Biochemical Pharmacology, Vol. 37, No. 23, pp. 4487–4493, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

OMEPRAZOLE, SCH 28080 AND DOXEPIN DIFFER IN THEIR CHARACTERISTICS TO INHIBIT H^+/K^+-ATPase DRIVEN PROTON ACCUMULATION BY PARIETAL CELL MEMBRANE VESICLES*

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(*Received* 5 *February* 1988; *accepted* 18 *June* 1988)

Abstract—The effects of omeprazole, SCH 28080 and doxepin were studied on H^+/K^+-ATPase mediated H^+ accumulation in parietal cell membrane vesicles. Omeprazole had no effect on the initial rate of H^+ accumulation and the initial steady state concentration of H^+; an inhibition was found after the vesicles were acidified. This inhibition was counteracted by the SH reducing agent dithioerythritol. SCH 28080 inhibited the initial rate of H^+ accumulation and the steady state H^+ concentration. The inhibitory effect of SCH 28080 was counteracted by KCl. Doxepin (3–100 μM) reduced the initial steady state H^+ concentration. Doxepin concentrations lower than 0.5 μM had no such effect but dissipated the proton gradient after the vesicles were fully acidified. This doxepin effect was partially counteracted by KCl and was also obtained in vesicles in which the pump reaction was stopped by EDTA. These data show that (i) omeprazole is an acid-activated compound which interferes with SH groups of the H^+/K^+-ATPase localized inside the vesicles; (ii) SCH 28080 interferes with the K^+ site of the H^+/K^+-ATPase; and (iii) doxepin interacts by a K^+ antagonistic activity at the H^+/K^+-ATPase site and in addition by intravesicular neutralization and/or a protonophoric mechanism with the process of H^+ formation.

Several antisecretory drugs such as the substituted benzimidazole omeprazole [1], the pyridyl (1, 2a) imidazole SCH 28080 [2–4], the neuroleptics and antidepressants trifluoperazine, doxepin and trimipramine [5, 6] interfere with the gastric proton pump, H^+/K^+-ATPase. The nature of the inhibitory reactions of these drugs with the H^+/K^+-ATPase can be described as follows: omeprazole itself is inactive, but it is converted at low pH into a compound which reacts with thiol groups of the H^+/K^+-ATPase [1, 7]. SCH 28080 interacts competitively with the luminal K^+ binding site of the enzyme [2, 3]. Conflicting results exist about the mechanism of action of neuroleptics. Im *et al.* [5] have reported that these drugs interfere competitively with the K^+ site of the H^+/K^+-ATPase, whereas we have found that these drugs, as well as antidepressants, interact by an allosteric mechanism at the K^+ site of the enzyme [6].

The *in vitro* test systems used for studying H^+/K^+-ATPase inhibitors range from intact gastric glands, isolated and enriched parietal cells to parietal cell membrane preparations. Parietal cell membranes containing H^+/K^+-ATPase activity can be prepared as leaky or intact vesicles. With intact vesicles drug effects can be studied on the transport function of the enzyme, the ATPase mediated H^+/K^+ exchange reaction. This approach may be superior to leaky membrane preparations since it resembles more the *in vivo* situation present in the intact parietal cell where the enzyme is localized at an asymmetric pH environment with the cytosolic site exposed to neutral pH and the luminal face exposed to a low pH. This report describes the nature of the action of omeprazole, SCH 28080 and doxepin on H^+/K^+-ATPase mediated H^+ accumulation on parietal cell membrane vesicles.

MATERIALS AND METHODS

Chemicals. Na_2-ATP, ionophores, acridine orange and trifluoperazine were from Sigma (Munich, F.R.G.). Omeprazole was kindly donated by Dr. E. Carlsson (AB Hässle, Mölndal, Sweden); SCH 28080 was generously supplied by Schering-Plough Corporation (Bloomfield, NJ); trimipramine was obtained from Rhône-Poulenc (Norderstedt, F.R.G.) and doxepin from Pfizer (Karlsruhe, F.R.G.).

Preparation of intact parietal cell membrane vesicles. Intact membrane vesicles were prepared from pig gastric mucosa. Scraped fundic mucosa was minced in 20 mM Tris–HCl buffer (pH 7.0) containing 0.25 M sucrose and 1 mM EDTA with a tissue-chopper (Moulinette). The minced material was filtered through a nylon mesh and was then homogenized by six up–down strokes with a Teflon–glass homogenizer. The 20,000 *g* supernatant of the homogenate was centrifuged for 60 min at 100,000 *g*. The resulting pellet was resuspended in buffer, flash frozen, and stored at −80°.

The ATPase activity in this crude microsomal fraction was 14.3 μmol P_i/mg protein per hr in the presence of $MgCl_2$ alone, 23.6, 33.7 and 79.9 μmol P_i/mg protein per hr in the presence of KCl (10 mM), KCl plus valinomycin (20 μg/ml) and KCl plus gramicidin (20 μg/ml) respectively. In the presence of 150 mM KCl the respective values were 21.8, 37.5 and 62.5 μmol P_i/mg protein per hr (values: mean

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from the two vesicle preparations used in this study). K^+ and K^+ plus valinomycin-stimulated ATPase activity remained unchanged when the KCl concentration was increased to 350 mM, whereas the K^+ plus gramicidin-stimulated ATPase activity was decreased to 49 μmol P_i/mg protein per hr. When 20 mM EDTA was present in the assay, no ATPase activity was detected.

ATPase activity was measured for 15 min at 37° in 1 ml samples containing 10 mM Pipes/Tris buffer (pH 7.3), 0.25 M sucrose, 0.1 mM EGTA, 2 mM $MgCl_2$, 2 mM ATP and 15 μg protein. When 150 mM or higher KCl concentrations were used, sucrose was omitted from the assay. P_i was determined according to the method of Carter and Karl [8] as described in detail previously [6].

Proton transport measurement. Vesicular accumulation of H^+ was studied at 37° in 2 ml samples containing 10 mM Pipes/Tris buffer (pH 7.0), 2 mM $MgCl_2$, 150 mM KCl, 120 μg membrane protein, 2 mM ATP and 10 μM acridine orange. Proton formation was initiated by addition of the K^+ ionophore valinomycin (30 μg) and was measured by the change in the fluorescence intensity of the dye acridine orange according to the method described by Lee and Forte [9]. Wavelengths used were 493 (excitation) and 530 nm (emission) in a SPF-500 Ratio Spectrofluorometer (American Instruments Company). All proton transport studies were done in duplicate with two different vesicle preparations. The individual variations found in the experiments with omeprazole and SCH 28080 were less than 5%; in contrast, the experiments with doxepin, trifluoperazine and trimipramine were more variable and dependent on the age of the vesicle (time after thawing). The experiments shown with doxepin were done within 2 hr after thawing the vesicles.

Protein determination. Protein was determined according to Lowry *et al.* [10] using bovine serum albumin as standard.

RESULTS

General properties of the vesicle preparation used. When vesicles were incubated in a $MgCl_2$, ATP and KCl containing buffer medium and the K^+ ionophore valinomycin was added, which allows access of KCl to the K^+ stimulatory site of the enzyme inside the vesicles, the initial fluorescence of acridine orange was increasingly quenched demonstrating intravesicular acidification. The vesicles used were able to maintain this H^+ gradient for about 45 min. When 1, 5 and 10 mM of the weak base aminopyrine were present in the incubation mixture, the original acridine orange quench was reduced by 20, 45 and 80%, respectively demonstrating intravesicular neutralization (data not shown). The effect of the K^+/H^+ ionophore nigericin is shown in Fig. 1. Nigericin (1 μM) totally abolished valinomycin induced H^+ accumulation (Fig. 1, curve D). At lower concentrations (0.05 μM) nigericin did not affect the initial steady state proton concentration but it slowly dissipated the H^+ gradient (Fig. 1, curve B).

Effects of omeprazole. Omeprazole (10, 30 and 50 μM) added to the vesicles before starting the pump reaction had almost no effect on the initial rate of proton accumulation and the initial steady state proton concentration. It inhibited the pump reaction after the vesicles were fully acidified (Fig. 2). Addition of 10 μM omeprazole to the vesicles at the steady state of proton concentration resulted in pump inhibition after a short lag period reaching a new steady state after approximately 30 min (Fig. 3). When the SH reducing agent dithioerythritol was

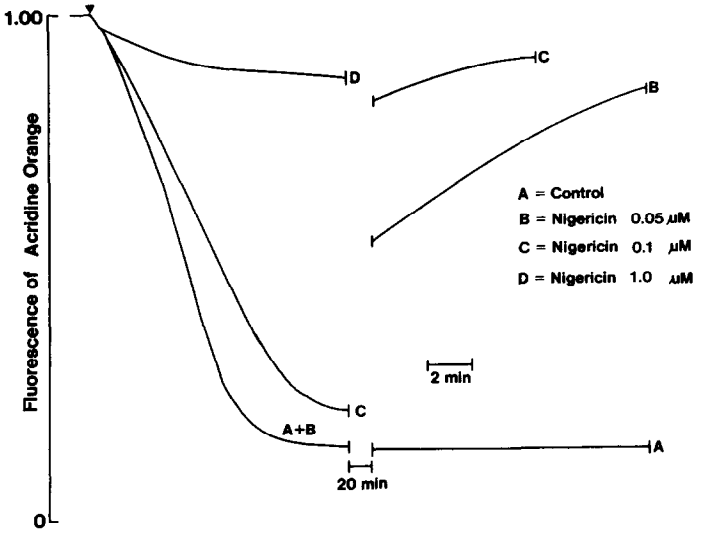

Fig. 1. Effects of different concentrations of the H^+/K^+ ionophore nigericin on H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. The incubation system was: 2 ml 10 mM Pipes/Tris buffer (pH 7.0), 2 mM $MgCl_2$, 150 mM KCl, 2 mM ATP, 0.12 mg membrane protein and 10 μM acridine orange. Nigericin was added to the vesicles in concentrations indicated before starting the pump reaction with 30 μg valinomycin (▼). Fluorescence quenching of acridine orange was measured as described in Materials and Methods.

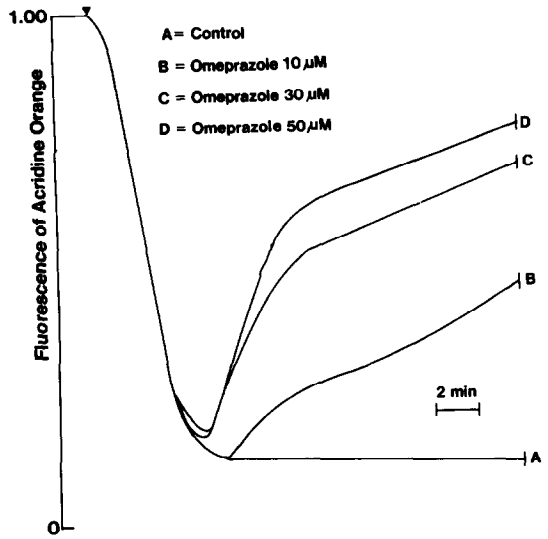

Fig. 2. Effect of increasing concentrations of omeprazole on H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 1. Omeprazole (dissolved in methanol) was added to the vesicles immediately before starting the pump reaction by valinomycin (▼).

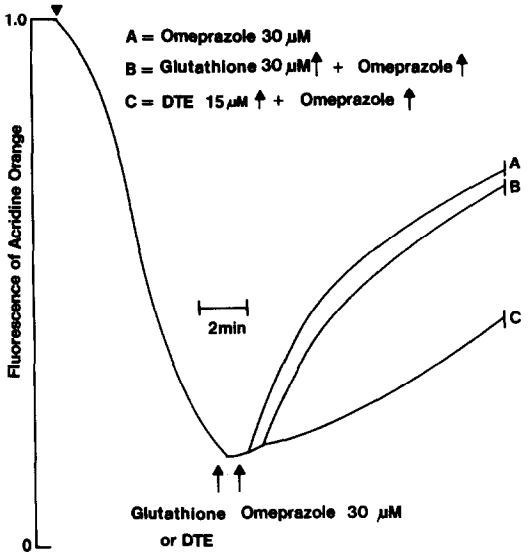

Fig. 4. Comparison of dithioerythritol and glutathione for their ability to prevent the effect of omeprazole on H^+/K^+-ATPase mediated performed H^+ gradient. Experimental conditions were as described in Fig. 1. DTE (15 μM) or glutathione (30 μM) were added before omeprazole (30 μM) as indicated by arrows.

added to the vesicles at this particular point in time, the pump function was restored (Fig. 3). In contrast, the less hydrophobic SH-reducing agents dithiothreitol and glutathione failed to restore the pump activity (data not shown). Furthermore, it was found that dithioerythritol (15 μM) but not glutathione (30 μM), could prevent omeprazole (30 μM) induced inhibition (Fig. 4). Fifty μM omeprazole produced a rapid and more pronounced inhibition when added to vesicles at the steady state of proton concentration (Fig. 5, curve B).

When omeprazole was added to vesicles in which maximal acidification was prevented by 5 mM aminopyrine, the pump became inhibited after a lag period and the degree of inhibition was reduced (Fig. 5, curve D). Addition of omeprazole up to 50 μM to vesicles, in which the pump reaction was stopped by EDTA (final concentration 20 mM) at the steady state proton concentration did not alter the rate of dissipation of the H^+ gradient, demonstrating that the drug did not act by intravesicular neutralization or as a protonophore (data not shown).

Effects of SCH 28080. SCH 28080 inhibited the initial rate of H^+ accumulation and steady state H^+ concentration (Fig. 6). Addition of 1 μM SCH 28080 at steady state H^+ concentration resulted in a rapid dissipation of the H^+ gradient which could be counteracted stepwise by increasing the medium KCl

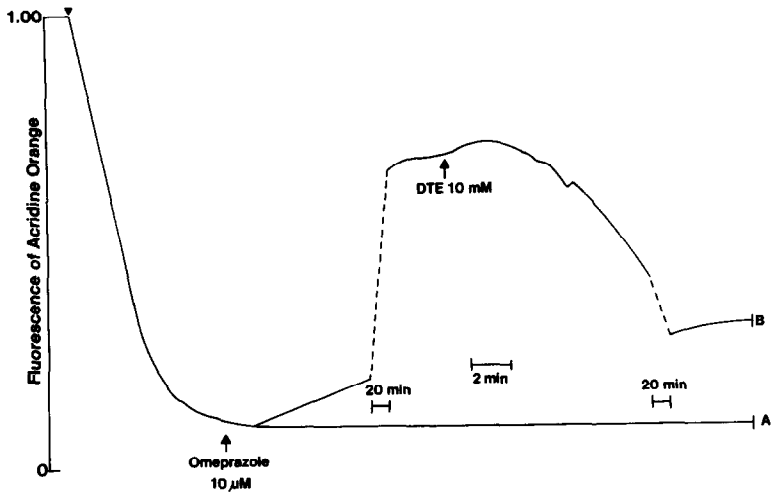

Fig. 3. Reversibility of the omeprazole-induced inhibition of H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 1. Omeprazole and the SH-reducing agent dithioerythritol (DTE) were added at the time indicated by arrows.

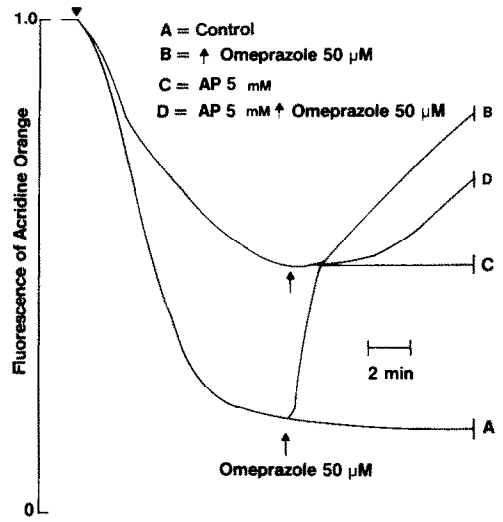

Fig. 5. Effect of 50 μM omeprazole added at the steady state level of H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 1, with the modification that 5 mM aminopyrine (AP) was present in samples C and D. Omeprazole was added at the time indicated by arrows.

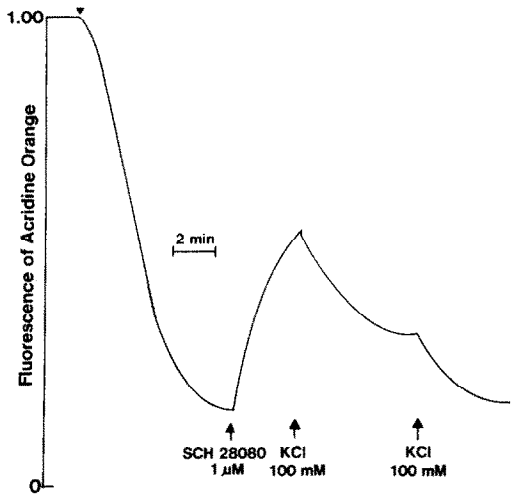

Fig. 7. Reversibility of SCH 28080-induced inhibition of H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 1. One μM SCH 28080 and KCl (so that the final KCl concentration was 250 and 350 mM in the medium) were consecutively added at the time indicated by arrows.

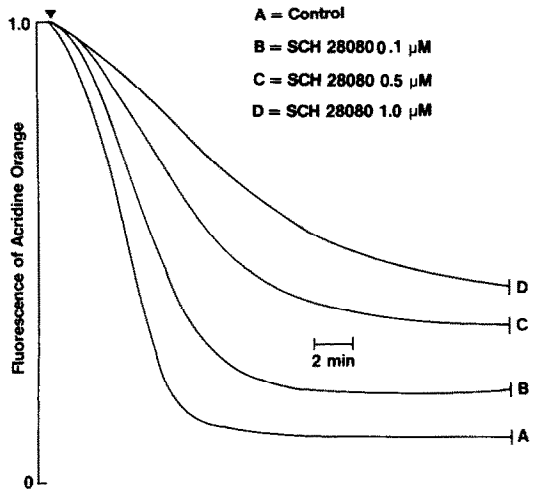

Fig. 6. Effects of different concentrations of SCH 28080 on H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 1. SCH 28080 (dissolved in dimethylsulphoxide) was added to the vesicles before starting the pump reaction by valinomycin (▼).

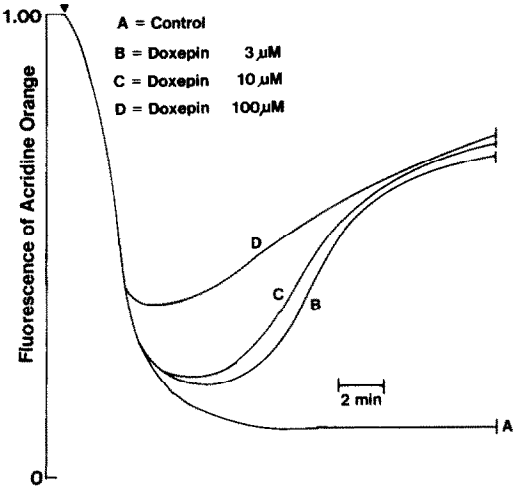

Fig. 8. Effect of different concentrations of doxepin (3, 10, 100 μM) on H^+/K^+-ATPase mediated H^+ accumulation in parietal cell vesicles. Experimental conditions were as described in Fig. 1. Doxepin (dissolved in H_2O) was added to the vesicles before starting the pump reaction by valinomycin (▼).

concentration to 250 and 350 mM, respectively (Fig. 7). Using vesicles in which the pump reaction was stopped by EDTA, the dissipation rate of the H^+ gradient was enhanced by SCH 28080, demonstrating that the drug has some protonophoric effect (data not shown).

Effects of doxepin. Doxepin at concentrations ranging from 3 to 100 μM reduced the initial steady state H^+ concentration (Fig. 8). At concentrations lower than 0.5 μM the drug failed to affect the initial steady state H^+ concentration but gradually dissipated the H^+ gradient (Fig. 9). Addition of doxepin to the vesicles at the steady state of H^+ concentration resulted in a rapid disruption of the H^+ gradient (Fig. 10). The H^+ gradient was partly and transiently restored when KCl was added immediately after application of doxepin (Fig. 10, curve B). When doxepin was allowed to react for longer the KCl effect was less pronounced (Fig. 10, curve C). Addition of doxepin to vesicles in which the pump reaction was stopped by EDTA resulted in a disruption of the preserved H^+ gradient, demonstrating

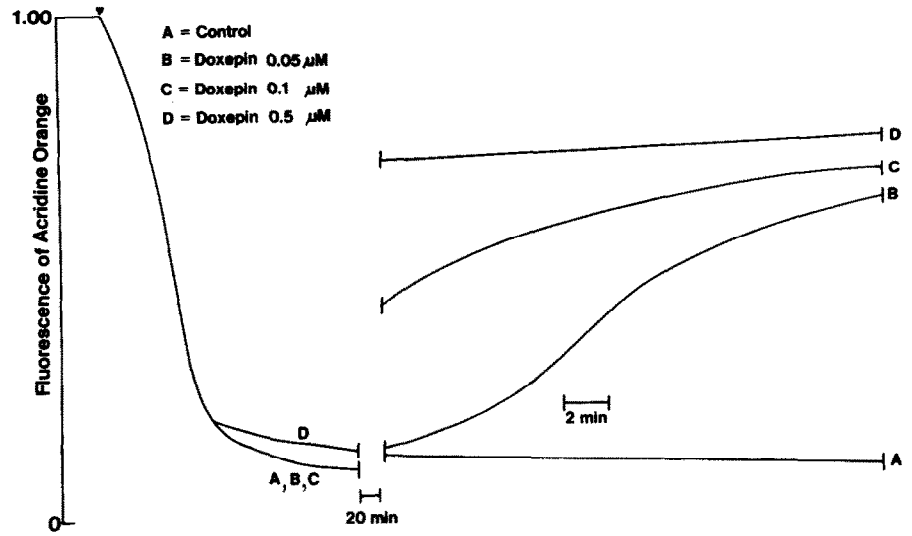

Fig. 9. Effect of doxepin (0.05, 0.1 and 0.5 μM) on H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 7.

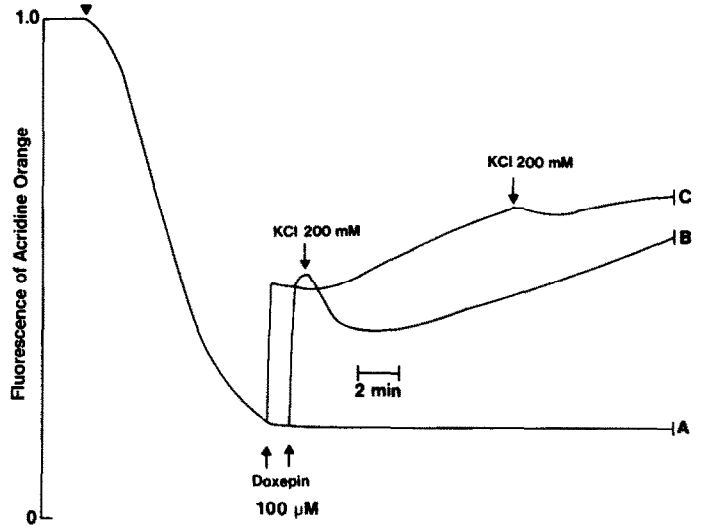

Fig. 10. Effect of 100 μM doxepin at the steady-state of H^+/K^+-ATPase mediated H^+ accumulation in intact parietal cell vesicles. Experimental conditions were as described in Fig. 1. Doxepin and KCl (final concentration 350 mM in the medium) were added at the time indicated by arrows.

that the compound—attributable to its physicochemical properties as a hydrophobic weak base—acts as a permeable buffer and/or by a protonophoric effect (Fig. 11). Identical results as shown for doxepin were found for the antidepressant trimipramine and the neuroleptic agent trifluoperazine (data not shown).

DISCUSSION

There is sufficient experimental evidence that omeprazole has antisecretory activity from blocking the H^+/K^+-ATPase and that the drug itself is not the active inhibitor. Thus it was found that the antisecretory potency of omeprazole under *in vivo* conditions is decreased when acid secretion was blocked by administration of cimetidine prior to omeprazole treatment [11]. In the isolated gastric mucosa, neutralization of the acid secretory membrane by weak buffers prevents omeprazole-induced inhibition of acid secretion (see ref. [17]). In leaky parietal cell membranes the inhibitory potency of omeprazole on H^+/K^+-ATPase activity was markedly increased when the enzyme was incubated together with the drug at slightly low pH (pH 6.1) or when the drug was dissolved in strong acid (pH 1.0 or 2.0) before adding to the enzyme buffered at pH 7.5 [1, 12–14]. In intact parietal cell vesicles the H^+/K^+-ATPase

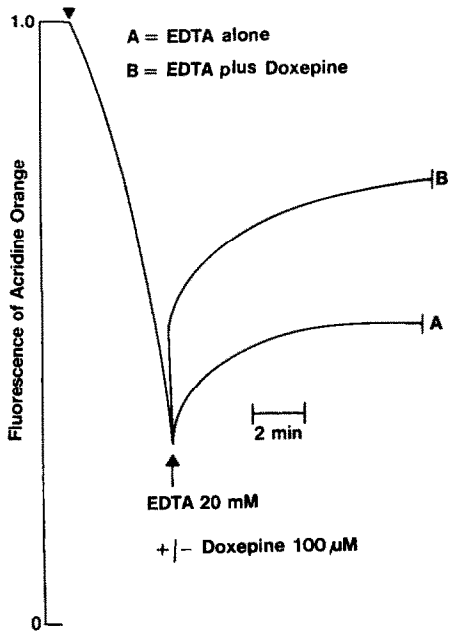

Fig. 11. Effect of doxepin on performed H^+ gradient in vesicles where ATPase reaction was stopped by addition of EDTA. Experimental conditions were as described in Fig. 1 with the modification that H^+/K^+-ATPase mediated H^+ accumulation was blocked by the addition of EDTA. EDTA alone (final concentration 20 mM) or EDTA plus doxepin (100 μM) were added as indicated by arrows.

activity was time and concentration dependently inhibited by omeprazole when acid was accumulated inside the vesicle, whereas under conditions in which no pH gradient was generated, no inhibitory effect on the H^+/K^+-ATPase was found [15, 16]. Recent studies investigating the acid-induced decomposition products of omeprazole have shown that the drug is rearranged at low pH into a cyclic sulphenamide [17, 18] and it was found that this compound interferes with enzymatic SH groups to form a disulphide [19]. The data presented here are in agreement with the concept that omeprazole is an acid-activated compound which is converted within the acid secretory membrane of the parietal cell into a compound which reacts with thiol groups of the H^+/K^+-ATPase. We have found that omeprazole inhibits the H^+/K^+-ATPase mediated H^+ generation in intact gastric vesicles only after the vesicles have reached maximal acidification (Fig. 2) and that inhibition was reduced in vesicles in which maximal acidification was prevented by the weak base aminopyrine (Fig. 5). Omeprazole-induced pump inhibition was reversed by the SH-reducing agent dithioerythritol (Fig. 3) but not by the less hydrophobic SH-reducing agents dithiothreitol and glutathione. Furthermore, proton transport was inhibited when glutathione was present in the medium whereas dithioerythritol was able to protect (Fig. 4). These data indicate that the acid-induced reaction product formed from omeprazole interacts with luminal SH groups of the H^+/K^+-ATPase.

The nature of interaction found with the pyridyl (1, 2a) imidazole SCH 28080 was quite different from that of omeprazole. The drug markedly inhibited the initial rate of H^+/K^+-ATPase mediated H^+ accumulation and the steady state proton concentration (Fig. 6). The data in Fig. 7 demonstrate that the drug interferes with the K^+ site of the enzyme in order to exert its effects. These findings are in complete accordance with the studies done in leaky vesicles in which the drug competitively and highly selectively interacted with the luminal K^+ site of the H^+/K^+-ATPase [2, 3, 20]. The observed protonophoric action is insufficient to explain the data shown in Fig. 7 but indicates that in the intact cell system the drug can interfere with H^+ production by an additional mechanism.

The experimental findings with doxepin were more complex. Previously we have reported that doxepin inhibits the K^+-stimulated ATPase activity with an IC_{50} value of 283 μM, whereas in the intact parietal cell preparation, histamine and dibutyryl-cAMP acid-induced secretion was inhibited by the drug with an IC_{50} value of 0.56 and 0.65 μM, respectively [6]. The higher potency of the drug in the cell preparation was partially explained by an accumulation of the drug due to its basic properties inside the acidic tubulo-vesicles and subsequent interaction at the K^+ site of the enzyme by an allosteric [6] or as proposed for neuroleptics by Im *et al.* a competitive mechanism [5]. The slow dissipation of the H^+ gradient as shown in Fig. 9 may support such an assumption but, as shown in Fig. 10, the doxepin action was counteracted only to some extent by KCl. Since doxepin destroyed the preserved proton gradient present in vesicles in which the pump reaction was stopped by EDTA (Fig. 11), we suggest that the main effect of the drug has to be attributed to the protonophoric or intravesicular neutralization capacity and that only part of the inhibitory action can be explained by the K^+ antagonistic activity at the enzyme.

In summary, the experiments on H^+/K^+-ATPase mediated H^+ formation accumulation in intact gastric vesicles have shown that omeprazole is an acid-activated compound which blocks essential SH groups of the enzyme localized inside the vesicle and that SCH 28080 interacts at the K^+ site of the enzyme. The antidepressant doxepin interacts with H^+ formation by intravesicular neutralization and/or by a protonophoric action and by a K^+ antagonistic activity at the H^+/K^+-ATPase.

We conclude that intact pumping vesicles are a useful *in vitro* model for studying the mechanism of action of H^+/K^+-ATPase inhibitors and that this *in vitro* model provides information about additional effects of a drug on H^+ formation at the apical parietal cell membrane.

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