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INHIBITIONS OF ACID SECRETION BY E3810 AND OMEPRAZOLE, AND THEIR REVERSAL BY GLUTATHIONE

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Abstract—A substituted benzimidazole 2{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl}-1*H*-benzimidazole sodium salt (E3810), is a gastric proton pump (H^+,K^+-ATPase) inhibitor. E3810 and omeprazole inhibited acid accumulation dose dependently as measured with aminopyrine uptake in isolated rabbit gastric glands, their IC_{50} values being 0.16 and 0.36 μM, respectively. The addition of exogenous reduced glutathione (GSH) to the gland suspension reactivated dose dependently the acid secretion which had been inhibited by 2 μM E3810 or omeprazole as a function of the incubation time. Furthermore, GSH at 1 and 3 mM reversed the antisecretory effect of E3810 more quickly than it did that of omeprazole. The antisecretory effect of E3810 was slightly greater than that of omeprazole in histamine-stimulated fistula dogs *in vivo*. The duration of the antisecretory activity of E3810 at concentrations of 2 and 4 mg/kg was shorter than that of omeprazole at the same concentrations in pentagastrin-stimulated fistula dogs. The reversal of the antisecretory activity of the inhibitors in dogs is suggested to be due to the action of endogenous extracellular GSH, in addition to *de novo* synthesis of the proton pump, because bullfrog gastric mucosae were found in the present study to secrete GSH into the mucosal solution at the rate of about 0.25 nmol/min/g tissue.

Two classes of compounds have been reported to inhibit specifically gastric H^+,K^+-ATPase i.e. the proton pump. These are reversible inhibitors such as SCH 28080 [1–4], scopadulcic acid [5], diacetyl scopadol [5] and SK&F 96079 [6], and irreversible inhibitors such as omeprazole [7–12], E3810‡ [13], HOE 731 [14, 15], BY 1023/SK&F 96022 [16], lansoprazole [17] and Ro 18-5364 [18]. The irreversible inhibitors do not react with H^+,K^+-ATPase directly; they must first be transformed into active compounds in an acidic environment such as the lumen of gastric glands or the intravesicular space of gastric vesicles [15, 19–23]. The transformation has actually been observed occurring in the acidic spaces [24]. Proteolysis studies have suggested that the activated omeprazole reacts with Cys^{321} of rat H^+,K^+-ATPase (and Cys^{322} of hog ATPase) [25].

One important question is how the parietal cell reactivates acid secretion to the normal level after the irreversible inhibition of the proton pump. There are two possible answers: (1) the enzyme–inhibitor complex may be dissociated slowly by an endogenous reducing compound such as the reduced form of glutathione (GSH) and (2) proton pumps are synthesized *de novo*. The half-life of the proton pump protein is about 30–48 hr so all of the pumps are replaced every 72–96 hr [26]. Omeprazole has a long duration of action *in vivo* [8] and, thus, it was postulated that recovery of the enzyme activity requires *de novo* enzyme synthesis. A recent study showed that the inhibitory action of HOE 731 in isolated parietal cells faded with increasing incubation time whereas inhibition by omeprazole remained unchanged with time. This indicates that the inhibitor–enzyme dissociation may occur with HOE 731 but not with omeprazole [15].

We reported previously that E3810 is more potent than omeprazole in inhibiting H^+,K^+-ATPase in hog gastric vesicles [13]. In this study we found that the addition of GSH to the suspension of rabbit gastric glands reversed the antisecretory effect of E3810 more quickly than it did that of omeprazole indicating that the dissociation of the inhibitor–enzyme complex is involved in the reactivation of the glands. This result is correlated with the present findings that the duration of the acid antisecretory effect of E3810 is shorter than that of omeprazole in dogs, and that GSH is secreted from frog gastric mucosae in the luminal solution.

MATERIALS AND METHODS

Chemicals. E3810 (Fig. 1) and omeprazole were synthesized by the Eisai Co., Ltd (Tokyo, Japan). Pentagastrin was obtained from ICI-Sumitomo (Osaka, Japan). Histamine dihydrochloride, GSH and rabbit albumin were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Collagenase type I, and *o*-phthaldialdehyde were obtained from Wako Pure Chemicals (Osaka, Japan), and Na_2-ATP from Oriental Yeast Co. (Osaka, Japan). Na-dibutyryl cAMP was obtained from the Daiichi Pharmaceutical Co. (commercial name: Actosin)

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‡ Abbreviations: GSH, reduced glutathione; E3810, 2{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl}-1*H*-benzimidazole sodium salt; AP, aminopyrine.

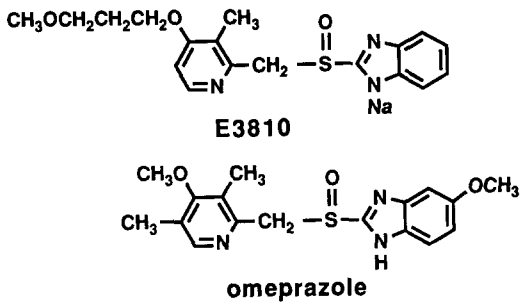

Fig. 1. Molecular structures of E3810 and omeprazole.

(Tokyo, Japan). [^{14}C]Aminopyrine was obtained from Amersham Japan (Tokyo, Japan). Other reagents used were of analytic grade.

Preparation of rabbit gastric glands. The glands were prepared from gastric mucosae of male Japanese white rabbits by collagenase digestion described elsewhere [27, 28]. Respiratory medium had the following composition in mM: 132.4 NaCl, 5.4 KCl, 5.0 Na_2HPO_4, 1.0 NaH_2PO_4, 1.2 $MgSO_4$, 1.0 $CaCl_2$, 10 mg/mL phenol red, 2 mg/mL bovine serum albumin and 2 mg/mL glucose, pH 7.4.

It is established that the [^{14}C]aminopyrine accumulation ratio (AP ratio: AP in intraglandular water/AP in incubation medium) is a good index of acid accumulation [29]. It is, therefore, used to determine parietal cell response to dibutyryl cAMP and proton pump inhibitor.

Samples of glands were incubated with the drug to be tested in the respiratory medium containing 0.065 μCi/mL [^{14}C]aminopyrine at 37° for 15 min in a shaking water bath. Then 1 mM dibutyryl cAMP was added followed by a 30 min incubation period. The glands were separated by a brief centrifugation. Wet and dry weights of the pellets were measured. Aliquots of the supernatant and the digested gland pellet were examined in a liquid scintillation counter and the AP ratio was calculated. The dry weight was multiplied by a factor of 2 (μL/mg) to approximate the total glandular water as determined by a previous study [27].

Time-course experiments were done over a total incubation period of up to 225 min. After preincubation of glands with 2 μM E3810 or omeprazole for 15 min at 37° 1 mM dibutyryl cAMP was added to the medium followed by an incubation period of 30 min. GSH (1 or 3 mM) was then added to the medium which was then incubated for up to the period indicated.

Gastric antisecretory effect in dogs stimulated by infusion of histamine. Experiments were performed in conscious male and female mongrel dogs, weighing 11–17 kg. These animals were provided with a conventional gastric fistula for sampling gastric juice. The gastric cannula was inserted in the corpus–antrum junction at the greater curvature. The animals were retained on a suspended sling (seating type; Natsume, Tokyo, Japan) when receiving tests. Experiments were started at least 3 weeks after surgery.

Gastric juice was drained through the implanted cannula into collection vessels. The volume of each sample collected over 20 min was determined. An aliquot of 1 mL was titrated to pH 7.0 with 40 mM NaOH. The acid output was calculated as: secretory volume × acid concentration.

Food was withdrawn 21 hr before each experiment, but the dogs had free access to water. All tests were started by checking that no spontaneous secretion was present. Acid secretion was stimulated by intravenous infusion of histamine via a catheter in the jugular vein for 3 hr at a rate of 0.1 mg/kg/hr. Collection of juice began immediately after the start of infusion of the stimulant and continued for the 3 hr period. Proton pump inhibitors were given to acid secreting dogs 60 min after the start of infusion of the stimulant: a solution of E3810 or a suspension of omeprazole in 0.2% $NaHCO_3$–0.5% methyl cellulose (0.1 mL/kg) was given intraduodenally through silicone tubing which had been inserted via the fistula on the previous day. Any drug remaining in the silicone tube was flushed into the duodenum with 2 mL of normal saline. In control experiments, the animals received the vehicle only. The acid output was expressed as mEq/20 min for each animal at each dose and time period. Experiments were performed once a week on each dog to avoid the cumulative inhibitory effect of drugs with a relatively long duration of action.

Duration of the antisecretory effect in dogs stimulated with pentagastrin. Six dogs with chronic gastric fistulas were separated into two groups of three. One group was tested initially with E3810 and with omeprazole one month later. The other group received the two tests in the inverse order. Experimental numbers were, therefore, six for each drug. E3810 or omeprazole was given to dogs (unstimulated) fasted for 21 hr at the doses indicated (see Results) as a single intraduodenal administration only on the first test day. At 1, 24 and 48 hr after drug administration 6 μg/kg of pentagastrin were injected intramuscularly to induce gastric secretion. Collection of gastric juice began immediately after the injection of pentagastrin and was continued for 2 hr. The total acid output was expressed as mEq/2 hr. Dogs were given food once a day after the end of each test. In a separate test the acid output induced by injection of pentagastrin in fistula dogs to which no drug had been given was determined as the pretreatment level.

Efflux study of GSH from bullfrog gastric mucosae in vitro. Bullfrog gastric mucosae were stripped of muscle layers and mucus on the mucosal surface was wiped off with paper. Then each mucosa was mounted as a flat sheet between fluid-filled Ussing chambers as described elsewhere [30, 31]. The mucosal area was 2 cm^2 and the volume of each chamber solution was 5 cm^3. Both solutions at 24° were stirred and gassed with 95% O_2–5% CO_2 during experiments. The serosal solution had the following composition in mM: 105 Na^+, 5 K^+, 1 Mg^{2+}, 2 Ca^{2+}, 97 Cl^-, 18 HCO_3^-, 1 $H_2PO_4^-$ and 11 glucose. The mucosal solution was 120 mM NaCl. The acid secretory rate and the transmucosal potential difference were measured for 3–4 hr as described elsewhere [30]. Every 20 min both mucosal

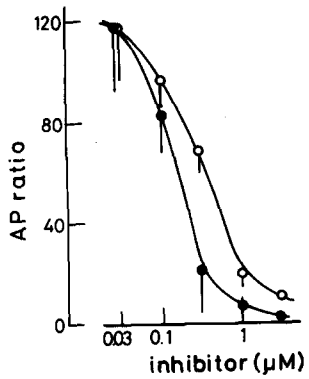

Fig. 2. Inhibitory effects of E3810 and omeprazole on [^{14}C]AP uptake in isolated rabbit gastric glands. The glands were incubated in the presence of E3810 (●) or omeprazole (○) at the indicated concentration for 15 min at 37°. Then, 1 mM dibutyryl cAMP was added followed by another incubation period of 30 min. Values are means ± SE (three different preparations).

and serosal solutions were exchanged for new solutions and the mucosal solutions were collected. To 2.7 mL of the mucosal solution 0.1 mL of 25% HPO_3 (phosphoric acid meta) was added immediately and the solution was stood on ice until used for the determination of GSH concentration.

The concentration of GSH in the mucosal solution was determined as described elsewhere [32]. The mucosal solution was neutralized by the addition of 0.2 mL of 1 M NaOH. The solution was then centrifuged at 1600 *g* for 30 min at 3° and the supernatant was collected. To 1 mL of the supernatant 0.9 mL of phosphate buffer containing 90 mM Na_2HPO_4, 10 mM NaH_2PO_4 and 5 mM Na_4-EDTA (pH 8.0) was added followed by addition of 0.1 mL of 0.1% *o*-phthaldialdehyde. The solution was incubated for 15 min at 25° and then transferred to a quartz cuvette. The fluorescence with excitation and emission wavelengths of 350 and 420 nm, respectively, was measured. GSH dissolved in the phosphate-EDTA buffer (pH 8.0) was used for obtaining a calibration curve.

Statistics. Data are expressed as means ± SE. ED_{50} values were calculated from the dose-inhibition relation by the method of least squares. Ninety-five per cent fiducial limits of ED_{50} were calculated according to Fieller's theorem [33]. The statistical significance of the difference between groups was determined by Student's *t*-test for paired values and differences were regarded as significant if $P < 0.05$.

RESULTS

Dose-dependent inhibition of [^{14}C]aminopyrine uptake in isolated rabbit glands by E3810 and omeprazole

Isolated gastric glands accumulate acid in the intracellular canaliculi of parietal cells when stimulated by histamine or dibutyryl cAMP. In the experiment shown in Fig. 2, glands were incubated with 1 mM dibutyryl cAMP for 30 min. Proton pump inhibitors such as E3810 and omeprazole accumulate in the acidic space, transform into active compounds and bind to a specific SH group of the H^+,K^+-ATPase on the luminal side, resulting in inhibition of the ATPase activity. Figure 2 shows dose-dependent inhibition of acid accumulation by E3810 and omeprazole. IC_{50} values for E3810 and omeprazole were 0.16 ± 0.03 and 0.36 ± 0.14 μM,

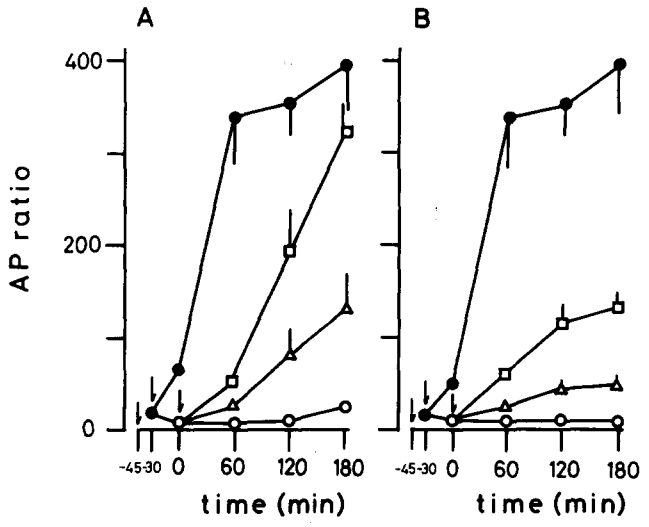

Fig. 3. Reversal of the antisecretory activities of E3810 and omeprazole in isolated rabbit glands. The incubation of glands was started (first arrow) in the presence (○, △, □) and absence (●) of 2 μM E3810 (A) or 2 μM omeprazole (B). Then, 1 mM dibutyryl cAMP was added (second arrow) to the gland suspension followed by another incubation period of 30 min. GSH, 1 (△) or 3 mM (□), was added (third arrow) followed by incubation for indicated periods. Values are means ± SE (three different preparations).

respectively, indicating that E3810 is more potent than omeprazole in inhibiting the proton pump in isolated rabbit gastric glands.

Reversal of the inhibitory effects of E3810 and omeprazole by GSH in gastric glands

In this experiment, we used E3810 and omeprazole at the same dose of 2 μM which almost completely inhibited the acid accumulation as measured using the AP ratio in rabbit gastric glands (Figs 2 and 3). When glands were incubated in the absence of inhibitors with 1 mM dibutyryl cAMP for 90–210 min, the AP ratio increased to 350–400 (Fig. 3). Figure 3A and B shows time-course profiles for reversal of the antisecretory effects of E3810 and omeprazole by exogenous GSH. GSH at 1 and 3 mM when added to the glands which had been exposed to omeprazole for 45 min reversed the antisecretory effect of the drug as a function of incubation time (Fig. 3B). But GSH at these concentrations did not fully restore the acid secretory activity of the omeprazole-treated glands to the same level as that of the untreated as previously reported [34].

On the other hand, GSH both at 1 and 3 mM reversed the antisecretory effect of E3810 more quickly than that of omeprazole (Fig. 3A). Within 3 hr, GSH at 3 mM fully restored the acid secretory rate of the E3810-treated glands to the same level as that of the untreated. Without exogenous GSH the reversal of the antisecretory effects of these inhibitors was not observed during the experimental period of 210 min.

Effects of E3810 and omeprazole on histamine-stimulated gastric acid secretion

We investigated the antisecretory activities of E3810 and omeprazole in dogs before determining whether the duration of the effect of E3810 was shorter than that of omeprazole.

Infusion of histamine induced gastric acid secretion. In the control experiment the maximal secretion was observed in the fourth 20-min collection period (Figs 4 and 5) and subsequently decreased slightly with time. The mean rate of secretion at zero time (Figs 4 and 5) (60 min after commencement of the histamine infusion) in the control dogs was 7.71 ± 0.76 mEq/20 min (N = 4) and the rate ranged from 6.03 to 9.09 mEq/20 min.

Figures 4 and 5 show that E3810 and omeprazole, respectively, dose-dependently inhibited the acid secretion. The drugs were administered intraduodenally 60 min after the start of the histamine infusion. The dose of 500 μg/kg of E3810 and that of 1000 μg/kg of omeprazole completely inhibited the acid secretion within 40 min. With the dose of 500 μg/kg of omeprazole almost complete inhibition was observed 90 min after administration. When the responses were measured 2 hr after drug administration the values of ED_{50} for E3810 and omeprazole were 62 (29–96, 95% fiducial limits) and 98 (30–166) μg/kg, respectively. These results indicate that the inhibitory potency of E3810 is slightly greater than that of omeprazole although the difference is not significant.

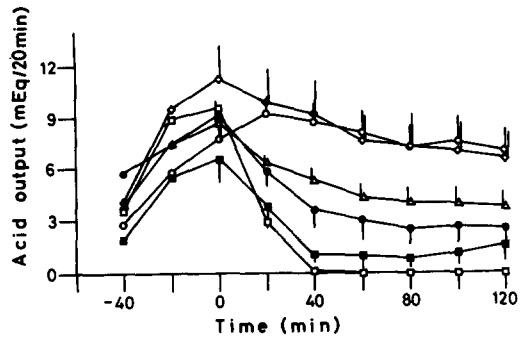

Fig. 4. Antisecretory effect of intraduodenal administration of E3810 on histamine-stimulated acid secretion in gastric fistula dogs. The acid output at 0 min, for example, represents the total output in juice collected during the period of −20 to 0 min. E3810 was administered at zero time. Infusion of histamine (100 μg/kg/hr) was started 1 hr before administration of E3810. Doses of E3810 were (○) 0; (◇) 31; (△) 63; (●) 125; (■) 250; (□) 500 μg/kg. Values were averages ± SE (N = 3–4).

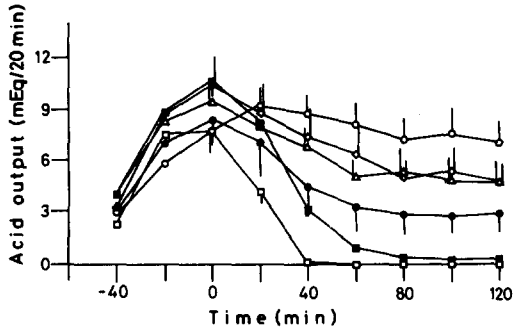

Fig. 5. Antisecretory effect of intraduodenal administration of omeprazole on histamine-stimulated acid secretion in gastric fistula dogs. Experimental details were as described in the legend of Fig. 4. Doses of omeprazole were (○) 0; (◇) 63; (△) 125; (●) 250; (■) 500; (□) 1000 μg/kg.

Duration of the antisecretory effect of inhibitor in pentagastrin-stimulated dogs

In the experiments shown in Figs 6 and 7 drug was given as a single intraduodenal administration to dogs fasted for almost 1 day. Then, pentagastrin was intramuscularly injected to induce acid secretion at the times indicated in the figures. The pretreatment level in pentagastrin-stimulated dogs was 15.6 ± 1.2 mEq/2 hr (N = 35). As previously reported [35], higher doses of proton pump inhibitor were required to induce a complete inhibition of acid secretion when the inhibitor was given to fasted (non secreting) dogs *in vivo*. We found that E3810 and omeprazole at doses greater than 2 mg/kg completely inhibited the acid secretion measured 1 hr after the administration of the drugs.

Figure 6 shows the effects of E3810 and omeprazole, at the same dose of 2.0 mg/kg, on acid output. One day after drug administration the acid

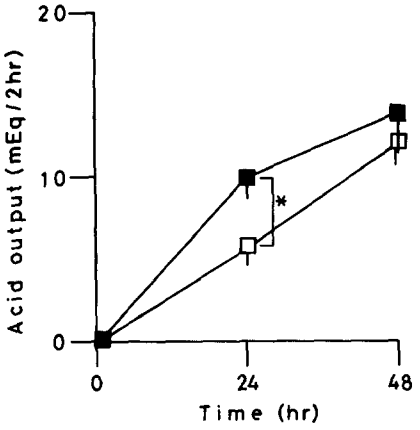

Fig. 6. Acid secretion stimulated by pentagastrin after single dose administrations of E3810 and omeprazole in gastric fistula dogs. Drug (2 mg/kg) was administered at 0 min. Collection of gastric juice was started 1, 24 and 48 hr after administration of drug and continued for 2 hr. Values are mean ± SE (N = 6). The pretreatment level in pentagastrin-stimulated dogs was 15.6 ± 1.2 mEq/2 hr (N = 35). (■) E3810; (□) omeprazole. * $P < 0.05$ between E3810 and omeprazole.

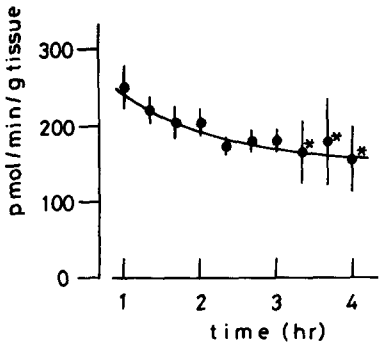

Fig. 8. The efflux of GSH from gastric mucosae of bullfrog into the mucosal solution. Each mucosa was set between Ussing chambers. The serosal and mucosal solutions were exchanged every 20 min for new solutions. The mucosal solution was collected and the concentration of GSH in the solution was determined by the method described elsewhere [34]. The time after the start of the chamber experiment is indicated. The experiments were done during the period from July to November. Values are means ± SE for 21–22 different frogs (7–8 different frogs for the values marked with *).

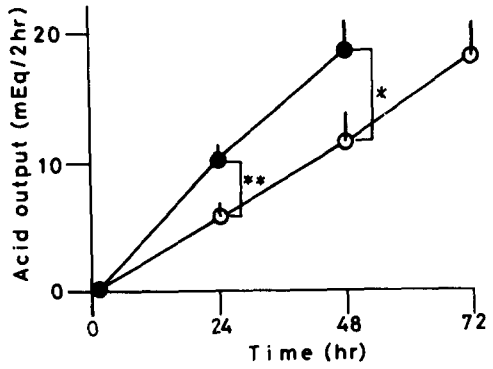

Fig. 7. Duration of antisecretory effects of E3810 and omeprazole at the same dose of 4.0 mg/kg. Experimental conditions were the same as those described in the legend of Fig. 6. Values are mean ± SE (N = 6). (●) E3810; (○) omeprazole. * $P < 0.05$ between E3810 and omeprazole. ** $P < 0.01$ between E3810 and omeprazole.

output was 10.2 ± 1.2 mEq/2 hr and 6.1 ± 1.3 mEq/2 hr, in the case of E3810 and omeprazole, respectively and the difference was significant ($P < 0.05$). At this dose, the acid output returned to the pretreatment level within 48 hr in both E3810- and omeprazole-treated dogs.

Figure 7 shows the duration of the antisecretory effects of E3810 and omeprazole at the same dose of 4.0 mg/kg. One day after drug administration the acid output in E3810- and omeprazole-treated dogs was 10.1 ± 1.2 and 5.8 ± 1.2 mEq/2 hr, respectively. This difference in acid output was significant ($P < 0.01$). Two days after administration, the corresponding values were 18.6 ± 2.4 and 11.5 ± 2.4 mEq/2 hr. This difference is also significant ($P < 0.05$). The acid output in E3810-treated dogs recovered to the pretreatment level within 2 days and that in omeprazole-treated dogs recovered within 3 days.

These results suggest that the recovery of pentagastrin-stimulated acid secretion in E3810-treated dogs was significantly faster than in omeprazole-treated dogs when compared at doses of 2 and 4 mg/kg.

Efflux of GSH from frog gastric mucosae into the mucosal solution

The proton pump inhibitors are activated in the acidic luminal space. The cationic activated compound binds to the specific cysteine residue on the luminal side of H^+,K^+-ATPase [25]. If there was a GSH efflux from gastric mucosae into the luminal space there would be a possibility that the extracellular GSH at loci dissociates the inhibitor–H^+,K^+-ATPase complex *in vivo* resulting in reactivation of the ATPase activity. Therefore, we investigated whether such an efflux occurred. We used bullfrog gastric mucosae because they maintain steady state conditions for a long time such as 8 hr at 25°. In mammalian experiments at an elevated temperature of 37° GSH secreted may be converted into oxidized glutathione at greater rates than in experiments at 25° [36]. Furthermore, isolated cells and gastric glands are not suitable models because the incubation solution mostly makes contact with the basolateral membranes of the cells and it is, thus, impossible to measure the efflux across only the luminal cell membranes.

Figure 8 shows that bullfrog gastric mucosae secrete GSH into the mucosal solution. The efflux rate of GSH at 1 hr after the start of the chamber experiment was 0.25 ± 0.03 nmol/min/g of wet tissue

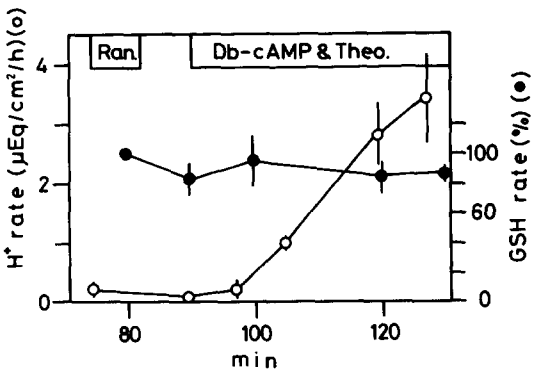

Fig. 9. Acid secretion (○) and GSH efflux (●) from bullfrog gastric mucosae into the mucosal solution. Each mucosa was set in Ussing chambers at zero time. The GSH efflux rate at 80 min was taken as 100%. The presence of 50 μM ranitidine (Ran.) or 1 mM dibutyryl cAMP (Db-cAMP) plus 1 mM theophylline (Theo.) is indicated by top bars. Values are mean ± SE for three frogs.

for 22 bullfrogs. Figure 9 shows that the efflux rate of GSH does not depend on the acid secretory rate. In the experiment, the spontaneously secreting rate of acid was low after 70 min incubation in the absence of secretagogues. When 50 μM ranitidine were added to the serosal solution acid secretion almost completely stopped. After washing out the ranitidine, 1 mM dibutyryl cAMP plus 1 mM theophylline were added which increased acid secretion to the normal level found previously [30]. During the period of this experiment, the efflux rate of GSH did not depend on the rate of acid secretion indicating that the efflux of GSH into the lumen is not linked to acid secretion.

DISCUSSION

E3810 is more potent than omeprazole in inhibiting the acid secretion in isolated rabbit glands (Fig. 2). The IC_{50} values were 0.16 and 0.36 μM for E3810 and omeprazole, respectively. In isolated gastric vesicles E3810 is about six times more potent than omeprazole under conditions that insured that these inhibitors were activated only in the intravesicular space [13].

Exogenous 3 mM GSH restored fully the acid secretory activity of isolated rabbit gastric glands treated with E3810 to the same level as that of untreated glands, but only partially restored the activity of omeprazole-treated glands (Fig. 3). Since GSH is a membrane-impermeable tripeptide exogenous GSH is not likely to penetrate into the cytosol of cells in the glands. Furthermore, the H^+,K^+-ATPase activity in gastric vesicles is completely inhibited by E3810 and omeprazole even in the presence of extravesicular (cytosolic) 1 mM GSH [13]. These results indicate that cytosolic GSH is not involved in the restoration of acid secretion in the glands.

It is reported that the inhibition caused by omeprazole in gastric vesicles is not reversed by cytosolic 2 mM GSH but the inhibition by Hoe 731 is reversed by cytosolic GSH [15]. The inhibitory action of Hoe 731 in isolated parietal cells decreased with incubation time whereas inhibition caused by omeprazole remained unchanged with time [14, 15]. Beil *et al.* [15] suggested that cytosolic GSH was involved in the reactivation of ATPase activity that has been inhibited by Hoe 731. These observations suggest that the GSH-induced reactivation of H^+,K^+-ATPase that has been inhibited by E3810 or omeprazole in isolated gastric glands is due to a different mechanism from that in the case of Hoe 731 inhibition. The reactive site of GSH in E3810- or omeprazole-treated glands is on the luminal side of the H^+,K^+-ATPase. Therefore, it is considered that a small amount of GSH slowly penetrates from the medium to the lumen of the secretory canaliculi through incompletely closed pathways, scavenges the activated inhibitor in the acidic space [34] and finally dissociates the inhibitor–enzyme complex resulting in the reactivation of the ATPase.

Before studying whether there is a difference between the duration of the antisecretory effects of E3810 and omeprazole in dogs, we measured the antisecretory effects of these inhibitors. The maximal rate of acid secretion induced by continuous intravenous infusion of histamine at 100 μg/kg/hr was 23 mEq/hr. E3810 and omeprazole dose-dependently inhibited the acid secretion and the ED_{50} values were 62 and 98 μg/kg, respectively. The corresponding value reported previously for omeprazole was 90 μg/kg [8].

The total acid output (0–2 hr) induced by a single intramuscular injection of pentagastrin (6 μg/kg) was 16 mEq and the corresponding value reported previously was 18 mEq [37]. The duration of antisecretory activity of E3810 was shorter than that of omeprazole in pentagastrin-stimulated fistula dogs when compared at the same drug doses of 2 (Fig. 6) and 4 mg/kg (Fig. 7). If the complete reactivation of acid secretion *in vivo* were due to only the *de novo* synthesis of H^+,K^+-ATPase the duration of the inhibitory effect of E3810 would be the same as that of omeprazole. The present finding indicates that dissociation of the inhibitor–H^+,K^+-ATPase complex also occurs *in vivo* and that E3810 dissociates from the H^+,K^+-ATPase more quickly than does omeprazole. For the reducing agent, endogenous GSH secreted from cells in the gastric mucosa may be considered because frog gastric mucosae *in vitro* secrete GSH into the lumen (Fig. 8). The efflux of GSH has been found in a variety of cells and organs [38] such as liver [35, 39], heart cells [40], fibroblasts [41] and lymphoid cells [42]. It is known that the efflux occurs independently of the subsequent enzymatic degradation. Thus, cells hitherto known to have a rapid turnover rate of GSH usually exhibit rapid export of GSH [43]. The extracellular GSH functions as a reducing agent which protects membranes by destroying reactive oxygen intermediates and free radicals formed physiologically [43]. GSH and other thiols are known to be present in mammalian and amphibian gastric mucosae and GSH is the predominant thiol [44, 45]. In the present study we found that GSH was secreted from the bullfrog gastric mucosae into the mucosal

solution at the rate of 0.25 nmol/min/g tissue. A similar extent of GSH efflux was observed in rat heart (0.37 nmol/min/g) [40]. Liver, however, secretes GSH into blood at the rate of 12.4 nmol/min/g and into bile at the rate of 3.4 nmol/min/g [39].

In the GSH efflux experiment the mucosal solution was exchanged every 20 min and collected. The GSH concentration in the collected solution was about 0.4 μM. GSH concentrations in the lumen of the secretory canaliculi are expected to be much higher than 0.4 μM. Thus, there is a possibility that the endogenous extracellular GSH slowly dissociates the inhibitor–H^+,K^+-ATPase complex under the assumption that (1) neither inhibitor affects the normal rate of pump resynthesis either directly or indirectly and (2) both compounds are completely cleared from the blood before the second period of pentagastrin stimulation.

Although we did not explore the possibility that thiols other than GSH dissociate the inhibitor–enzyme complex it should not be excluded from consideration that, for example, γ-glutamyl-glutathione which is predominantly extracellular exhibits reducing properties at the site [46].

We conclude that E3810 is an effective inhibitor of gastric acid secretion in dogs with a shorter duration of antisecretory activity than that of omeprazole. *De novo* synthesis of H^+,K^+-ATPase and the dissociation of the inhibitor–enzyme complex by endogenous extracellular GSH are suggested as contributing to the reversal of the antisecretory activity in dogs.

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