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THE INHIBITORY EFFECT OF HOE 731 IN ISOLATED RABBIT GASTRIC GLANDS

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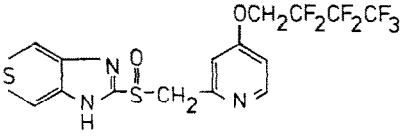
Abstract—HOE 731, a substituted thienoimidazole derivative, was studied on [^{14}C]aminopyrine uptake and oxygen consumption in isolated rabbit gastric glands. HOE 731 caused a concentration-dependent inhibition of [^{14}C]aminopyrine uptake during histamine and dbcAMP stimulation. The inhibition during dbcAMP stimulation was in accordance with its proton-pump inhibiting properties, which has already been reported. (Herling *et al.*, *Gastroenterology* **96**: A206, 1989). IC_{50} values were during histamine stimulation 0.8 ± 0.3 μM and during dbcAMP stimulation 1.3 ± 0.4 μM. The inhibition was reversible after addition of dithioerythritol and was of a non-competitive type. Omeprazole caused similar inhibitory effects in the same concentration-range. During time-course studies in glands, the inhibitory effect on [^{14}C]aminopyrine uptake of 0.1 μM HOE 731 already appeared after 10 min of incubation but decreased with increasing incubation time, while 0.1 μM omeprazole caused an unchanged inhibition which started after 30 min of incubation. The concentration of 3 μM of HOE 731 and omeprazole caused a comparable constant inhibition. After pre-incubation for 135 min under basal conditions with subsequent stimulation of the glands with dbcAMP, the inhibitory effect of 10 μM HOE 731 also decreased in contrast to omeprazole. During stimulation for 4 hr, the inhibition of both compounds remained constant. In oxygen consumption studies HOE 731, at 100 μM, caused a strong inhibition down to basal values. This inhibitory effect could be prevented totally when 10 mM imidazole was added to neutralize the acidic compartment of the parietal cell during stimulation. It is concluded that HOE 731 needs acid-activation like omeprazole to inhibit the proton pump, but probably due to its chemical differences (stability, pH for conversion of HOE 731 to its active form) it shows a different inhibitory profile (faster transformation into its active moiety with faster onset of a partially reversible inhibition) as compared to omeprazole.

Omeprazole, a substituted benzimidazole, is a specific and effective inhibitor of gastric acid secretion in experimental animals [1] and man [2]. Its inhibitory effect is caused by interference with the gastric proton-pump (H^+,K^+-ATPase), localized on the apical membrane of the parietal cell [3].

To characterize a new compound as a proton-pump inhibitor it has to fulfil certain pharmacological criteria, which are now known since the experience with omeprazole. The mechanism of the inhibitory effect of omeprazole on gastric acid secretion differs from that of other receptor antagonists (H_2-receptor antagonists, anticholinergics). *In vitro*, omeprazole inhibits the gastric H^+,K^+-ATPase [3, 4], as well as dbcAMP-stimulated [^{14}C]aminopyrine uptake in parietal cell [5] or gastric gland preparations [4, 6]. *In vivo*, omeprazole caused an inhibition of gastric acid secretion with similar ID_{50} values, irrespective of the secretagogues (histamine, gastrin, carbachol) used [7] or if basal secretion was studied [6].

The aim of this study was to define in more detail the acid inhibitory effect of HOE 731, a substituted thienoimidazole derivative (Fig. 1), in isolated rabbit gastric glands. For an earlier member of this chemical class, S 1924, the above mentioned criteria have been fulfilled [6]. It has also been reported that HOE 731 inhibits H^+,K^+-ATPase activity after preincubation at pH 6 and that it is effective in inhibiting gastric acid secretion in rats and dogs [8].



Hoe 731

Fig. 1. Chemical structure of HOE 731.

MATERIALS AND METHODS

Compounds. Collagenase (150–200 units/mg), dibutyryladenosin 3:5-cyclic monophosphate (dbcAMP) and dithioerythritol (DTE) were from Sigma Chemical Co. (Deisenhofen, F.R.G.) and histamine from Merck (Darmstadt, F.R.G.). [^{14}C]Aminopyrine (AP) (90–120 mCi/mmol) were from New England Nuclear (Dreieich, F.R.G.). Omeprazole and HOE 731 (2-((4-(2,2,3,3,4,4,4-heptafluoro-butoxy)-2-pyridylmethyl) sulfinyl)-1*H*-thieno[3,4-*d*]imidazole) were synthetized at HOECHST AG (Frankfurt/Main, F.R.G.). All chemicals used were of analytical grade or of the highest purity available.

Gastric gland preparation. Rabbits (2–3 kg) were killed by cervical fracture/dislocation during anaesthesia. High pressure perfusion of rabbit stomachs was carried out as previously described [9]. The gastric mucosa of the corpus was scraped off and minced with a pair of scissors. Pieces of mucosa were incubated in a collagenase (1 mg/mL) containing medium for 30–45 min at 37°. The medium contained

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in mM: 100.0 NaCl, 5.0 KCl, 0.5 NaH_2PO_4, 1.0 Na_2HPO_4, 1.0 $CaCl_2$, 1.5 $MgCl_2$, 20.0 $NaHCO_3$ 20.0 Hepes, 2 mg/mL glucose and 1 mg/mL rabbit albumin, pH was adjusted to 7.4 with 1 M Tris. The glands were filtered through a nylon mesh to remove coarse fragments and rinsed three times in incubation medium. The glands were diluted to a final concentration of 2–4 mg dry weight/mL.

[^{14}C]*Aminopyrine (AP) accumulation*. The ability of gastric glands to form acid was measured based on AP accumulation [10]. Samples of 1.0 mL gland suspension were equilibrated in 1.0 mL medium containing 0.1 μCi/mL [^{14}C]AP at 37° in a shaking water bath together with the agent to be tested. After 20 min either histamine or dbcAMP was added, followed by a 30 min incubation period for histamine and a 45 min incubation period for dbcAMP. The glands were then separated from the medium by brief centrifugation. Aliquots of the supernatant and the digested gland pellet were examined in a liquid scintillation counter. The AP accumulation was calculated as the ratio between AP in intraglandular water and AP in the incubation medium [11]. All determinations were done in triplicate.

Studies to determine the reversibility of the inhibitory effect of HOE 731 were performed with the membrane permeable mercaptane, dithioerythritol (DTE). This was added to the glands 30 min after addition of dbcAMP and subsequently incubated for 15 min until the reaction was terminated by centrifugation.

Time–course experiments were performed over an incubation period of up to 4 hr. In one series of experiments HOE 731 and omeprazole were incubated at 0.1 and 3 μM for 60 min together with dbcAMP for stimulation. In a second series of experiments HOE 731 and omeprazole were pre-incubated at 10 μM with glands up to 195 min under basal conditions and subsequently stimulated with dbcAMP for 45 min. In a third series HOE 731 and omeprazole were added to the glands together with dbcAMP for stimulation over an incubation period of up to 4 hr.

Respiratory studies. Glandular oxygen consumption was measured at 37° using a Warburg respirometer and air as the gas phase [10]. The flasks (15 mL) had a central cup containing a CO_2 absorber, 20% KOH solution on a filter paper. The flask contained 1 mL medium to which 1 mL of gland suspension was added. Imidazole was added to the glands from the beginning. After 20 min equilibration, HOE 731 and dbcAMP were added and the oxygen consumption was measured at 15 min intervals for the following 45 min. Corrections were made for ambient temperature and atmospheric pressure.

Statistics. IC_{50} values were calculated by probit analysis. Statistical differences ($P < 0.05$) were assessed by Student's *t*-test, N being the number of different gland preparations.

RESULTS

Inhibition of [^{14}C]*AP uptake*

Histamine and dbcAMP stimulated acid formation in isolated rabbit gastric glands concentration-dependently. Maximum stimulation was achieved with 100 μM histamine and 0.3–1 mM dbcAMP [12]. The inhibitory effect of HOE 731 was studied on histamine- and dbcAMP-induced [^{14}C]AP accumulation. In concentrations ranging from 0.1 to 30 μM HOE 731 produced a concentration-dependent inhibition of [^{14}C]AP accumulation down to values below basal. IC_{50} values for HOE 731 during both kinds of stimulation are summarized in Table 1. Studies to define the type of inhibition caused by HOE 731 in glands were performed during dbcAMP stimulation. Concentrations of 0.3 and 1 μM of HOE 731 were added to the dbcAMP concentration–response curve (50–250 μM). HOE 731 caused a non-competitive type of inhibition (Fig. 2).

Table 1. IC_{50} values of HOE 731 on histamine- and dbcAMP-stimulated [^{14}C]aminopyrine accumulation in isolated rabbit gastric glands

Stimulation	N	IC_{50} (μM)
Histamine	4	0.8 ± 0.3
dbcAMP	3	1.3 ± 0.4

Values are means ± SE, N = number of different gland preparations. HOE 731 was studied in six concentrations from 0.1 to 30 μM. Basal AP-ratio was 10.7 ± 0.7 and stimulated AP-ratios were 29.7 ± 3.6 with 100 μM histamine and 56.7 ± 13.7 with 0.3 mM dbcAMP.

The inhibitory effect of 10 μM HOE 731 on dbcAMP-stimulated AP accumulation was dose-dependently reversed by 0.1 and 1 mM dithioerythritol DTE (Fig. 3).

In time–course experiments HOE 731 was studied in concentrations of 0.1 and 3 μM over 60 min during dbcAMP stimulation. Even at 0.1 μM of HOE 731 there was an inhibitory effect already detectable after 10 min of incubation. This inhibition of HOE 731 appeared as a concave curve, so that the inhibition decreased with increasing incubation time (Fig. 4a). In contrast, the inhibition of 0.1 μM omeprazole did not appear until 30 min after incubation but it then remained constant (Fig. 4a). Both compounds at 3 μM caused a comparable inhibition, which remained constant over the complete observation period (Fig. 4b).

In contrast to omeprazole, in time–course studies, when HOE 731 was preincubated with glands under basal conditions with subsequent stimulation, the inhibitory effect of HOE 731 decreased after preincubation for 135 minutes (Fig. 5a). During stimulation over 4 hr HOE 731 and omeprazole caused an unchanged inhibition (Fig. 5b).

Respiratory studies. The oxygen consumption of isolated gastric glands correlated with parietal cell function [10]. HOE 731 at a concentration of 100 μM caused a strong inhibition of dbcAMP-induced respiration down to basal values. In the presence of 10 mM imidazole this inhibitory effect was totally prevented thus oxygen consumption was not different from control values for imidazole alone (Fig. 6).

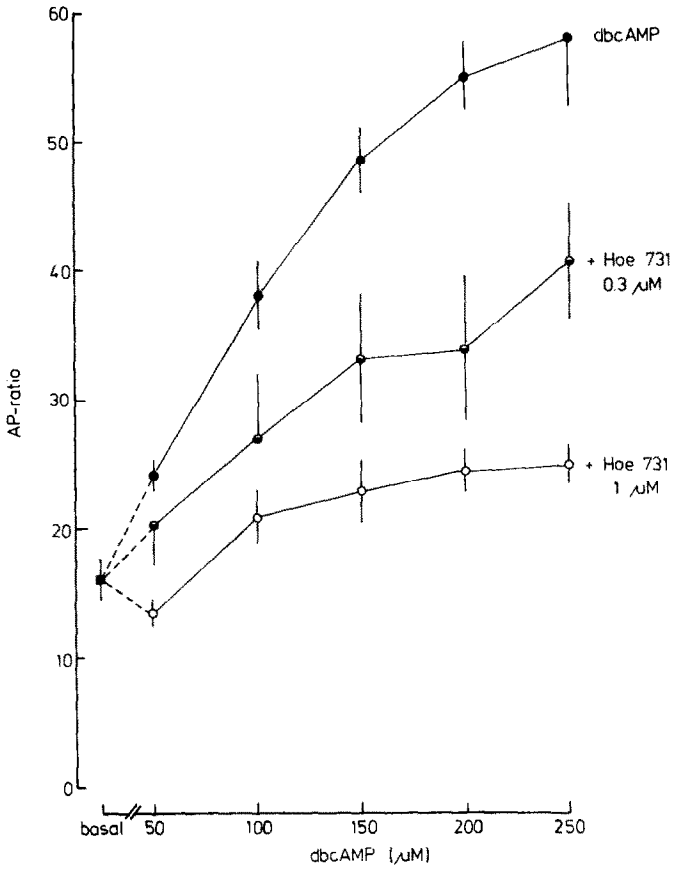

Fig. 2. Inhibitory effect of HOE 731 on dbcAMP-responsiveness on [^{14}C]AP uptake in isolated rabbit gastric glands. Values are means ± SE, N = 5 different gland preparations.

DISCUSSION

Substituted thienoimidazoles are potent H^+,K^+-ATPase inhibitors, as previously shown for HOE 731 [8] and S 1924 [6]. They inhibited the gastric proton pump with a lower IC_{50} value after preincubation at pH 6.0 compared to pH 7.4. This behaviour has already been established for omeprazole. Omeprazole was defined as acid-sensitive [13, 14] and is transformed in acidic environments into active transformation products [15]. Therefore, the inhibition during preincubation at pH 6.0 resulted in a lower IC_{50} value as during preincubation at pH 7.4 [4, 6]. A similar behaviour was observed for substituted thienoimidazoles [6, 8]. It seems very likely that thienoimidazole derivatives share a similar acid-catalysed transformation (acid-activation), as it has already been supposed [16]. HOE 731 shows a pH-dependent reduction of its chemical stability ($T_{1/2}$ for HOE 731 in 0.1 M phosphate buffer at pH 9, 4.5 and 2 of 16.2 hr, 27 min and 3 min, respectively; $T_{1/2}$ for omeprazole in 0.1 M phosphate buffer at pH 4.5 and 2 of 45 min and 11 min, respectively). More detailed results of its chemistry and its inhibitory effect on ATPase activity and proton transport of gastric vesicles will be published separately [17, 18].

In gastric glands HOE 731 inhibited histamine- as well as dbcAMP-induced AP accumulation in the micromolar range. The inhibition during dbcAMP stimulation was in accordance with its proton-pump inhibiting properties. In contrast, H_2-receptor antagonists did not inhibit dbcAMP-stimulated [^{14}C]AP accumulation [4, 12].

It has been reported that IC_{50} values for omeprazole are in the micromolar range for [^{14}C]AP uptake in rabbit gastric glands irrespective of the stimulant [4, 6]. HOE 731 and omeprazole seem to be equally effective on gastric acid secretion *in vitro*. The type of inhibition caused by HOE 731 was non-competitive. The reversibility of the inhibitory effect of HOE 731 by dithioerythritol (DTE) revealed that SH-groups are involved in the inhibition caused by HOE 731. Similar results have already been described for omeprazole [5, 19].

The time–course of the inhibitory effect of 0.1 µM HOE 731 resulted in a concave curve. The inhibition appeared with a faster onset for HOE 731 than for omeprazole. However, in the case of HOE 731 the inhibition decreased with increasing incubation time. In contrast, the inhibition caused by 0.1 µM omeprazole remained unchanged. From these data it seems very likely that HOE 731 was transformed quicker into its active moiety during stimulation in glands than omeprazole.

The inhibitory effect of pre-incubated HOE 731 in glands during time–course experiments differed significantly from that of omeprazole after 135 and

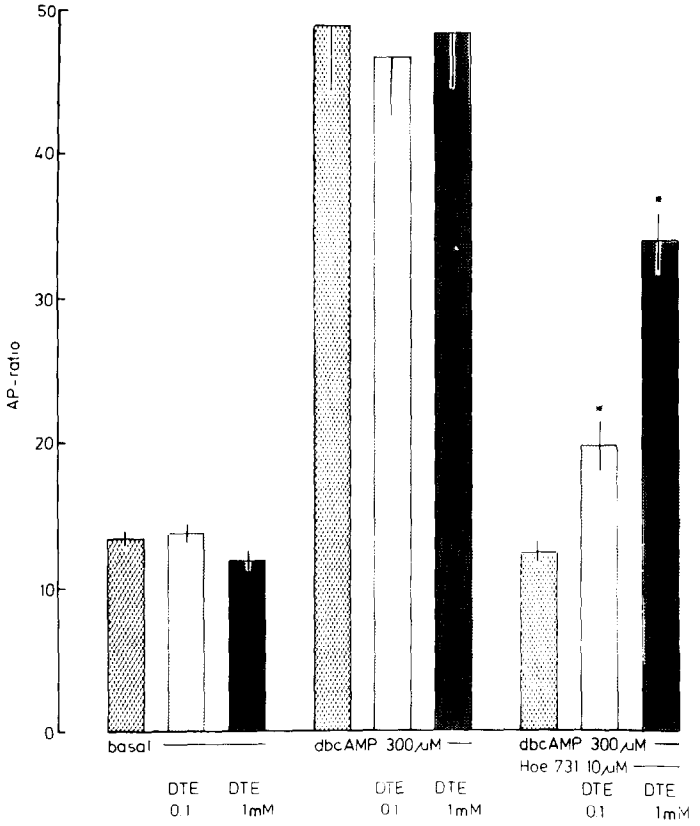

Fig. 3. Reversibility of the inhibitory effect of HOE 731 by dithioerythritol (DTE) in gastric glands. Values are means ± SE, N = 4 different gland preparations. Asterisks show significant differences ($P < 0.05$) compared to the inhibitory effect of 10 µM HOE 731 alone.

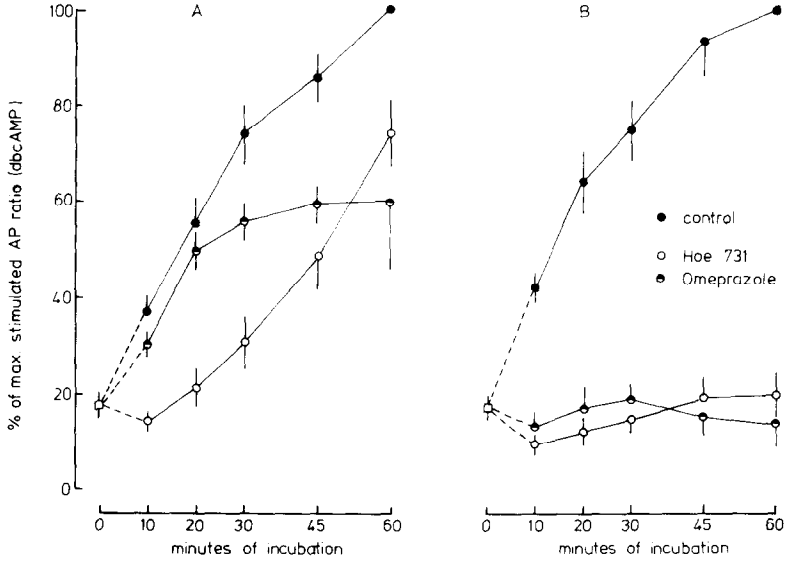

Fig. 4. Time-courses of the inhibitory effects of 0.1 µM (A) and 3 µM (B) HOE 731 and omeprazole on dbcAMP-stimulated [^{14}C]AP accumulation in glands. Values are means ± SE, N = 3–5 different gland preparations. Maximal stimulated AP-ratios after 0.3 mM dbcAMP were 86.3 ± 7.9 for (A) and 65.6 ± 3.8 for (B), respectively.

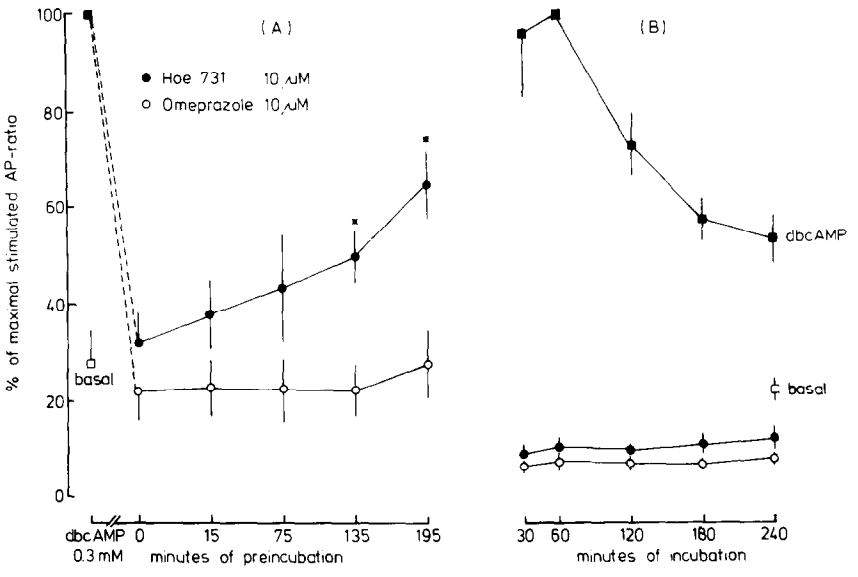

Fig. 5. Time–courses of the inhibitory effects of 10 μM HOE 731 and omeprazole after pre-incubation and subsequent dbcAMP stimulation for 45 min (A) and after incubation over 240 min together with dbcAMP (B) on [^{14}C]AP accumulation in glands. Values are means ± SE, N = 4–6 different gland preparations. Asterisks show significant differences ($P < 0.05$) compared to omeprazole. Maximal stimulated AP-ratios after 0.3 mM dbcAMP were 48.1 ± 12.6 for (A) and 79.6 ± 7.2 for (B), respectively.

195 min of pre-incubation. After 135 min the inhibitory effect of HOE 731 decreased in contrast to that caused by omeprazole. When HOE 731 was incubated with stimulated glands for up to 4 hr, it caused a constant inhibition similar to omeprazole. The fading profile during pre-incubation was probably due to a higher chemical instability in neutral buffer of HOE 731 in comparison to omeprazole. However, if HOE 731 accumulated immediately within the secretory channel during stimulation and subsequent transformation and binding to the proton pump, its inhibitory effect remained constant.

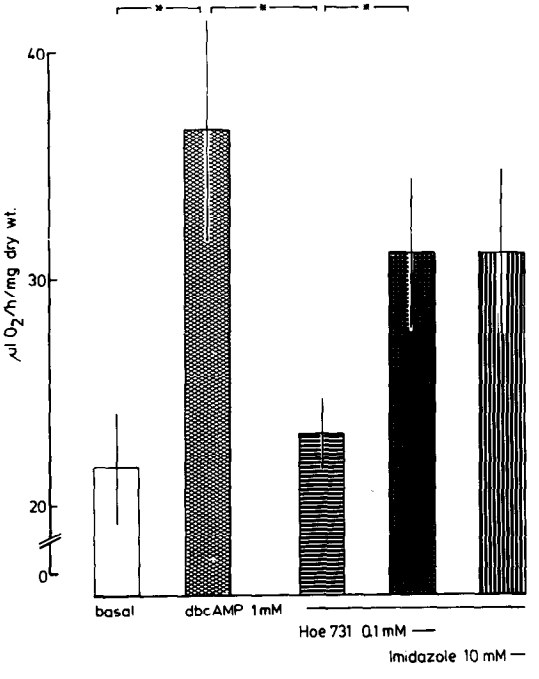

Fig. 6. Oxygen consumption studies with HOE 731 in the presence and absence of 10 mM imidazole. Values are means ± SE, N = 7 different gland preparations. Asterisks show significant differences ($P < 0.05$).

Oxygen consumption studies with glands were performed in the presence and absence of imidazole, in order to get some information whether HOE 731 needs acid-activation for binding to the proton pump. Imidazole accumulates as a weak base ($pK_a = 6.0$) within the secretory channel of the parietal cell and neutralizes the secreted protons [20]. Therefore, the accumulation of HOE 731 as a weak base ($pK_a = 3.7$) in the acidic compartment of the parietal cell and the subsequent acid-catalysed transformation could not appear under this condition. The inhibitory effect of HOE 731 was completely prevented by the presence of imidazole. Therefore, an acidic compartment seems to be a prerequisite for the efficacy of HOE 731.

It can be speculated that probably due to its chemical differences (stability, pH for conversion of HOE 731 into its active form) HOE 731 showed a different inhibitory profile (faster transformation into its active moiety with a faster onset of inhibition) as compared to omeprazole. Hijden *et al.* [21] studied the effect of omeprazole on H^+,K^+-ATPase containing vesicles, which were absorbed to a planar lipid membrane by measuring electrical currents. Omeprazole caused, in an almost perfectly buffered system, an increasing inhibition the more the pump was activated. These observations raised the question if omeprazole, in addition to its efficacy at low pH, can be activated at high pH by the H^+,K^+-ATPase itself during the stimulatory process of the ATPase by exposing extremely acidic groups to the

membrane interface. Beil *et al.* [18] observed in proton transport studies that the inhibitory action of HOE 731 could be prevented by DTE and in contrast to omeprazole additionally in part by glutathione. These observations indicate, that the reactive species formed from HOE 731 inside the vesicles have the ability to cross the membrane or that sufficient amounts of the active inhibitor are formed in the apical membrane as observed already for omeprazole [21]. Therefore, it seems likely that faster activated HOE 731 binds to some other groups of the proton pump than activated omeprazole does and these groups are probably later partially accessible to cytosolic glutathione for reactivation.

In animal studies with HOE 731 and omeprazole [22], which will be published in detail later, we observed that also *in vivo* the inhibitory profile of HOE 731 differed from that of omeprazole in high doses.

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