Br. J. Pharmac. (1986), **88**, 389–395

The sulphoxide moiety of substituted benzimidazoles is essential for inhibition of parietal cell K^+/H^+-ATPase

W. Beil*, M. Eltze, K. Heintze, K. Klemm, R. Riedel, C. Schudt, K.-Fr. Sewing[1]* & A. Simon

Abteilung Allgemeine Pharmakologie*, Medizinische Hochschule Hannover, D-3000 Hannover and Byk Gulden Research Laboratories, D-7750 Konstanz, F.R.G.

1 The antisecretory action of the benzimidazole sulphoxide derivative B 823-10, 2[(4-methoxy-3-methyl-2-pyridylmethyl)-sulphinyl]-5-trifluoromethyl(1H)-benzimidazole, was compared with the effect of the corresponding sulphide B 823-08 in several *in vivo* and *in vitro* test systems.

2 The sulphide B 823-08 and the sulphoxide B 823-10 were found to be equipotent in the Shay rat. The sulphide was found to inhibit H^+ secretion in intact rabbit gastric glands and enriched guinea-pig parietal cells with lower potency than the corresponding sulphoxide. The relative potency in antisecretory activity (sulphide/sulphoxide) decreased in the following rank order: Shay rat: gastric glands: parietal cells.

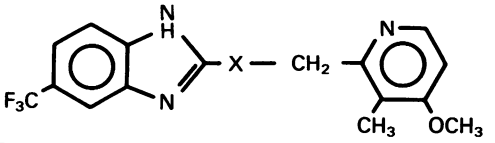
3 Purified K^+/H^+-ATPase was not blocked by the sulphide, whereas the sulphoxide inhibited the overall as well as the partial reactions of this enzyme.

4 In all *in vitro* systems tested, inhibition of H^+ secretion and enzyme activity by the sulphoxide, but not by the sulphide, was antagonized by SH-compounds such as dithiothreitol.

5 It is concluded that *in vivo* sulphoxidation of the sulphide plays an important role in acid inhibition. *In vitro* an additional inhibitory mechanism of the sulphide has to be considered.

Introduction

Substituted benzimidazoles are potent inhibitors of gastric H^+ secretion in experimental animals (Larsson *et al.*, 1983) and in man (Lind *et al.*, 1983). In several *in vitro* preparations, such as isolated gastric mucosa of the guinea-pig (Sjöstrand *et al.*, 1978), isolated gastric glands of the rabbit (Wallmark *et al.*, 1983) and enriched guinea-pig parietal cells (Sewing *et al.*, 1983), these compounds inhibit H^+ secretion induced by histamine and dibutyryl cyclic AMP suggesting that they act on a step in the acid-secretory process, distal to the parietal cell receptor. Studies on the K^+/H^+-ATPase, which can be regarded as the proton pump in the secretory membrane of parietal cells have shown that substituted benzimidazoles inhibit both the release of inorganic phosphate from ATP and H^+ transport (Fellenius *et al.*, 1981). These observations suggest that the inhibitory effect of substituted benzimidazoles on gastric acid secretion has to be attributed to inhibition of the parietal cell proton pump, the K^+/H^+-ATPase. Omeprazole, the most extensively studied benzimidazole derivative, was reduced to its corresponding sulphide during incubation with isolated gastric glands of the rabbit (Larsson *et al.*, 1983). It was of interest to find out whether or not these drugs in their reduced form are also effective inhibitors of acid secretion due to the attack on K^+/H^+-ATPase. Therefore, the inhibitory action of a new benzimidazole derivative B 823-10 (2[(4-methoxy-3-methyl-2-pyridylmethyl)-sulphinyl]-5-tri-fluoromethyl(1H)-benzimidazole), a sulphoxide, was compared with the corresponding sulphide B823-08 in several *in vivo* and *in vitro* test systems. The structural formulae of the two compounds used in the study are given in Figure 1.



B823-08 X = S (sulphide)
B823-10 X = S→O (sulphoxide)

Figure 1 Structural formula of B 823–10 and B 823–08.

[1]Author for correspondence

Methods

Pylorus-ligated rats

Studies were performed in rats with pylorus ligation (Shay *et al.*, 1954). After a 22 h fast with free access to drinking water, the test compounds were administered via gastric tube at a volume of 10 ml kg^{-1}. One hour later, acetylsalicylic acid (ASA) 100 mg kg^{-1}, suspended in tylose (10 ml kg^{-1}), was given orally under ether anaesthesia. ASA was used to obtain reproducible lesions of the mucosa. The data together with results of other ulcer models will be reported separately. The abdomen was opened and the pylorus ligated. Four hours later, the abdomen was opened again; the stomach was ligated on either end, excised, and the stomach content assayed for volume, pH and H^+ concentration.

Isolated lumen-perfused stomach of mouse

The effect of both drugs on spontaneous acid secretion was investigated in the isolated, distended stomach of the mouse by a technique similar to that described by Wan (1977) and Szelenyi (1981). Male mice (NMRI, Ivanovas, Kisslegg; 23–28 g) with free access to food and water were anaesthetized with ether. The stomach was exposed, cannulated at the pyloric and oesophageal end, washed, and rapidly dissected. It was placed in a 35 ml organ bath containing a buffered serosal solution (mM: NaCl 118.1, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 0.6, KH_2PO_4 1.0, Na_2HPO_4 16.0 and glucose 31.6) kept at 37°C and gassed vigorously with 95% O_2 and 5% CO_2. The stomach lumen of four parallel preparations was perfused via the pyloric cannula with oxygenated, unbuffered mucosal solution (mM: NaCl 135.8, KCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 1.3, and glucose 31.6) at a rate of 1 ml min^{-1}. The perfusate was passed over a flow-type pH electrode system raised 18 cm above the stomach level to distend the organ. Changes in pH were recorded continuously via pH-meters on a multichannel recorder. Spontaneous H^+ secretion was allowed to stabilize for 30 min before the drug was added to the serosal solution. Responses were calculated from the change in pH, converted into nmol H^+ min^{-1} and expressed as percentage change in acid secretion compared with the value immediately before drug administration. Acid secretion was then monitored at 5 min intervals for 1 h.

Isolated gastric glands

Gastric glands were prepared from New Zealand white rabbits according to the method of Berglindh & Öbrink (1976). Following vascular perfusion of the rabbit stomach, the mucosal layer was scraped off, minced and digested by collagenase (150 u ml^{-1}) for 60 min. Acid formation was determined on the basis of the uptake of [^{14}C]-aminopyrine into the glands. Glands were incubated for 30 min at 37°C with dibutyryl cyclic AMP 1 mM and [^{14}C]-aminopyrine 1 μM (sp. act. 101 mCi $mmol^{-1}$) in a medium consisting of (mM): NaCl 132, KCl 5.4, $MgSO_4$ 1.2, $CaCl_2$ 1.2, NaH_2PO_4 1, Na_2HPO_4 5, glucose 11, phenol red 10 μg ml^{-1}, and rabbit serum albumin 1 mg ml^{-1} pH 7.4. After incubation, the glands were spun down and the supernatant was recovered. The glands were dried, weighed and dissolved in 1 N NaOH. The distribution of radioactivity between supernatant and glands was used to calculate the aminopyrine ratio. For inhibition studies, the test compounds were transferred into the incubation medium before addition to the glands.

Isolated and enriched guinea-pig parietal cells

The method of preparation of isolated and enriched guinea-pig parietal cells was in principle that of Soll (1978), with the modifications described in detail by Sewing *et al.* (1983). Acid secretion in parietal cells was determined from cellular uptake of [^{14}C]-aminopyrine (Sewing *et al.*, 1983). For inhibition studies, parietal cells were preincubated with the test compounds for 30 min at 22°C. Thereafter, histamine 1 mM was added and the secretory response was determined after incubation for 20 min at 37°C. For studies with dithiothreitol (DTT), parietal cells were incubated with B 823-10 1 μM or B 823-08 10 μM for 30 min at 22°C. Thereafter, DTT 1 mM was added to the cells and allowed to react for another 30 min. Then the cells were stimulated with histamine (10^{-7}–10^{-3} M) for 20 min at 37°C.

Preparation of K^+/H^+-ATPase

Tubulo-vesicular membranes containing K^+/H^+-ATPase were prepared from guinea-pig parietal cells by differential and density gradient centrifugation as described by Beil & Sewing (1984).

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

The assay medium contained $MgCl_2$ 2 mM, Tris/HCl buffer 50 mM pH 7.5, membrane protein 5–8 μg with or without KCl 10 mM in a final volume of 1 ml. The reaction was started by addition of ATP 2 mM and stopped after incubation for 15 min at 37°C with 1 ml of 20% trichloroacetic acid (TCA). Liberated inorganic phosphate (P_i) was analysed by the method of Sanui (1974). Reaction rates were about 8 μmol P_i mg^{-1} protein h^{-1} in the absence, and about 50 μmol P_i mg^{-1} protein h^{-1} in the presence of K^+. For inhibition experiments, the substituted benzimidazoles were dissolved in dimethylsulphoxide

(DMSO) and preincubated at appropriate concentrations in the assay medium together with the gastric membranes for 30 min at 22°C. The final DMSO concentration in the assay was 1%, which had no effect on K^+/H^+-ATPase activity.

Phosphorylation of K^+/H^+-ATPase

K^+/H^+-ATPase-containing membranes (20 μg) were preincubated for 30 min at 22°C in 0.2 ml of Tris/HCl buffer 50 mM, pH 7.5. Phosphorylation was started by addition of [γ-^{32}P]-ATP 10 μM (500 c.p.m. pmol^{-1}) and $MgCl_2$ 2 mM and stopped 15 s later with 0.2 ml of 10% TCA containing Tris-ATP 5 mM and K_2HPO_4 5 mM. The protein precipitate formed was collected on Millipore membrane filters (type HAWP, 0.45 μm) and washed with 30 ml of 5% TCA containing K_2HPO_4 5 mM. Under control conditions, 647 ± 109 pmol phosphoenzyme per mg membrane protein was found.

p-*Nitrophenylphosphatase assay*

p-Nitrophenylphosphatase (p-NPPase) activity was assayed in a medium containing $MgCl_2$ 6 mM, *p*-nitrophenylphosphate 6 mM, Tris/HCl buffer 50 mM pH 7.5 and 10–15 μg membrane protein with or without KCl 10 mM in a total volume of 1 ml for 20 min at 37°C. The reaction was stopped by addition of 1 ml of 1N NaOH. *p*-Nitrophenol was measured at 410 nm. The reaction rate was about 30 μmol *p*-nitrophenol mg^{-1} protein h^{-1}in the presence of Mg^{2+} and K^+.

Metabolism of B 823-08 in rat liver microsomes

Excised livers from female rats (180–220 g, strain SIV 50), pretreated with phenobarbitone 80 mg kg^{-1} for three days, were homogenized (Potter, 860 r.p.m., 6 strokes) in Tris/HCl buffer 100 mM, pH 7.4 supplemented with KCl 150 mM, and microsomal fractions were obtained by centrifuging the post 10,000 *g* supernatant at 100,000 *g* for 60 min. Microsomes (1.5 mg) were incubated in a medium consisting of Tris/HCl buffer 100 mM, pH 7.4, $MgCl_2$ 1 mM and B 823-08 300 μM in a total volume of 5.0 ml. The reaction was started by addition of $NADPH_2$ 1 mM. After appropriate time intervals, 1 ml samples were taken and frozen in liquid nitrogen. B 823-08 and metabolites were analysed by h.p.l.c. using the precolumn switching technique (Huber *et al.*, 1982). Separation was performed on a Hypersil RP-18 column (125 × 4 mm). The samples were eluted with 43/75% methanol in 0.01 M ammonium phosphate buffer pH 7.0 at a flow rate of 1 ml min^{-1} and detected at 280 nm.

Protein determination

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

Drugs and radiochemicals

The drugs used and their sources were: collagenase, 125–230 units mg^{-1}; Na_2-ATP; Tris-ATP; histamine dihydrochloride; dithiothreitol (Sigma, Munich); Pronase E (70.000 PUK g^{-1}); acetylsalicylic acid (Merck, Darmstadt); dibutyryl cyclic AMP, monosodium salt (Boehringer, Mannheim); bovine serum albumin, (Serva, Heidelberg); [^{14}C]-aminopyrine (sp. act. 60–120 mCi mmol^{-1}; New England Nuclear Corp., Dreieich); [γ-^{32}P]-adenosine triphosphate (sp. act. 10 Ci mmol^{-1}; Amersham, Braunschweig); B 823-10 (2[(4-methoxy-3-methyl-2-pyridylmethyl)-sulphinyl]-5-trifluoromethyl-(1H)-benzimidazole) and its corresponding sulphide B 823-08 (Byk Gulden, Konstanz).

Results

Shay rat

In pylorus-ligated rats, both B 823-10 and B 823-08 produced a dose-dependent inhibition of acid output after oral administration. The ED_{50} values obtained were (with 95% confidence limits in parentheses): 1.5 (0.6–3.6) μmol kg^{-1} for B 823-10 and 1.8 (1.1–3.4) μmol kg^{-1} for B 823-08. ED_{50} values were calculated from at least four doses using 8 to 24 animals per dose.

Isolated lumen-perfused stomach of the mouse

The isolated, lumen-perfused stomach of the mouse produced a stable spontaneous acid output of 126 ± 8 nmol H^+ min^{-1} (mean ± s.e., $n = 70$) over 30 min which slowly decreased by an average of 26% during the subsequent 1 h observation period. The sulphoxide B 823-10 induced a concentration- and time-dependent inhibitory response. The IC_{50} value calculated from data obtained 30 min after addition of the drug was 38 μM ($n = 8$ animals per dose). In contrast, the sulphide B 823-08 did not inhibit acid secretion, but proved to be a weak stimulator with a peak secretory response between 20 and 30 min after drug administration. The maximum effect of about 30% stimulation was obtained at a concentration of 10 μM (Figure 2).

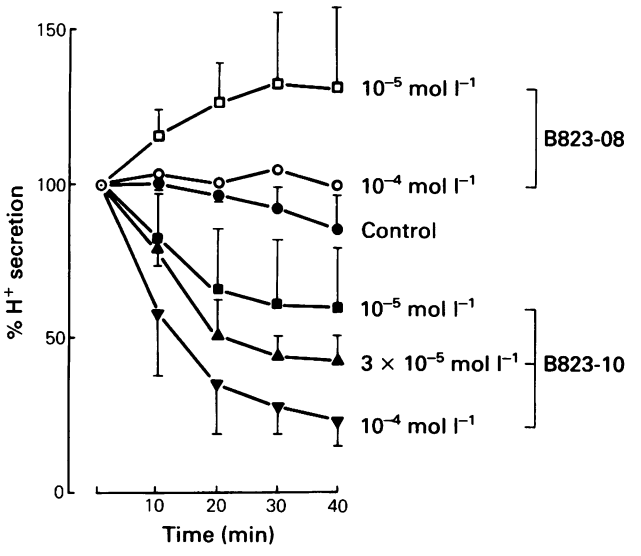


Figure 2 Time course in inhibition of H^+ secretion in the isolated, lumen-perfused stomach of the mouse after administration of B 823–10 and B 823–08. Each point represents mean of $n = 8$ for each drug concentration, vertical lines indicate s.e.

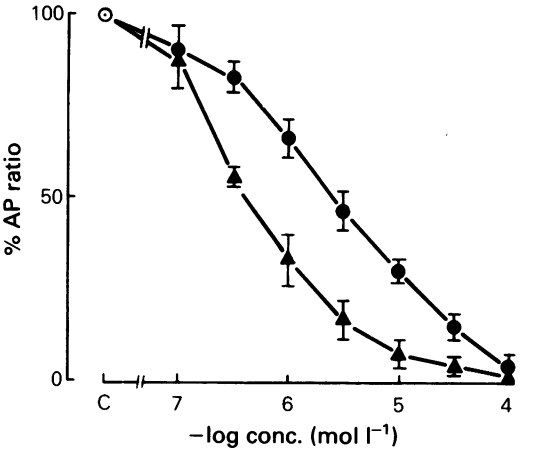


Figure 3 Inhibitory action of B 823–10 (▲) and B 823–08 (●) on dibutyryl cyclic AMP-stimulated (1 mM) aminopyrine accumulation in rabbit gastric glands. A ratio of 61 for aminopyrine accumulation under stimulated control conditions was taken as 100%. Values are means for three different gland preparations; vertical lines indicate s.e.

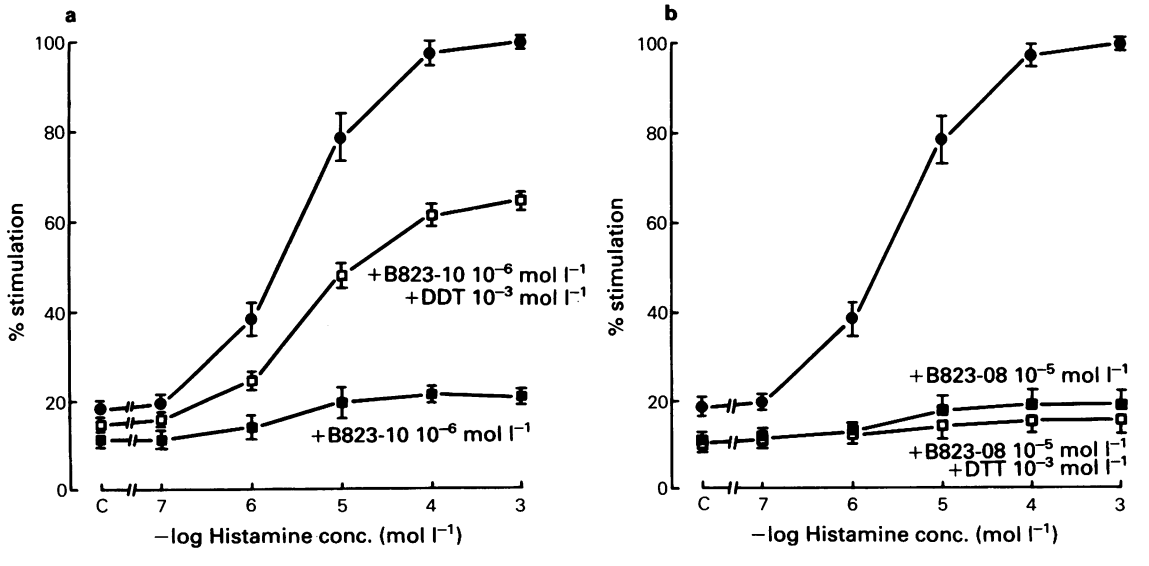


Figure 4 Effect of dithiothreitol (DTT) on inhibition of aminopyrine accumulation by B 823–10 (a) and B 823–08 (b). Enriched parietal cells from guinea-pigs were incubated for 30 min with B 823–10 1 μM or B 823–08 10 μM. Thereafter, DTT 1 mM was added and allowed to react for a further 30 min. Then histamine was added in the concentration indicated and aminopyrine (AP) uptake was measured after an additional 20 min. AP uptake in the presence of histamine 1 mM alone was taken as 100%. Values are means for three different cell preparations, vertical lines indicate s.e. mean.

Studies with isolated glands

Both compounds inhibited [^{14}C]-aminopyrine uptake, reflecting H^+ formation in response to dibutyryl cyclic AMP 1 mM, in a concentration-dependent manner (Figure 3). The IC_{50} values were: $0.41 \pm 0.07\,\mu M$ for B 823-10 and $3.3 \pm 0.57\,\mu M$ for B 823-08 ($n = 3$ different gland preparations for either compound).

Studies with isolated and enriched guinea-pig parietal cells

Histamine-stimulated (1 mM) [^{14}C]-aminopyrine accumulation was inhibited by both compounds with IC_{50}values of 0.17 ± 0.04 and $3.1 \pm 0.3\,\mu M$ for B 823-10 and B 823-08, respectively ($n = 3$ different cell preparations for either compound). It was found that the about 95% inhibition induced by B 823-10 $1\,\mu M$ could be antagonized by 60% when adding dithiothreitol 1 mM. In contrast, dithiothreitol did not antagonize the inhibitory action of equally potent concentrations of the sulphide B 823-08 (Figures 4a and 4b).

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

The sulphoxide B 823-10 inhibited isolated and purified K^+/H^+-ATPase activity ($IC_{50} = 1.03 \pm 0.19\,\mu M$, $n = 4$, different enzyme preparations), whereas the sulphide B 823-08 in concentrations up to $100\,\mu M$ failed to inhibit the enzyme by more than 20% (Figure 5). Both compounds were further tested for their inhibitory effect on partial reactions of ATPase,

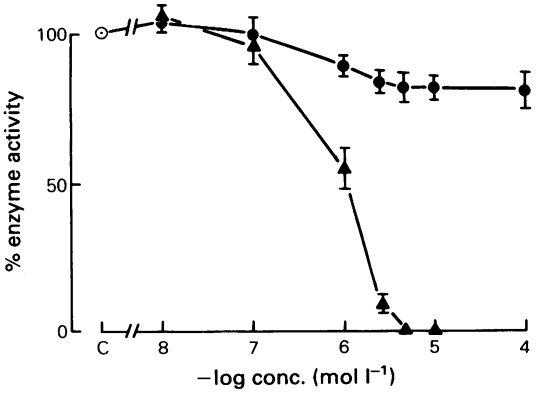


Figure 5 Concentration-response curve for B 823-10 (▲) and B 823-08 (●) on K^+/H^+-ATPase from guinea-pigs. K^+/H^+-ATPase was pre-incubated for 30 min at 22°C with different drug concentrations. Thereafter, enzyme activity was determined. Uninhibited enzyme activity was $50 \pm 8\,\mu mol\ P_i\,mg^{-1}$ protein h^{-1} (mean ± s.e.) and was set to 100% for each of the enzyme preparations.

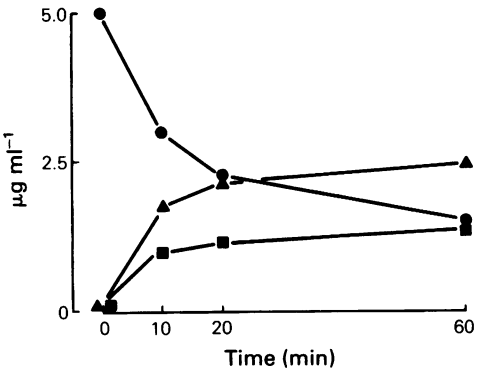


Figure 6 Transformation of B 823–08 by rat liver microsomes. Rat liver microsomes were incubated with B 823–08. At the times indicated, samples were taken and assayed for B 823–08 (●) and B 823–10 (▲) content by h.p.l.c.; (■) unidentified metabolite. Ordinate scale: μg drug ml^{-1} microsomal suspension containing 0.3 mg protein.

i.e. the phosphoenzyme steady-state formation and *p*-nitrophenylphosphatase activity. The sulphoxide B 823-10 inhibited phosphoenzyme formation as well as *p*-NPPase in a concentration-dependent manner with IC_{50} values of 20.3 ± 1.9 and $3.8 \pm 1.1\,\mu M$ ($n = 3$ different enzyme preparations), respectively. The sulphide B 823-08 up to a concentration of $100\,\mu M$ failed to influence both reactions.

Metabolism of B 823-08 in rat liver microsomes

The sulphide B 823-08 was transformed by rat liver microsomes into the sulphoxide B 823-10 and into a second major metabolite not yet identified (Figure 6). The transformation process occurred rapidly, with a new steady state between sulphide and sulphoxide being reached within 20 min.

Discussion

Of the two benzimidazole derivatives investigated, the inhibitory action of the sulphoxide B 823-10 was found to differ in many respects from that of the corresponding sulphide B 823-08, both *in vitro* and *in vivo* (Table 1). In general it can be stated that, the more integrated the test system used was, the more the sulphide B 823-08 gained in inhibitory potency relative to the sulphoxide B 823-10. The only exception to this rule is the mouse isolated stomach; possible reasons for that will be discussed below.

The failure of the sulphide to inhibit the K^+/H^+-ATPase including its partial reactions indicates that the sulphoxide moiety is essential for K^+/H^+-ATPase

inhibition. The gain in relative potency of the sulphide from the isolated enzyme over isolated cells and glands to the intact animal can be explained by an increasing rate of sulphoxidation of the sulphide. The most likely enzyme responsible for that is the microsomal P-450 system in the liver and less effectively in the gastric mucosa. Rat liver microsomes which have been shown to convert the sulphide in the sulphoxide contain approximately 1 nmol cytochrome P 450 per mg protein, whereas in the rat stomach 0.03 nmol cytochrome P 450 per mg microsomal protein was found (Güngerich & Mason, 1979). If the difference were the same in the guinea-pig, this would explain the relatively low but demonstrable inhibitory potency of the sulphide in gastric glands, even if no sulphoxide was detectable by h.p.l.c. and t.l.c. after incubation of glands with the sulphide (data not shown). The quantities possibly formed may have been below the detection limit. Although the P-450 system is the most likely enzyme involved in sulphoxidation it is also possible that other enzymes can convert the sulphide in the sulphoxide. The capacity of DTT and β-mercaptoethanol to antagonize the inhibitory effect of the sulphoxide but not that of the sulphide in guinea-pig isolated parietal cells is identical with that found for omeprazole and its sulphide (Beil *et al.*, 1985). These results indicate that SH-groups are involved in the inhibitory mechanism of substituted benzimidazole sulphoxides as pointed out repeatedly (Wallmark *et al.*, 1984; Im *et al.*, 1985) and may indicate reversibility of the action. However, the failure of β-mercaptoethanol to antagonize the inhibitory effect of the sulphide suggests that an additional mechanism independent of the inhibition of K^+/H^+-ATPase exists, by which the sulphide inhibits acid secretion. This mechanism is so far totally unknown.

The mouse isolated stomach does not seem to fit totally into the system. Although the sulphide appears to have a direct inhibitory component, in guinea-pig isolated parietal cells without conversion in the sulphoxide it failed to inhibit in this tissue but rather acted as a weak stimulant of acid secretion. This peculiarity is difficult to explain. The failure of inhibition requires two prerequisites: absence or extremely low activity of an enzyme system that converts the sulphide in the sulphoxide and the ability of the sulphide itself to inhibit acid secretion directly. These two features in conjunction with a phosphodiesterase inhibition as shown for several substituted benzimidazoles (unpublished data) could explain the

Table 1 Summary of effects of B 823-10 and B 823-08 in different *in vivo* and *in vitro* preparations

Test model	*n**	*ED_{50} (μmol kg^{-1}) or IC_{50} (μM)* B 823–10	B 823–08	*Relative potency* B 823–08/ B 823–10
Acid output in Shay rats	8–24	1.5	1.8	1.2
Acid secretion in mouse isolated stomach	8	38	Weak stimulation	
AP-accumulation in rabbit fundic glands	3	0.41	3.3	8
AP-accumulation in guinea-pig parietal cells	3	0.17	3.1	18
K^+/H^+-ATPase in tubulovesicular membranes	4	1.03	>100	>97
Phosphoenzyme formation	3	20.3	>100	>5
p-NPPase	3	3.8	>100	>26
Ability of DTT to reverse inhibition of AP-accumulation in rabbit glands and guinea-pig parietal cells	3	Yes	No	

AP = aminopyrine; DTT = dithiothreitol; *p*-NPPase = *p*-nitrophenylphosphatase.
*Number of animals (preparations) per dose (concentration).

weak stimulation of H^+ secretion in the mouse isolated stomach.

In summary the data have shown that: (1) the sulphoxide moiety of substituted benzimidazoles is an essential factor for inhibition of gastric K^+/H^+-ATPase; (2) *in vivo* the sulphide B 823-08 can be regarded as a prodrug since it is very likely that the same hepatic biotransformation which has been shown *in vitro* also occurs *in vivo*; (3) an additional inhibitory effect of the sulphide B 823-08 on the acid secretory process in the parietal cell has to be taken into account.

This work was supported by BMFT Grant No. 0385075.

References

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

BEIL, W., MÄDGE, S., HANNEMANN, H. & ZIMMER, K. O. (1985). Characteristics of K^+/H^+-ATPase inhibition by omeprazole and omeprazole sulphide. *Hepato-Gastroenterology*, **32,** 47.

BERGLINDH, T. & ÖBRINK, K.J. (1976). A method for preparing isolated glands from the rabbit gastric mucosa. *Acta physiol. scand.*, **96,** 150–159.

FELLENIUS, E., BERGLINDH, T., SACHS, G., OLBE, L., ELANDER, B., SJÖSTRAND, S.E. & WALLMARK, B. (1981). Substituted benzimidazoles inhibit acid secretion by blocking (K^+/H^+) ATPase. *Nature*, **290,** 159–161.

GÜNGERICH, F.P. & MASON, P.S. (1979). Immunological comparison of hepatic and extrahepatic cytochrome P-450. *Molec. Pharmac.*, **15,** 154–164.

HUBER, R., ZECH, K., WÖRZ, M., KRONBACH, TH. & VÖLTER, W. (1985). Direct injection of biological fluids: application of HPLC-analysis with precolumn enrichment to the pharmacokinetic studies of lonazolac. *Chromatographia*, **16,** 233–236.

IM, W.B., BLAKEMAN, D.P. & SACHS, G. (1985). Reversal of antisecretory activity of omeprazole by sulphhydryl compounds in isolated rabbit gastric glands. *Biochim. biophys. Acta*, **845,** 54–59.

LARSSON, H., CARLSSON, E., JUNGGREN, U., OLBE, L., SJÖSTRAND, S.E., SKANBERG, J. & SUNDELL, G. (1983). Inhibition of gastric acid secretion by omeprazole in the dog and rat. *Gastroenterology*, **85,** 900–907.

LIND, T., CEDERBERG, C., EKENVED, G., HAGLUND, U. & OLBE, L. (1983). Effect of omeprazole – a gastric proton pump inhibitor – on pentagastrin stimulated acid secretion in man. *Gut*, **24,** 270–276.

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

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

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

SHAY, H., SUN, D.C. & GRUENSTEIN, M. (1954). A quantitative method for measuring spontaneous gastric secretion in the rat. *Gastoenterology*, **26,** 906–913.

SJÖSTRAND, S.E., RYBERG, B. & OLBE, L. (1978). Stimulation and inhibition of acid secretion in the isolated guinea-pig gastric mucosa. In *Gastric Ion Transport*. ed. Öbrink, K.J. & Flemström, G. pp. 181–187. *Acta physiol. scand.* spec. suppl.

SOLL, A.H. (1978). The action of secretagogues on oxygen uptake by isolated mammalian parietal cells. *J. clin. Invest.*, **61,** 370–380.

SZELENYI, I. (1981). An isolated mammalian stomach preparation for studying the effect of substances on gastric acid secretion. *Arzneim.-Forsch./Drug Res.*, **31,** 998–1000.

WALLMARK, B., JARESTEN, B.M., LARSSON, H., RYBERG, B., BRANDSTRÖM, A. & FELLENIUS, E. (1983). Inhibition of gastric acid secretion by omeprazole, cimetidine, and SCN^-. Differentiation of their inhibition characteristics *in vitro*. *Am. J. Physiol.*, **243,** G64–G71.

WALLMARK, B., BRÄNDSTRÖM, A. & LARSSON, H. (1984). Evidence for acid-induced transformation of omeprazole into an active inhibitor of (H^+K^+)-ATPase within the parietal cell. *Biochim. biophys. Acta*, **778,** 549–558.

WAN, B.Y.C. (1977). Metiamide and stimulated acid secretion from isolated non-distended and distended mouse stomach. *J. Physiol.*, **266,** 327–346.

(Received June 27, 1985.
Revised February 11, 1986.
Accepted February 27, 1986.)