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STUDIES ON THE MECHANISM OF ACTION OF THE GASTRIC MICROSOMAL ($H^+ + K^+$)-ATPase INHIBITORS SCH 32651 AND SCH 28080

CYNTHIA K. SCOTT,* ERIN SUNDELL and LORRAINE CASTROVILLY
Division of Biological Research, Research Laboratories, Ortho Pharmaceutical Corp., Raritan, NJ 08869, U.S.A.

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Abstract—The novel antisecretory agents SCH 32651 and SCH 28080 were evaluated for their antisecretory activities *in vitro* as well as for their abilities to inhibit the ($H^+ + K^+$)-ATPase enzyme activity in preparations of microsomal membranes from rabbit fundic mucosa. SCH 32651 and SCH 28080 inhibited both the histamine- and dibutyryl cAMP-stimulated uptake of [^{14}C]-aminopyrine into isolated parietal cells with IC_{50} values of about 1.5 and 0.02 μM respectively. SCH 32651 and SCH 28080 competitively inhibited the K^+-stimulated hydrolysis of ATP catalyzed by the ($H^+ + K^+$)-ATPase with K_i values of 16.3 and 0.12 μM respectively. The inhibition of the enzyme by both compounds was not affected by the addition of the sulfhydryl reducing agents dithiothreitol or β-mercaptoethanol, was readily reversible by dilution or washing, and was dependent upon the concentration of KCl used to stimulate the enzyme. These data suggest that SCH 32651 and SCH 28080 are reversible, competitive inhibitors of the K^+-stimulated hydrolysis of ATP.

SCH 32651 [3-amino-2-methyl-8-(phenylmethoxy)imidazo[1,2-a]pyrazine HCl · 1/3 H_2O] and a prototype compound, SCH 28080 (2-methyl-8-(phenylmethoxy)imidazo[1,2-a]pyridine-3-acetonitrile), are novel antiulcer agents with both antisecretory and cytoprotective activities [1, 2]. Studies in isolated guinea pig fundic mucosa indicate that the antisecretory activity involves a direct action on the parietal cell distal to either cholinergic or H_2-histaminergic receptor stimulation and at a site beyond the formation of cyclic AMP [3, 4]. Compounds with this profile, e.g. omeprazole, have been reported to inhibit the enzyme which mediates H^+ transport out of the parietal cell, ($H^+ + K^+$)-ATPase [5]. We previously reported that SCH 32651 and a prototype compound of this series, SCH 28080, inhibit the ($H^+ + K^+$)-ATPase activity in preparations of rabbit microsomal membranes with IC_{50} values of 4.0 and 200.0 μM respectively [6].

In the present work we continue to investigate SCH 32651 and SCH 28080 for the nature of their interactions with the isolated ($H^+ + K^+$)-ATPase. These compounds were also examined for their antisecretory activities *in vitro* in isolated rabbit parietal cells using the accumulation of the radiolabeled marker [^{14}C]-aminopyrine as an indirect measurement of parietal cell stimulation [7]. For comparative purposes, the standard ($H^+ + K^+$)-ATPase inhibitor omeprazole was included in many of these studies. The structures of these compounds are shown in Fig. 1.

* Author to whom all correspondence should be sent.

METHODS

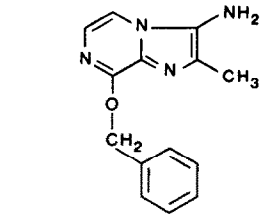
Chemicals

Omeprazole was supplied by Merck Sharp & Dohme Research Laboratories, West Point, PA. SCH 28080 and SCH 32651 were supplied by Schering Plough Corp., Bloomfield, NJ.

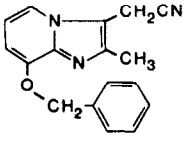
Isolated rabbit parietal cell assay

The basic procedures were adapted from Batzri and Gardner [8] and Soll [9]. Parietal cells were isolated from the fundic mucosa of rabbit stomachs by a four-stage collagenase digestion process. The supernatant fraction from the last two stages of this process contained the individual parietal cells. This cell suspension was centrifuged and reconstituted in a modified Hanks' buffer [BME Vitamin solution; L-glutamine and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffer added to Hanks' buffer] to contain 2–3 $\times$ 10^6 cells/ml. The cells in the suspension were then evaluated for their ability to accumulate [^{14}C]-aminopyrine ([^{14}C]AP), a weak base that accumulates in acidic environments such as the parietal cell [7].

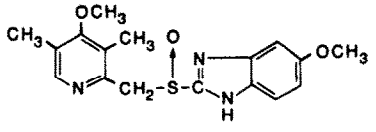
Parietal cells were incubated in a flask containing ~0.23 μCi [^{14}C]AP, 1 $\times$ 10^{-6} M histamine or 3 $\times$ 10^{-4} M dibutyryl cAMP, 1 $\times$ 10^{-5} M isobutylmethylxanthine, and test compound in a 0.02 ml volume of dimethyl sulfoxide (DMSO). The flasks were incubated in a shaking water bath at 37° for 20 min. Two 0.50-ml aliquots were then taken from each flask, and cell pellets were collected by centrifugation. Pellets were solubilized with Protosol (New England Nuclear) and radioactivity was determined using liquid scintillation spectrometry. The



SCH 32651



SCH 28080



OMEPRAZOLE

Fig. 1. Chemical structures of SCH 32651, SCH 28080 and omeprazole.

concentrations of histamine (1×10^{-6} M) and dibutyryl cAMP (3×10^{-4} M) used in the antagonism studies were chosen as they consistently produced 40–50% of maximal response (10 to 20-fold increase in [^{14}C]aminopyrine accumulation compared to basal accumulation).

Data for the antagonism studies are expressed as IC_{50} values, the concentration of drug required to inhibit the histamine or dibutyryl cAMP response by 50%.

To investigate the reversibility of the SCH 28080, SCH 32651 and omeprazole inhibition of histamine-stimulated acid secretion *in vitro* in isolated rabbit parietal cells, the following experiment was conducted. Various preparations of parietal cells (1×10^6 cells/ml) were incubated with either DMSO, 10 μM SCH 28080, 100 μM SCH 32651 or 10 μM omeprazole (final concentration). These concentrations of drugs inhibited the 10^{-6} M histamine-stimulated response in the parietal cell assay by 100%. After incubation while stirring for 30 min at 24°, the cell suspensions were centrifuged at 1000 *g* for 10 min at 4°. After resuspension in fresh, drug-free medium, the cells were incubated for an additional 10 min at 24° while stirring. The cell suspensions were recentrifuged as specified above, and the cells were resuspended in fresh medium at the same concentration. The various cell preparations were then assayed for their response to histamine stimulation following the procedure outlined above. The results are expressed as a percentage of the maximal response to histamine.

($H^+ + K^+$)-ATPase

Vesicle preparation. Gastric ($H^+ + K^+$)-ATPase was prepared from rabbit stomachs using a modification of the method of Tanisawa and Forte [10]. Briefly, membrane vesicles were prepared by differential centrifugation followed by separation using modified sucrose gradients. Fundic mucosa from New Zealand White rabbits were homogenized in a modified Tris buffer consisting of 250 mM sucrose, 0.2 mM EDTA, and 5.0 mM Tris adjusted to pH 7.4 with HCl. The homogenate was centrifuged at 8000 *g* for 5 min with two washes with the same buffer. The pooled supernatant fractions were centrifuged at 35,000 *g* for 30 min. The resulting supernatant was layered over a 37% sucrose solution and centrifuged at 100,000 *g* for 5 hr. The light microsomal membrane fraction was collected, resuspended in buffer, and recentrifuged at 100,000 *g* for 90 min. The final pellet was resuspended in homogenization buffer and stored at −20°.

Assay procedure. ($H^+ + K^+$)-ATPase activity was measured as described by Forte *et al.* [11] in a 1-ml incubation volume containing 50 mM Tris, pH 7.4, 2 mM $MgCl_2$, 2 mM Na_2ATP, with or without various concentrations of KCl, and vehicle control (0.01 ml DMSO or 0.01 N H_2SO_4) or compound added in a 0.01 ml volume. The usual order of addition was Tris buffer, $MgCl_2$, KCl, compound or vehicle, and enzyme. In the reducing agent studies, dithiothreitol or β-mercaptoethanol was added prior to the addition of compound and enzyme. Typically, membrane protein (20 μg) was added, and the tubes were preincubated with drug for 10 min at 37°. SCH 28080 and SCH 32651 were solubilized in DMSO. As omeprazole requires exposure to an acidic environment to be converted to the active inhibitor of the ($H^+ + K^+$)-ATPase, in the isolated enzyme studies omeprazole was dissolved in 0.01 N H_2SO_4 and used as quickly as possible following dissolution. This solution was referred to as the "omeprazole degradation mixture". Substrate, Na_2ATP, was then added and the tubes were incubated for 15 min at 37°. The pH of the reaction volume was 7.4. The reaction was stopped by the addition of 1 ml of 14% trichloroacetic acid (TCA), and the samples were centrifuged at 2000 *g* for 10 min. The amount of inorganic phosphate (P_i) present in an aliquot of supernatant fraction was determined by the method of Eibl and Lands [12]. ($H^+ + K^+$)-ATPase activity was determined after correcting for basal (Mg^{2+} only) enzyme activity present in the membrane preparation and is expressed as μmoles P_i/mg protein/hr. The mean enzyme activities for three enzyme preparations were as follows (micromoles of ATP hydrolyzed per mg protein per hr ± SEM): Mg^{2+}-stimulated ATPase, 15.1 ± 0.42; K^+-stimulated ATPase, 32.5 ± 2.2. None of the compounds, at the highest concentrations tested, had any significant effect on the basal (Mg^{2+} only) ATPase activity.

($Na^+ + K^+$)-ATPase assay

($Na^+ + K^+$)-ATPase activity was measured as described by Wallmark *et al.* [13] in a 1-ml incubation volume containing 50 mM Tris, pH 7.4, 2 mM $MgCl_2$, 2 mM Na_2ATP, with or without various concentrations of 100 mM NaCl plus 10 mM KCl and vehicle control (0.01 ml DMSO or 0.01 N H_2SO_4) or compound added in a 0.01-ml volume. ($Na^+ + K^+$)-

ATPase from dog kidney was obtained from the Sigma Chemical Co. with an approximate activity of 1.35 units/mg protein. One unit liberates 1.0 μmole P_i from ATP/min at pH 7.4 at 37° in the presence of Na^+, K^+ and Mg^{2+}. Typically, 20 μg enzyme was added per tube. The tubes were pre-incubated with drug for 10 min at 37°. SCH 28080 and SCH 32651 were solubilized in DMSO, and omeprazole in 0.01 N H_2SO_4. Substrate, Na_2ATP, was then added, and the tubes were incubated for an additional 15 min at 37°. The reaction was stopped by the addition of 1 ml of 14% TCA, and the samples were centrifuged at 2000 *g* for 10 min. The amount of P_i present in an aliquot of supernatant fraction was determined as described for the $(H^+ + K^+)$-ATPase assay. In the absence of K^+ and Na^+, reaction rates were about 20.5 μmoles P_i/mg protein/hr. In the presence of Na^+ and K^+, the reaction rates were about 64.4 μmoles P_i/mg protein/hr. None of the compounds, at the highest concentrations tested, had any significant effect on the basal ATPase activity.

RESULTS

Inhibition of parietal cell stimulation

SCH 32651 inhibited both the histamine and dibutyryl cAMP stimulation of [^{14}C]AP uptake into isolated rabbit parietal cells with IC_{50} values of 1.45 and 1.63 μM respectively (Table 1). Omeprazole and SCH 28080 were more potent than SCH 32651 at inhibiting the histamine-induced response with IC_{50} values of 0.25 and 0.029 μM respectively. Both compounds displayed similar potencies when dibutyryl cAMP was used as the stimulant (Table 1). At higher concentrations, 10 and 100 μM, all three compounds inhibited the basal accumulation of [^{14}C]AP into unstimulated parietal cells.

In these experiments all three compounds were solubilized in DMSO. When omeprazole was dissolved in 0.01 N H_2SO_4, similar to its solubilization for the isolated enzyme assay, the compound displayed similar potency ($IC_{50} = 0.11 \pm 0.01$ μM, N = 3) at inhibiting the histamine accumulation of [^{14}C]AP into isolated rabbit parietal cells. The potency of the "omeprazole degradation mixture" to inhibit this response decreases with increasing time of exposure to 0.01 N H_2SO_4. The "degradation mixture" was completely inactive after 60–90 min of exposure to acid.

Inhibition of $(H^+ + K^+)$-ATPase

Kinetic analysis. SCH 32651 was examined for its ability to inhibit the generation of inorganic phosphate induced by various concentrations of KCl. Increasing concentrations of SCH 32651 produced parallel shifts in the KCl concentration curves, and maximal enzyme activity was reestablished with higher concentrations of KCl, suggesting competitive antagonism (Fig. 2A). A Lineweaver–Burk plot of these data indicated that SCH 32651 was a competitive inhibitor of the K^+-stimulated enzyme reaction (Fig. 2B). The K_a for K^+-stimulation was 0.43 ± 0.07 mM (N = 3). The data were regraphed as the slope of the reciprocal plots versus inhibitor concentration, and the K_i for SCH 32651, determined from linear regression analysis, was 16.3 μM (Fig. 3).

A similar type of interaction was observed for SCH 28080 (Fig. 4, A and B). Increasing concentrations of SCH 28080 produced a parallel and surmountable shift in the KCl concentration curve, and Lineweaver–Burk analysis indicated that SCH 28080 competitively inhibited the K^+-stimulated hydrolysis of ATP. The K_i value for SCH 28080 inhibition of the enzyme was determined to be 0.12 μM (Fig. 3).

In contrast, the addition of 3.3 and 6.7 μM omeprazole solubilized in 0.01 N H_2SO_4 greatly decreased the reaction velocity, V_{max}, from a control value of 16.8 units of activity (μmoles P_i/mg protein/hr, 37°) to 6.4 and 2.1 units respectively (Fig. 5). In addition, the inhibition of the enzyme by this mixture was not overcome by increasing the concentration of KCl.

Decreasing the concentrations of KCl (from 10.0 to 0.2 mM) used to stimulate the hydrolysis of ATP resulted in a 6-fold increase in the potency of SCH 32651 (Table 2). Decreasing the KCl concentration also increased the potency of SCH 28080, while having no effect on the potency of omeprazole to inhibit the $(H^+ + K^+)$-ATPase.

Reversibility of enzyme inhibition

To investigate whether the inhibition of the $(H^+ + K^+)$-ATPase by SCH 28080 and SCH 32651 was reversible, the K^+-stimulated ATPase activity was measured before and after dilution and washing of the microsomal membrane preparation in drug-free medium. Concentrated microsomal preparations were incubated with 10 μM SCH 28080 or

Table 1. Activities of SCH 32651, SCH 28080 and omeprazole in isolated rabbit parietal cells

Compound	Inhibition of histamine-stimulated [^{14}C]aminopyrine uptake IC_{50} (μM)	Inhibition of dibutyryl cAMP-stimulated [^{14}C]aminopyrine uptake IC_{50} (μM)
SCH 32651	1.45 ± 0.17	1.63 ± 0.24
Omeprazole	0.25 ± 0.07	0.28 ± 0.07
SCH 28080	0.029 ± 0.001	0.018 ± 0.002

All values are the mean ± SEM for N = 3–4 experiments. Each experiment utilized a different parietal cell population and was conducted in duplicate.

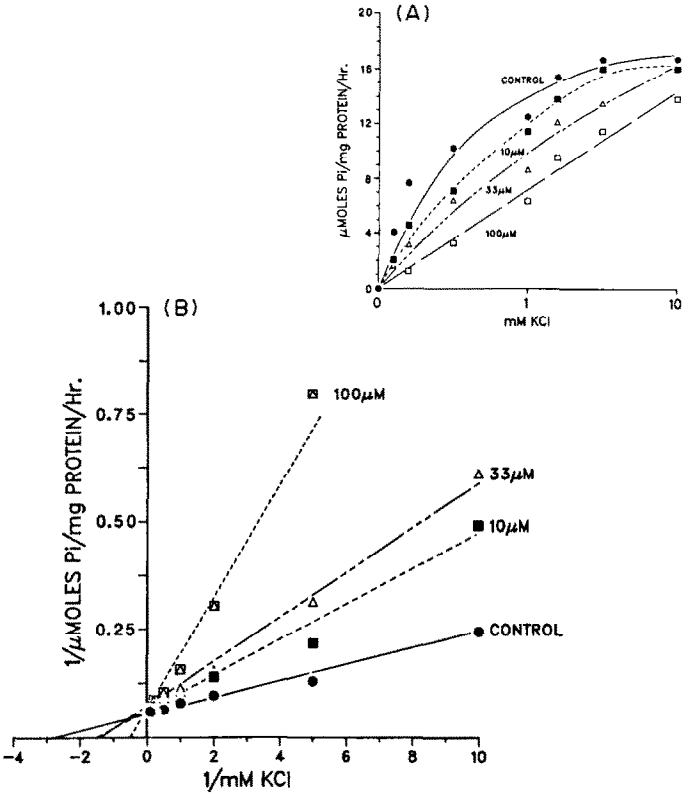


Fig. 2. (A) K^+-stimulated hydrolysis of ATP in the presence of 10, 33 and 100 μM SCH 32651. The points are experimentally determined enzymatic rates and are the mean of three experiments, each conducted in duplicate. (B) Double-reciprocal plot of K^+-stimulated ATP hydrolysis and the effect of 10, 33 and 100 μM SCH 32651. The lines are computer-generated linear regression "best fit" lines ($r \geq 0.98$ for all lines).

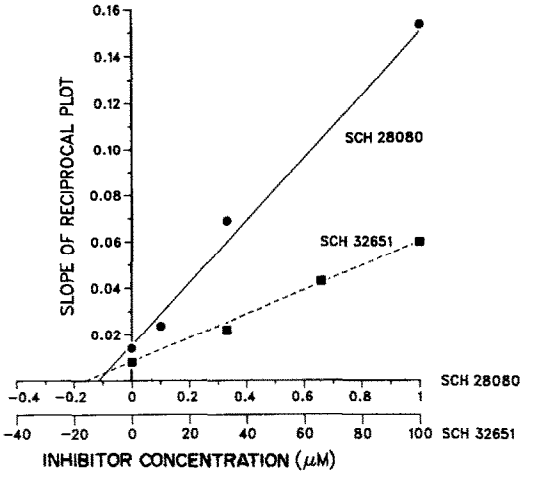


Fig. 3. Determination of K_i for SCH 28080 and SCH 32651. The slopes of the double-reciprocal plots of K^+-stimulated ATP hydrolysis in the presence of various concentrations of SCH 28080 and SCH 32651 were plotted versus inhibitor concentration. Computer-generated linear regression "best fit" lines were generated ($r = 0.99$ for both lines).

100 μM SCH 32651 (final concentration). Upon dilution (1:50) of these membrane preparations for assay, the enzyme activities of SCH 32651, SCH 28080, and vehicle control-treated microsomes were similar (17.5, 17.8 and 21.5 μmoles ATP hydrolyzed/mg protein/hr respectively). These membrane preparations were also diluted 24-fold, washed in drug-free medium, and recovered by centrifugation at 200,000 *g* for 90 min. The enzyme activities of washed microsomal preparations were 16.9, 16.4 and 21.2 μmoles ATP hydrolyzed/mg protein/hr for vehicle control, SCH 28080 and SCH 32651 treated microsomal preparations respectively. These experiments indicated that the inhibition of the $(H^+ + K^+)$-ATPase by SCH 32651 and SCH 28080 was readily reversed by dilution and/or washing.

In contrast, the inhibition of the $(H^+ + K^+)$-ATPase by "the omeprazole-degradation mixture" was not reversed by dilution or washing. The K^+-stimulated activity in microsomal membrane preparations before washing was 16.3 and 3.4 μmoles ATP hydrolyzed/mg protein/hr for control and 100 μM omeprazole-treated preparations respectively. After a 24-fold dilution and subsequent washing and recovery by centrifugation (200,000 *g* for 90

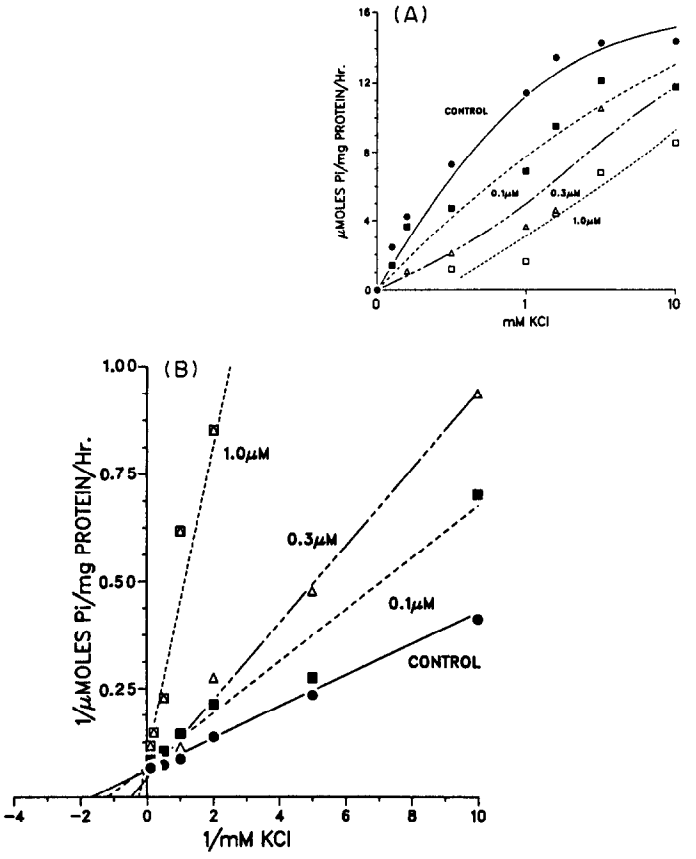


Fig. 4. (A) K^+-stimulated hydrolysis of ATP in the presence of 0.1, 0.3 and 1.0 μM SCH 28080. The points are experimentally determined enzymatic rates and are the mean of three experiments, each conducted in duplicate. (B) Double-reciprocal plot of K^+-stimulated ATP hydrolysis and the effect of 0.1, 0.3 and 1.0 μM SCH 28080. The lines are computer-generated linear regression "best fit" lines ($r \geq 0.98$ for all lines).

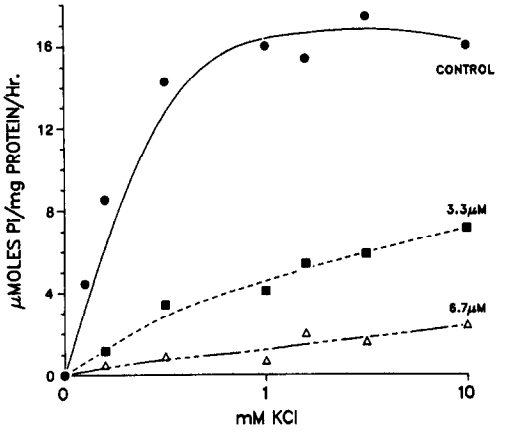


Fig. 5. K^+-stimulated hydrolysis of ATP in the presence of 3.3 and 6.7 μM omeprazole. The points are experimentally determined enzymatic rates and are the mean of two experiments each conducted in triplicate.

min), the activities were 14.9 and 2.2 μmoles for control and 100 μM omeprazole-treated preparations respectively.

Reversibility of acid secretion in the isolated parietal cell

Using parietal cells treated with vehicle prior to washing, increasing concentrations of histamine caused a dose-dependent increase in the amount of [^{14}C]AP accumulation into the parietal cell. Parietal cells preincubated with 100 μM SCH 32651 followed by washing responded to histamine in a manner similar to control (DMSO-treated) cells. Preincubation of the cells with 10 μM omeprazole followed by washing caused about a 40% reduction in their ability to respond to histamine. Parietal cells exposed to 10^{-5} M SCH 28080 prior to washing lost their ability to respond to histamine, even at histamine concentrations as high as 100 μM (data not shown).

Table 2. Effect of KCl concentration on the inhibition of $(H^+ + K^+)$-ATPase by SCH 32651, SCH 28080 and omeprazole

Compound	IC_{50} (μM) KCl concentration 0.2 mM	1.0 mM	10.0 mM
SCH 32651	37.5 ± 2.5	65.0 ± 15.0	235.0 ± 15.0
SCH 28080	0.24 ± 0.11	0.35 ± 0.5	2.95 ± 0.55
Omeprazole	1.75 ± 0.25	2.05 ± 0.05	2.20 ± 0.20

All values are the mean ± one-half the range for two preparations each conducted in duplicate.

Effect of reducing agents on the inhibition of the $(H^+ + K^+)$-ATPase enzyme by SCH 32651, SCH 28080 and omeprazole

The effects of various concentrations of the disulfide bond reducing agents dithiothreitol (DTT) or β-mercaptoethanol on the inhibition of the $(H^+ + K^+)$-ATPase enzyme by SCH 32651, SCH 28080 and omeprazole were investigated. Addition of 0.1 mM DTT or 0.1 mM β-mercaptoethanol to the incubation mixture prior to the addition of the enzyme abolished the ability of omeprazole to inhibit the $(H^+ + K^+)$-ATPase, while having no effect on the ability of SCH 32651 or SCH 28080 to inhibit the enzyme (data not shown).

Inhibition of $(Na^+ + K^+)$-ATPase

The standard $(Na^+ + K^+)$-ATPase inhibitor, ouabain, inhibited the Na^+ plus K^+-stimulated enzyme activity in preparations of dog kidney with an IC_{50} of 0.90 μM. Neither SCH 28080 nor SCH 32651 inhibited this enzyme at concentrations as high as 100 μM. The omeprazole "degradation mixture" inhibited this enzyme with an IC_{50} of 10.0 μM.

DISCUSSION

SCH 32651 and SCH 28080 are reported to be novel, orally effective antiulcer compounds with both cytoprotective and antisecretory activities [1, 2]. Studies in guinea pig isolated fundic mucosa indicate that the compounds act at a site distal to the histamine or cholinergic receptors and at a site beyond the formation of cAMP [3, 4]. Data reported in this study confirm the antisecretory activities of SCH 32651 and SCH 28080 in isolated rabbit parietal cells. The stimulation of [^{14}C]aminopyrine uptake into rabbit parietal cells by either histamine or dibutyryl cAMP was inhibited in a concentration-dependent manner by both SCH 32651 and SCH 28080. SCH 28080 was about fifty times more potent than SCH 32651 at inhibiting the histamine-induced response. The $(H^+ + K^+)$-ATPase inhibitor omeprazole also inhibited both the histamine and dibutyryl cAMP responses in isolated parietal cells with a potency about nine times greater than SCH 32651. This suggests that omeprazole, SCH 28080 and SCH 32651 act at a site in the parietal cell distal to histaminergic or cAMP stimulation, possibly affecting the gastric $(H^+ + K^+)$-ATPase.

SCH 32651 and SCH 28080 inhibit the $(H^+ + K^+)$-ATPase activity in preparations of rabbit gastric microsomes [6]. The results of the studies reported here demonstrate that these compounds were competitive inhibitors of the K^+-stimulated hydrolysis of ATP. SCH 28080 appeared to be more potent than SCH 32651, with a K_i value of 0.115 versus 16.3 μM respectively. The degree of inhibition of the enzyme by SCH 32651 and SCH 28080 was dependent upon the concentration of potassium used to stimulate the enzyme. Data from these experiments suggest that lower concentrations of KCl increased the potency of the Schering compounds. Since the cytosolic K^+-concentration in the parietal cell can be over 100 mM and the K^+ concentration in the gastric juice is about 10 mM, it appears that a potency difference could exist depending on the K^+ concentration at the site where these agents interact with the enzyme, i.e. at the intra- or extracellular face of the membrane. Further studies to investigate the effect of K^+ concentration on both the $(H^+ + K^+)$-ATPase enzyme activity and the inhibition of this activity by the Schering compounds need to be conducted. In contrast, omeprazole did not display a competitive interaction with the K^+-stimulated reaction. The inhibition of the enzyme by omeprazole was not overcome by increasing concentrations of KCl and was independent of the concentration of KCl used to stimulate the enzyme.

Omeprazole has been reported to interact with the $(Na^+ + K^+)$-ATPase from dog kidney [14]. We confirmed that acid-solubilized omeprazole inhibited the $(Na^+ + K^+)$-ATPase activity, exhibiting an IC_{50} of 10.0 μM. It was still about three times more potent against the $(H^+ + K^+)$-ATPase, IC_{50} of 3.3 μM. In contrast, neither SCH 32651 nor SCH 28080 inhibited this enzyme at concentrations as high as 100 μM.

Recent reports in the literature suggest that the inhibition by omeprazole of the $(H^+ + K^+)$-ATPase may involve its irreversible incorporation into the ATPase enzyme where it may oxidize key sulfhydryl groups [15, 16]. Published data have shown that the gastric ATPase, inactivated by omeprazole *in vivo* or *in vitro*, recovers its K^+-dependent ATP hydrolyzing activity upon incubation with mercaptoethanol, a disulfide bond reducing agent [15, 17]. Additional studies by Im *et al.* [16] indicate that omeprazole does not inhibit the ATPase in dog or rat gastric microsomal membranes that are prepared in the presence of 1 mM DTT. We confirmed these data

and found that 0.1 mM DTT or β-mercaptoethanol, added prior to the addition of the omeprazole degradation mixture, completely abolished the ability of this mixture to inhibit the rabbit microsomal ($H^+ + K^+$)-ATPase while having no effect on the ability of either SCH 32651 or SCH 28080 to inhibit the enzyme. These data suggest a possible competitive interaction between DTT and the omeprazole degradation mixture for the sulfhydryl groups of the ($H^+ + K^+$)-ATPase.

The results of the reversibility experiments conducted here have shown that the inhibition of the isolated ($H^+ + K^+$)-ATPase by omeprazole was not reversible by dilution and subsequent washing. In contrast, the enzyme activities of SCH 28080 and SCH 32651 washed membrane preparations were similar to control enzyme activities, indicating that the interaction of the Schering compounds with the enzyme is readily reversible. Reversibility studies conducted in the isolated parietal cell assay yielded different results. The inhibition of the histamine-induced uptake of [^{14}C]AP into isolated parietal cells by 100 μM SCH 32651 was completely reversed by washing the cells after incubation. The inhibition by 10 μM omeprazole was restored to 60% of the control histamine response after washing. This is in contrast to data by Im *et al.* [18] and Sewing *et al.* [19] who found that washing omeprazole-exposed glands free of the extracellular drug leads to a recovery of the acid secretory response. Under similar conditions we achieved only a 60% recovery of the histamine or the dibutyryl cAMP response in our cell system. Interestingly, under the conditions used in these experiments, incubation of the parietal cells with 10 μM (final concentration) SCH 28080 led to an inhibition of the histamine response that was not recovered by washing. The discrepancy in these findings between SCH 28080 and SCH 32651 was unexpected and is presently under further investigation.

It should also be noted that all of the compounds tested were at least ten times more potent at inhibiting the histamine-stimulated uptake of [^{14}C]AP than at inhibiting the isolated ($H^+ + K^+$)-ATPase enzyme. This has also been observed in previously published reports. Sewing *et al.* [19] found the IC_{50} for omeprazole to inhibit the histamine-stimulated accumulation of [^{14}C]aminopyrine into isolated guinea pig parietal cells to be 0.13 ± 0.03 μM. Beil and Sewing [20] found that the IC_{50} for omeprazole to inhibit the $H^+ + K^+$-ATPase isolated from guinea pig parietal cell is 1.8 ± 0.5 μM. This difference may be due to several factors. One possible explanation is that, in the physical preparation of the isolated enzyme from the crude rabbit gastric mucosa, the orientation of the enzyme in its membrane environment was altered compared to its orientation in the intact parietal cell membrane, thereby reducing its activity. The increase in potency of omeprazole in the cell system cannot be explained by the different methods of solubilization as both DMSO and H_2SO_4 solubilized omeprazole were equipotent in inhibiting both the histamine and the dibutyryl cAMP responses in isolated parietal cells. In the isolated enzyme system, where there are no mechanisms for acid activation of omeprazole, the compound must be dissolved in acid to induce the conversion to the active enzyme inhibitor. Although acidification induces activation, the activated omeprazole has a very short half-life ($T_{1/2}$ = 3 min) [21] so that progressively less material is available to inhibit the enzyme in both the isolated enzyme and the isolated parietal cell system. Another explanation for the difference in potency by omeprazole in these two systems is the observation by Im *et al.* [18] that omeprazole's inhibition of acid secretion in the isolated parietal cell is dependent upon the involvement of intracellular glutathione. It is possible that the increased potency of omeprazole as well as its reversibility in the parietal cell could be due to the modification of the enzyme by changing intracellular glutathione levels. The difference in potency for the two Schering compounds in these systems is more difficult to explain and could be the result of decreased sensitivity of the isolated enzyme versus the enzyme in the intact parietal cell. It is also possible that there is some as yet unknown additional action of these compounds in the intact cell system.

These data suggest that SCH 32651 and SCH 28080 may inhibit the gastric ($H^+ + K^+$)-ATPase by a mechanism different from that of omeprazole. SCH 32651 and SCH 28080 appeared to be reversible, competitive inhibitors of the K^+-stimulated ATP hydrolysis, whereas omeprazole was not a competitive inhibitor of this reaction. The mechanism of action of omeprazole may involve oxidation of key sulfhydryl groups in the gastric ($H^+ + K^+$)-ATPase enzyme, whereas these studies showed that oxidation of sulfhydryl groups with DTT or β-mercaptoethanol had no effect on the ability of SCH 32651 and SCH 28080 to inhibit the isolated enzyme. SCH 28080 and SCH 32651 did not inhibit the ($Na^+ + K^+$)-ATPase, whereas omeprazole had the ability to inhibit this enzyme, especially under acidic conditions. Isolated parietal cell studies indicated that these compounds inhibit both the histamine and dibutyryl cAMP stimulation of [^{14}C]uptake with a relative potency order of SCH 28080 > omeprazole > SCH 32651.

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