.0 mM in the presence of 3 and 10 μM SCH 28080 (Figure 6). Calculated K_i value for the *p*-NPPase was 2.4 μM.

Unlike the Mg^{2+}-dependent part of the K^+/H^+-ATPase, the basal activity of the *p*-NPPase was not affected by SCH 28080 up to a concentration of 100 μM (data not shown).

Effect of SCH 28080 on Na^+/H^+-*ATPase*

Na^+/K^+-ATPase activity measured in the presence of 20 mM KCl and 100 mM NaCl was slightly depressed in the presence of 100 μM SCH 28080 to 80 ± 5.5% of the control value (data not shown).

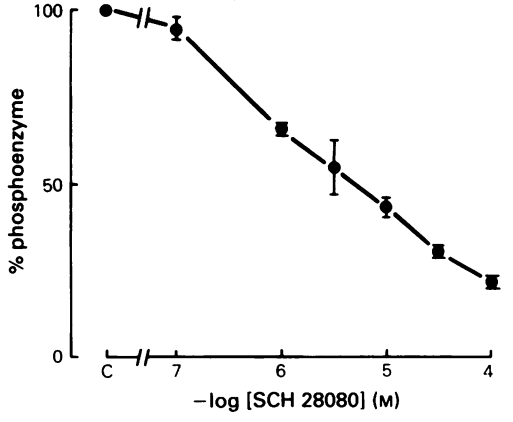

Figure 5 Effect of SCH 28080 on steady-state phosphoenzyme formation of K^+/H^+-ATPase. Purified K^+/H^+-ATPase-containing membranes (20 μg protein) were preincubated for 30 min with SCH 28080 at concentrations indicated in the figure. Phosphorylation reaction was carried out for 15 s at 22°C in the presence of 2 mM $MgCl_2$ and 10 μM [γ-^{32}P]-ATP. Control level was 718 ± 144 pmol phosphoenzyme mg^{-1} protein. This value was set as 100%. Values are mean, with vertical lines indicating s.e.mean, of 2 different enzyme preparations.

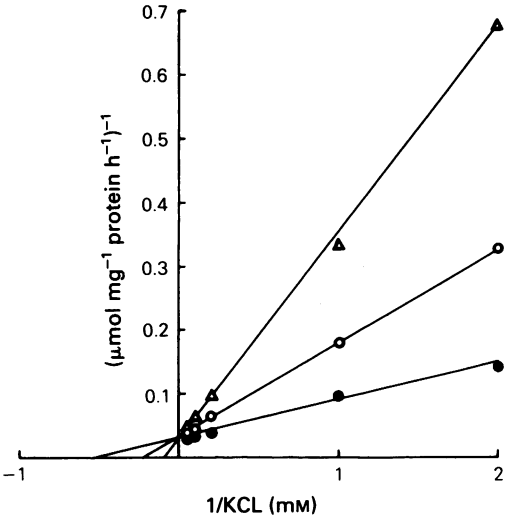

Figure 6 Lineweaver-Burk plots of the hydrolysis rate of *p*-nitrophenylphosphate by K^+/H^+-ATPase vs concentration of KCl (0.5 to 20 mM) in the absence (●) or presence of 3 (○) and 10 (△) μM SCH 28080.

Discussion

We have demonstrated that SCH 28080 inhibits acid formation in isolated and enriched parietal cells stimulated by histamine and K^+. These findings indicate a direct effect of SCH 28080 on the parietal cell and suggest a site of action near or at the K^+/H^+-ATPase. Kinetic studies with the isolated K^+/H^+-ATPase indicated a competitive inhibition of the gastric ATPase with respect to K^+. There are two K^+-sites within the domain of the K^+/H^+-ATPase accessible for K^+ from the luminal site of the enzyme. One is of high affinity ($K_m < 1$ mM) and is responsible for the generation of protons by hydrolysis of ATP. The other is a low affinity K^+-site responsible for the transport of generated H^+ across the apical membrane (Nandi & Ray, 1982). The type of inhibition of K^+/H^+-ATPase induced by SCH 28080 suggests that the compound primarily competes at the high affinity site with the low K_m for K^+.

The drug also inhibited phosphoenzyme formation and the K^+-dependent *p*-nitrophenylphosphatase activity of the ATPase. Again, inhibition of *p*-nitrophenylphosphatase was in competition with K^+. The K^+-site of the phosphatase is located on the cytoplasmic site of the K^+/H^+-ATPase (Saccomani *et al.*, 1975) and the phosphatase is involved in the dephosphorylation reaction. However, the calculated K_i values were 0.02 μM for the ATPase and 2.4 μM for the *p*-NPPase, suggesting that inhibition of the phosphatase is inferior to the proposed interaction of the drug at the luminal K^+-site of the ATPase.

Competitive inhibition by interaction with the K^+-site of the K^+/H^+-ATPase has been described for different drugs, such as trifluoperazine, verapamil (Im *et al.*, 1984) and nolinium bromide (Nandi *et al.*, 1983). The only common functional group of these different drugs is a tertiary amine (Im *et al.*, 1984). SCH 28080 fulfils this criterion since it bears a pyridine moiety within its molecule.

The sequence of potency for enzyme inhibition by SCH 28080 was: K^+/H^+-ATPase > *p*-NPPase > Na^+-ATPase. Despite the fact that different K^+ concentrations were used in the different enzyme assays, the observed weak potency of SCH 28080 at inhibiting the Na^+/K^+-ATPase had to be attributed to a low affinity of the drug for the K^+-site of this enzyme.

Therefore, in conclusion, SCH 28080 appears to act with high selectivity on the parietal cell K^+/H^+-ATPase, establishing its antisecretory effect by interaction with the high affinity K^+-site of the gastric ATPase.

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References

ALBINUS, M. & WINNE, D. (1983). Subclasses of muscarinic receptors in isolated gastric mucosal cells: Receptor characterization and parietal cell function. *Eur. J. Pharmac.*, **94**, 281–295.

BEIL, W. & SEWING, K.-FR. (1984). Inhibition of partially purified K^+/H^+-ATPase from guinea-pig isolated and enriched parietal cells by substituted benzimidazoles. *Br. J. Pharmac.*, **82**, 651–657.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSON, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature*, **236**, 385–390.

CHIU, P.J.S., CASCIANO, C., TETZLOFF, G., LONG, J.F. & BARETT, A. (1983). Studies on the mechanisms of the antisecretory and cytoprotective actions of SCH 28080. *J. Pharmac. exp. Ther.*, **226**, 121–125.

IM, W.B., BLAKEMAN, D.P., MENDLEIN, J. & SACHS, G. (1984). Inhibition of $(H^+ + K^+)$-ATPase and H^+ accumulation in hog gastric membranes by trifluoperazine, verapamil and 8-(N,N-diethylamino) octyl-3,4,5-trimethoxybenzoate. *Biochim. biophys. Acta*, **770**, 65–72.

LONG, J.F., CHIU, P.J.S., DERELANKO, M.J. & STEINBERG, M. (1983). Gastric antisecretory and cytoprotective activities of SCH 28080. *J. Pharmac. exp. Ther.*, **226**, 114–120.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDELL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

NANDI, J. & RAY, T.K. (1982). Mechanism of action of gastric secretory inhibitors: Effects of SCN^-, OCN^-, NO_2^- and NH_4^+ on $(H^+ + K^+)$-ATPase-mediated transport of H^+ inside gastric microsomal vesicles. *Arch. Biochem. Biophys.*, **216**, 259–271.

NANDI, J., WRIGHT, M.V. & RAY, T.K. (1983). Mechanism of gastric antisecretory effect of nolinium bromide. *Gastroenterology*, **85**, 938–945.

SACCOMANI, G., SHAH, G., SPENNY, J.G. & SACHS, G. (1975). Characterization of gastric mucosal membranes. VIII. The localization of peptides by iodination and phosphorylation. *J. biol. Chem.*, **250**, 4802–4809.

SACHS, G., CHANG, H.H., RABON, E., SCHACKMANN, R., LEWIN, M. & SACCOMANI, G. (1976). A nonelectrogenic H^+ pump in plasma membranes of hog stomach. *J. biol. Chem.*, **251**, 7690–7698.

SANUI, H. (1974). Measurement of inorganic orthophosphate in biological materials: Extraction properties of butyl acetate. *Anal. Biochem.*, **60**, 489–504.

SEWING, K.-FR., HARMS, P., SCHULZ, G. & HANNEMANN, H. (1983). Effect of substituted benzimidazoles on acid secretion in isolated and enriched guinea pig parietal cells. *Gut*, **24**, 557–560.

WALLMARK, B., SACHS, G., MARDH, S. & FELLENIUS, E. (1983). Inhibition of gastric $(H^+ + K^+)$-ATPase by the substituted benzimidazole, picoprazole. *Biochim. biophys. Acta*, **728**, 31–38.

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