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Cimetidine and Other H_2-Receptor Antagonists as Inhibitors of Human E3 Aldehyde Dehydrogenase

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SUMMARY

The histamine H_2-receptor antagonists have been identified as inhibitors of human liver aldehyde dehydrogenase (EC 1.2.1.3) isozymes, E1, E2, and E3. Inhibition was strongest with the E3 isozyme, whose substrates include γ-aminobutyraldehyde, the aldehyde metabolites of polyamines, and betaine aldehyde. Burimamide, metiamide, cimetidine guanidine, cimetidine, and tiotidine were competitive with aldehyde substrates and noncompetitive with the coenzyme, binding to both the free E3 isozyme and the enzyme·coenzyme binary complex. Cimetidine and tiotidine were the best inhibitors, with K_i values of 1.1 ± 0.2 μM and 1.0 ± 0.0 μM, respectively; both are the first ever described potent and selective inhibitors of the E3 isozyme. Examination of the H_2-receptor antagonist structures for insight into the moieties accounting for E3 isozyme inhibition pointed to the side-chain polar groups as strongly influencing inhibition, with the cyanoguanidine side chain of cimetidine and tiotidine having the strongest influence. The K_i value of the E3 isozyme for cimetidine was the same as the *in vitro* dissociation constant for the H_2-receptor.

The role of the biogenic amine histamine is not clearly understood, but its release from various tissues of the human body is frequently associated with the inflammatory state. Histamine is also involved in gastric secretory activity and, thus, plays a role in gastric ulcer formation. The effects of histamine are brought about through the activation of the histamine H_1-, H_2-, and H_3-receptors. These receptors are distinguishable on the basis of their differing sensitivities to agonists and antagonists (1–4). Some tissues have predominantly one type of receptor, whereas others contain a mixture of the receptors. Gastric acid secretion is mediated almost exclusively through H_2-receptor activation (2). In the last 20 years, H_2-receptor antagonists, such as cimetidine (Tagamet), famotidine (Pepcid), and ranitidine (Zantac), have been widely used clinically in the treatment of peptic ulcers.

The metabolism of biogenic amines usually proceeds through aldehyde intermediates. Aldehyde dehydrogenase (EC 1.2.1.3) catalyzes the NAD^+-linked dehydrogenation of aldehydes to acids and has a broad substrate specificity. Naturally occurring substrates include aldehyde metabolites of histamine, putrescine, and dopamine (5). Three isozymes of aldehyde dehydrogenase, the cytoplasmic E1 and E3 isozymes and the mitochondrial E2 isozyme, have been purified from human liver (6, 7), and their cDNAs have been cloned (8, 9). The genes coding for these isozymes have been chromosome-localized: the *ALDH1* of E1 on chromosome 9; the *ALDH2* gene of E2 on chromosome 12, and the *ALDH9* gene of E3 on chromosome 1 (10, 11). Although all three isozymes exhibit broad substrate specificity, the activity with aminoaldehydes at low concentrations is confined to the E3 isozyme. The E3 isozyme catalyzes the conversion of γ-aminobutyraldehyde to γ-aminobutyric acid (K_m = 5–14 μM) (7, 12) and aldehyde metabolites of spermidine and spermine to corresponding carboxylic acids (12). More recently, it was also identified as a betaine aldehyde dehydrogenase (13). It appears that E3 isozyme may play a role in intermediary metabolism of putrescine, polyamines, and choline.

Study of this enzyme in more complex systems is hindered by the fact that up to the present time no inhibitors have been identified. Here, we report the potent inhibition of the E3 isozyme by the histamine H_2-receptor antagonists. The structures of the H_2-receptor antagonists used during this investigation are shown in Fig. 1. All are based on the chemical structure of histamine and consist of a basic substituted 5-membered ring (imidazole, thiazole, or furan) with a 4-atom side chain at position 4 of the ring. Each side chain bears a polar group, such as a thiourea, guanidine, or cyanoguanidine.

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ABBREVIATION: NHD^+, nicotinamide hypoxanthine dinucleotide.

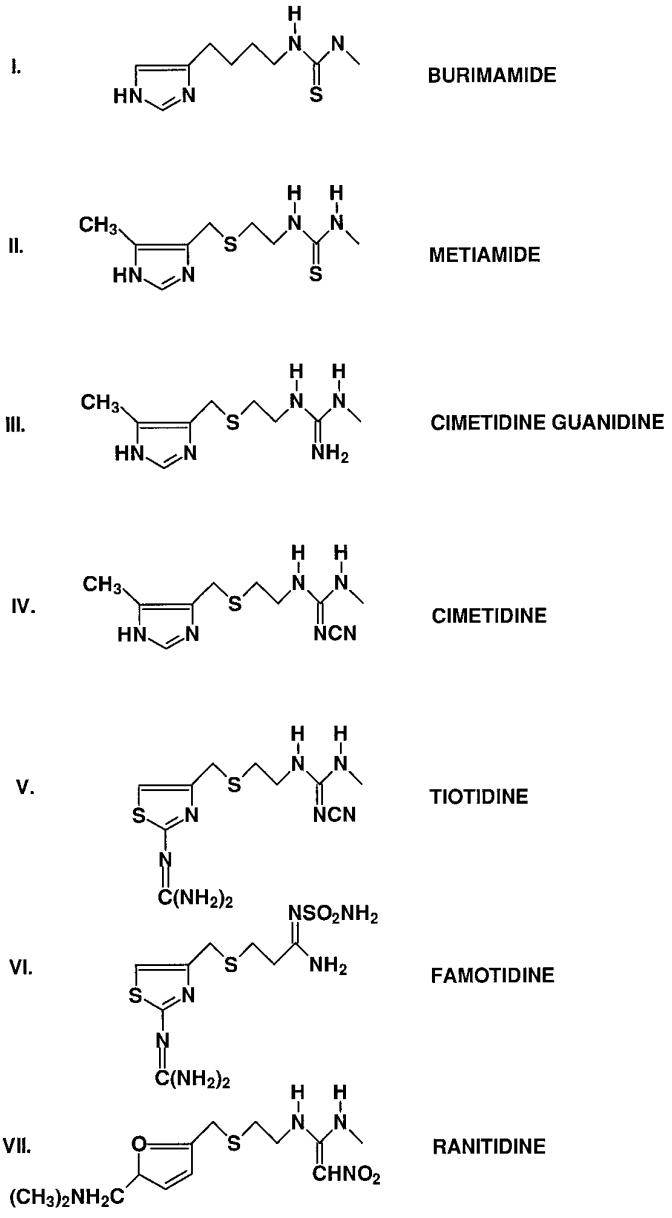

Fig. 1. Chemical structures and nomenclature of the histamine H_2-receptor antagonists used during this investigation. Each structure consists of a substituted 5-membered ring, with a flexible 4-atom side chain that bears a polar group at position 4 of the ring.

Experimental Procedures

Reagents. Aminodecyl agarose, aminoguanidine, cimetidine, 4-(dimethylamino)cinnamaldehyde, famotidine, furan, glycolaldehyde, histamine, histidine, nicotinamide hypoxanthine dinucleotide (NHD^+), ranitidine hydrochloride, and thiazole were obtained from Sigma Chemical (St. Louis, MO). Guanidine hydrochloride was from United States Biochemicals (Cleveland, OH). Burimamide (SKF 91923), cimetidine guanidine (SKF 92408), and metiamide (SKF 92058) were a gift from SmithKline Beecham Pharmaceuticals (Philadelphia, PA). Cyanoguanidine and imidazole were obtained from Aldrich (Milwaukee, WI); dimaprit (*S*-[3-(*N*,*N*-dimethylamino)propyl]-isothiourea) dihydrochloride and cimetidine were from Research Biochemicals International (Natick, MA); and NAD^+ (grade 1) was from Boehringer Mannheim (Indianapolis, IN). Tiotidine maleate was a gift from Dr. T. O. Yellin, SmithKline Beecham Pharmaceuticals, King of Prussia, PA. Tiotidine was also obtained from Tocris Cookson (St. Louis, MO). Stock solutions of the compounds were made up in water, 10 mM HCl, or 0.05 M sodium phosphate buffer, pH 7.4. Tiotidine was also dissolved in dimethyl sulfoxide and famotidine in *N*,*N*-dimethylformamide, both solvents having no effect at 1% v/v concentrations on E3 isozyme activity.

Enzymes. E1, E2, and E3 isozymes were purified from human liver as previously described (6, 7) and stored in 30% glycerol, at 4°, under nitrogen. Before use, the enzymes were extensively dialyzed against 30 mM sodium phosphate buffer, pH 7.0, 1 mM EDTA, to remove glycerol and β-mercaptoethanol. The protein concentration was determined as described by Goa (14), using bovine serum albumin as a standard. Protein was also assayed spectrophotometrically at 280 nm, using an extinction coefficient of 1.0 $(1\ mg/ml)^{-1}\ cm^{-1}$ (7). E3 enzyme stability was checked using an assay mixture of 0.1 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, 500 μM NAD^+ and 0.1 M γ-aminobutyraldehyde as substrate (7). When partial loss of activity occurred, experimental results were adjusted to the maximal activity of 1.6 μmol/min/mg (7).

Kinetic studies. All assays were carried out in 0.05 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, glycolaldehyde as substrate and either NAD^+ or NHD^+ as coenzyme, at 25°. NADH (or NHDH) formation was monitored at 340 nm using a Gilford spectrophotometer. An extinction coefficient of 6.22 $mM^{-1}\ cm^{-1}$ for NADH (and NHDH) was used for the calculation of reaction rates. It was also used in the spectrophotometric determination of the concentration of glycolaldehyde stock solutions, as previously described (12). Inhibition experiments were performed in two ways: (i) for competition versus substrate, glycolaldehyde concentrations were varied at different fixed test-inhibitor concentrations, at a single NAD^+ concentration (500 μM); (ii) for competition versus coenzyme, NHD^+ concentrations were varied at different fixed test-inhibitor concentrations, at a single nonsaturating glycolaldehyde concentration. Reactions were initiated by the addition of enzyme. Reaction rates were determined by tangents to initial velocities. Each point represents duplicate determinations (which did not differ from each other by more than 5%) of the reaction rates. Kinetic data were obtained using the SlideWrite Plus Program, according to the method of Lineweaver and Burk (15), employing the linear least squares regression fit of reciprocals of the reaction rates versus reciprocals of substrate concentration. Regression coefficients were between 0.993 and 0.999 for all data.

Results

Effect of H_2-receptor antagonists on the E1, E2, and E3 isozymes of human aldehyde dehydrogenase. The effect of compounds I-VI on the catalytic activity of the three isozymes of liver aldehyde dehydrogenase (E1, E2, E3) is shown in Table 1. All the compounds (1 mM) had the greatest

TABLE 1

Comparison of the effect of the H_2-antagonists on the E1, E2, and E3 isozymes of human aldehyde dehydrogenase

Enzyme activity was measured at 25° in 0.05 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, 500 μM NAD, and K_m concentrations of glycolaldehyde for each of the three isozymes (330 μM for E1, 50 μM for E2, and 220 μM for E3), in the presence or absence of 1 mM compound. The reaction was started by addition of enzyme.

Compound	Enzyme activity		
	E1	E2	E3
		%	
Burimamide	102	31	20
Metiamide	110	72	19
Cimetidine guanidine	111	71	23
Cimetidine	80	81	0
Tiotidine	43	36	0
Famotidine	134	71	60

effect on the E3 isozyme; cimetidine and tiotidine completely abolished E3 isozyme catalytic activity. The compounds had less effect on the other two aldehyde dehydrogenase isozymes. However, tiotidine abolished more than 50% of the catalytic activity of both the E1 and E2 isozymes and burimamide had a similar effect on the E2 isozyme. It is interesting to note that metiamide, cimetidine guanidine, and especially famotidine produced a slight but reproducible activation of the E1 isozyme. The effect of ranitidine (compound VII) could not be determined because of its high absorbance in the range of NADH absorbance.

Inhibition studies of the E3 isozyme with glycolaldehyde as the varied substrate. NAD^+ was used as the fixed substrate at a saturating concentration (500 μM) with glycolaldehyde (K_m = 221 μM, see footnote to Table 2). Thus, the K_i values shown in Table 2 represent dissociation constants of H_2-receptor antagonists from E3·NAD·inhibitor ternary complex. This concentration of NAD also approximates that in mammalian liver. Compounds I–VI (Fig. 1) inhibited the E3 isozyme in a competitive manner versus aldehyde substrate (as shown for cimetidine, Fig. 2A), allowing K_i values to be obtained from the slope replots (Fig. 2A, *inset*) which were all linear. K_i values shown in Table 2 are mean values from triplicate determinations. Although K_i values for cimetidine and tiotidine were in the low micromolar range, those for burimamide, metiamide, and cimetidine guanidine were larger by about 2 orders of magnitude, and that for famotidine was larger, by almost 4 orders of magnitude.

Inhibition studies of the E3 isozyme with NHD^+ as the varied substrate

Studies with NHD^+ were conducted to determine points of inhibitor binding to the E3 isozyme. The K_m for NAD^+ for the E3 isozyme is low (4 μM), which made variation of coenzyme concentration difficult even at the highest sensitivity of our instruments. Therefore, the NAD^+ analog NHD^+ (K_m = 203 μM, see footnote to Table 3) was used in the kinetic studies where coenzyme was varied. Compounds I–V (Fig. 1) were shown to inhibit the E3 isozyme in a noncompetitive manner, producing both slope and intercept effects (see Fig. 2B, which shows cimetidine guanidine), versus NHD^+. $K_{i\ slope}$ and $K_{i\ intercept}$ values (Table 3) were obtained from secondary plots (as shown in Fig. 2B, *inset*). $K_{i\ intercept}$ values are modified by the fixed concentration of glycolaldehyde used in the experiment. True K_i values were, therefore, calculated using the following equation: $K_i = K_{i\ intercept}/(1 + [\text{concentration of glycolaldehyde}]/K_m\ \text{glycolaldehyde})$.

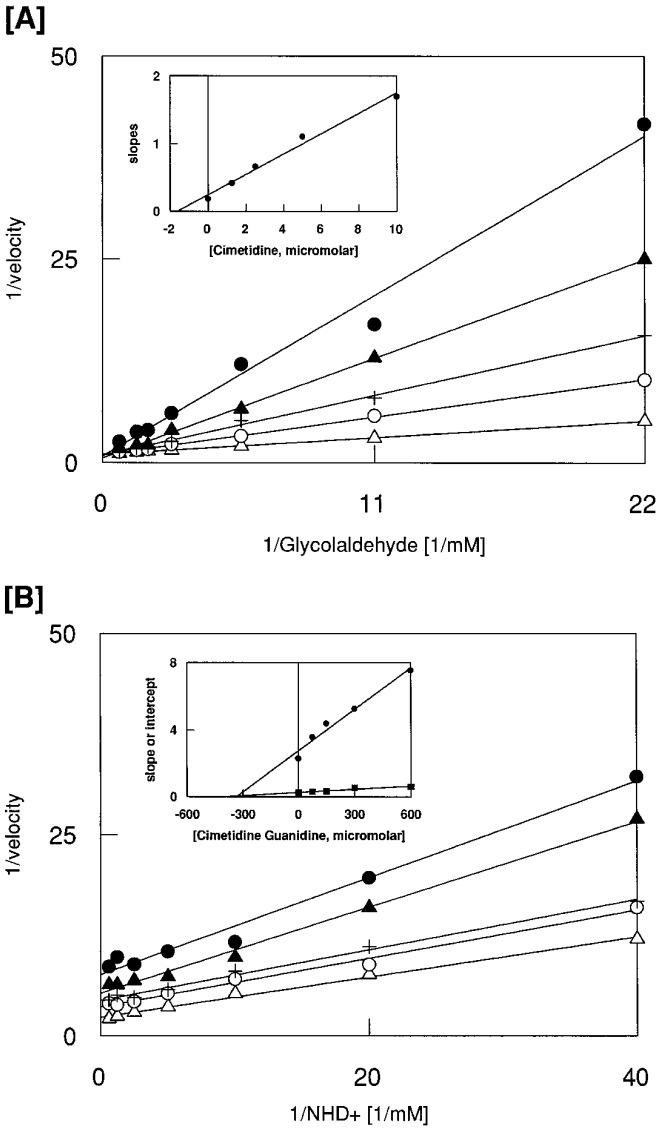

Fig. 2. Lineweaver-Burk plots of cimetidine and cimetidine guanidine inhibition of E3 isozyme activity. The reaction velocity is expressed as micromoles of NADH or NHDH/min/mg of enzyme protein. A, Cimetidine inhibition of E3 isozyme activity at varied glycolaldehyde concentrations and fixed cimetidine concentrations of 0 μM (△), 1.25 μM (○), 2.5 μM (+), 5 μM (▲), and 10 μM (●). *Inset,* slope replot of data represented in A. B, Cimetidine guanidine inhibition of E3 isozyme activity at varied NHD^+ concentrations and fixed cimetidine guanidine concentrations of 0 μM (△), 75 μM (○), 150 μM (+), 300 μM (▲), and 600 μM (●). *Inset,* slope and intercept replots of data represented in B; ■, slopes; ●, intercepts.

Structurally related compounds as inhibitors of the E3 isozyme. Imidazole, thiazole, and cyanoguanidine (Table 4) at 1 mM concentration had no effect on E3 isozyme activity. Dimaprit (*S*-[3-(*N*,*N*-dimethylamino)propyl]-isothiourea), an H_2-receptor agonist, had only slight inhibitory effect. Inhibi-

TABLE 2

H_2 receptor antagonist inhibition of the E3 isozyme with glycolaldehyde as the varied substrate

Enzyme activity was measured at 25° in 0.5 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA and 500 μM NAD^+. Inhibition pattern was competitive in all cases. Values presented as mean ± standard error are representative of three separate experiments. The K_m for glycolaldehyde for the E3 isozyme from 13 experiments was 221.0 ± 20 μM and the V_{max} of 6 experiments was 0.9 ± 0.006 μmol/min/mg. K_B values were determined *in vitro* on guinea pig right atrium against histamine or dimaprit stimulation.

Inhibitor (range)	Varied substrate (glycolaldehyde) range	K_i (mean ± standard error)	In vitro K_B for H_2-receptors[a]
(μM)	*mM*	*μM*	*μM*
Burimamide (0–600)	0.1–8	202 ± 46	7.8
Metiamide (0–600)	0.1–8	122 ± 23	0.92
Cimetidine guanidine (0–600)	0.03–2	76 ± 8	16
Cimetidine (0–10)	0.03–2	1.1 ± 0.2	0.79
Tiotidine (0–12)	0.05–1.5	1.0 ± 0.0	0.015[b]
Famotidine (0–5000)	0.05–1	3400.0 ± 630	0.01[c]

[a] Brimblecombe *et al.* (16).
[b] Yellin *et al.* (17).
[c] Ganellin *et al.* (4).

TABLE 3

H_2 receptor antagonist inhibition of the E3 isozyme with NHD^+ as the varied substrate

K_i values were calculated using the equation $K_i = K_{i\ app}$ (1 + [concentration of aldehyde-substrate]/K_m aldehyde substrate). Inhibition pattern was noncompetitive in all cases. The K_m values for NHD^+ for the E3 isozyme at 100 μM, 300 μM, and 2 mM were similar, with a K_m of 203 ± 31 (mean ± standard error) for five determination

Inhibitor (range)	Varied substrate (NHD^+) range	Fixed substrate (glycolaldehyde)	K_i slope	K_i intercept	K_i calculated from K_i intercept
(μM)	mM	μM	μM	μM	μM
Burimamide (0–600)	0.125–2	100	20	270	190
Metiamide (0–600)	0.125–2	100	150	315	220
Cimetidine guanidine (0–600)	0.05–2	300	390	405	180
Cimetidine (0–10)	0.05–2	300	4	7	3
Tiotidine (0–12)	0.25–20	100		3	2

TABLE 4

Effect of compounds structurally related to the H_2-receptor antagonists on E3 isozyme activity

Activity in the absence of inhibitor was 100%. Assays were carried in 0.05 M sodium phosphate buffer, pH 7.4, 1 mM EDTA, containing 500 μM NAD^+, and 300 μM glycolaldehyde.

Inhibitor	E3 activity remaining at concentration of inhibitor			
	1 mM	5 mM	10 mM	100 mM
			%	
Furan	100		100	100
Imidazole	100		41	20
Thiazole	100		82	23
Aminoguanidine	100			
Cyanoguanidine	100		100	45
Guanidine			100	100
Dimaprit	95	55		

tion of E3 isozyme by imidazole and thiazole was not observed at concentrations below 10 mM. Cyanoguanidine did not exhibit any inhibitory properties up to a concentration of 100 mM.

Comparison of inhibition constants for the E3 isozyme with those for the histamine H_2-receptor. Comparison of the K_i values for the E3 isozyme with the *in vitro* dissociation constants, K_B, of compounds I–V for the histamine H_2-receptor (4, 16–18) showed that the K_i value of cimetidine for the E3 isozyme was indistinguishable from that for the H_2-receptor (Table 2). The K_i values of cimetidine guanidine were also similar, whereas those of tiotidine, burimamide, and metiamide for the E3 isozyme were 2 orders of magnitude higher than for the H_2-receptor. Interestingly, famotidine, which was a poor inhibitor of the E3 isozyme, had an extremely low (17 nM) K_i value for the H_2-receptor.

Discussion

The histamine H_2 receptor antagonists (compounds I–VI, Fig. 1) inhibited or activated all three isozymes of human aldehyde dehydrogenase to varying degrees (Table 1). With all the compounds, inhibition was strongest of the E3 isozyme. Cimetidine and tiotidine proved to be potent inhibitors with K_i values of ~ 1 μM (Table 2). Although still in the micromolar range, the K_i values of burimamide, metiamide, and cimetidine guanidine were 1–2 orders of magnitude larger than those of cimetidine and tiotidine. Thus, H_2-receptor antagonists are the first ever reported potent and selective inhibitors of the E3 isozyme. Only famotidine was a poor inhibitor with a K_i value in the millimolar range.

The K_i values (Table 2) were used to identify the inhibiting moieties of the histamine H_2-receptor antagonists. The ring moieties of the structures were considered first. Metiamide and burimamide, have similar structures. The imidazole ring of metiamide, however, has a methyl substitution at position 5. Metiamide also contains a sulfur in the side chain. It is, therefore, not clear whether the slightly lower K_i value for metiamide for the E3 isozyme is due to the ring substitution or to the presence of sulfur in the side chain. Cimetidine and tiotidine, too, have similar structures, the only difference being that the methyl-imidazole ring of cimetidine is replaced by a guanidinothiazole in tiotidine. However, their K_i values for the E3 isozyme were identical (Table 2), which suggests that replacement of the methylimidazole by a guanidinothiazole ring did not affect inhibition. Comparison of the side chains of the compounds revealed that the removal of the cyano group of the cyanoguanidino side chain of cimetidine resulted in a loss of inhibitory properties. This is seen in the 68-fold increase in the K_i value for cimetidine guanidine. An even higher loss of inhibitory properties (110-fold increase in the K_i value) occurred with the replacement of the cyanoguanidino group (cimetidine) by a thiourea group (metiamide). Replacement of the methyl cyanoguanidino group of tiotidine by a sulfonylamido group (famotidine) increased the K_i value by 3 orders of magnitude. From this, it appears that the side-chain polar groups strongly influence inhibition, with the methyl cyanoguanidino group having the strongest influence. When cyanoguanidine was tested (Table 4) for inhibition of the E3 isozyme; however, it proved to be a poor inhibitor. It thus appears that spatial configuration and a 5-membered heterocyclic ring is important; cyanoguanidine is a potent inhibitor only as a part of the side chain of cimetidine or tiotidine. Although the side chain of cimetidine guanidine has structural resemblance to a known substrate of E3 isozyme, γ-guanidinobutyraldehyde (8), substrates resembling the methyl cyanoguanidine-containing side chain of cimetidine and tiotidine have not yet been identified.

The K_i values of the H_2-receptor antagonists for the E3 isozyme were also compared with their K_i values for the histamine H_2-receptor (Table 2). Although the K_i values of cimetidine were identical, the K_i values of the other H_2-receptor antagonists for the E3 isozyme were larger than those for the H_2-receptor. The most notable difference was with famotidine, whose K_i value for the E3 isozyme was 5 orders of magnitude larger than that for the H_2-receptor. The recognition of these compounds by the E3 isozyme must, therefore, occur in a manner different from that by the histamine H_2 receptor. From data in Table 2 it appears that the side-chain polar groups influence the inhibition of the E3 isozyme by the H_2-receptor antagonists more strongly than the ring moieties. In contrast, the ring moieties of these

compounds are more important for H_2-receptor recognition, and act cooperatively with the side-chain polar groups to confer receptor binding (4, 16, 19). This is demonstrated by the fact that replacement of the methylimidazole ring of cimetidine by a thiazole ring (tiotidine and famotidine) or a furan ring (ranitidine, H_2-receptor, K_i = 0.063 μM) resulted in more potent antagonists of the H_2-receptor (Table 2) (4, 19).

All six compounds inhibited the E3 isozyme in a noncompetitive manner versus varied NHD^+, producing both slope and intercept effects (Fig. 2B). Only an intercept effect versus the varied coenzyme would be expected if the H_2-receptor antagonists bound solely to the E3·coenzyme binary complex (20). The slope effect with varied NHD^+ shows that the H_2 receptor antagonists can also bind before the coenzyme in the reaction sequence, binding to the free enzyme. This binding is prevented when coenzyme is saturating (Table 2). Cimetidine was found to specifically elute the E3 isozyme from an affinity column (to be published elsewhere as a part of improved purification procedure), confirming that cimetidine can bind to the free enzyme. Because of the use of two different coenzymes, the ternary complexes shown in Tables 2 and 3 are different, the one represented by the K_i values in Table 2 is from the E3·NAD^+·I complex and that represented by $K_{i\ intercept}$ values in Table 3 is from the E3·NHD^+·I complex. Despite these differences, the $K_{i\ slope}$ values in Table 2 and values calculated from the $K_{i\ intercept}$ in Table 3 are similar and possibly identical within the experimental error of the procedure employed. The dissociation constants for the E·I binary complex (represented by the $K_{i\ slope}$ values, Table 3), except for burimamide, were also similar to those of E3·coenzyme·I ternary complexes (K_i values in Table 2 and calculated K_i values in Table 3), differing by only a factor of 2 at the most. For the E·I binary complex, the dissociation constant (Table 3, $K_{i\ slope}$) for burimamide was 10-fold lower than that for the E3·NAD^+·I ternary complex (Tables 2 and 3). This suggests that, with the exception of burimamide, the inhibitors and the coenzyme bind independently to the enzyme.

H_2-receptor antagonists are the first ever described potent and selective inhibitors of the E3 isozyme. Their immediate use is envisaged in the purification of the E3 isozyme. They could also be valuable in further studies of the E3 isozyme both *in vitro* and in intact animals. The low K_i values of the E3 isozyme with cimetidine and tiotidine (Table 2) suggest that it may be inhibited *in vivo* during treatment for stomach ulcer, where concentrations of cimetidine are known to rise to $\sim$ 200 μM (21). Such inhibition could result in altered metabolism of polyamines, γ-aminobutyric acid and betaine which are important in cell growth, differentiation, neurotransmission, and osmoregulation.

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References

1. Ash, A. S. F., and H. O. Schild. Receptors mediating some actions of histamine. *Br. J. Pharmacol. Chemother.* **27:**427–439 (1966).
2. Black, J. W., W. A. M. Duncan, G. J. Durant, C. R. Ganellin, and M. E. and Parsons. Definition and antagonism of histamine H_2-receptors. *Nature (London)* **236:**385–390 (1972).
3. Arrang, J. M., M. Garbarg, and J. C. Schwartz. Autoinhibition of histamine release mediated by a novel class (H_3) of histamine receptor. *Nature (Lond.)* **302:**832–837 (1993).
4. Ganellin, C. R. Pharmacochemistry of H_1 and H_2 receptors. *Recept. Biochem. Methodol.* **16:**1–56 (1992).
5. Pietruszko, R. Aldehyde dehydrogenase (EC 1.2.1.3), in *Biochemistry and Physiology of Substance Abuse* (R. Watson, ed.). Vol. 1. CRC Press, Boca Raton, FL, 89–127 (1989).
6. Greenfield, N. J., and R. Pietruszko. Two aldehyde dehydrogenases from human liver. Isolation via affinity chromatography and characterization of the isozymes. *Biochim. Biophys. Acta* **483:**35–45 (1977).
7. Kurys, G., W. Ambroziak, and R. Pietruszko. Human aldehyde dehydrogenase: purification and characterization of a third isozyme with low K_m for γ-aminobutyraldehyde. J. *Biol. Chem.* **264:**4715–4721 (1989).
8. Hsu, L. C., K. Tani, K. Kurachi, and A. Yoshida. Cloning of cDNAs for human aldehyde dehydrogenases 1 and 2. Proc. *Natl. Acad. Sci. USA* **82:**3771–3775 (1985).
9. Kurys, G., P. Shah, A. Kikonyogo, D. Reed, W. Ambroziak, and R. Pietruszko. Human aldehyde dehydrogenase: cDNA cloning and primary structure of the enzyme that catalyzes dehydrogenation of γ-aminobutyraldehyde. *Eur. J. Biochem.* **218:**311–320 (1993).
10. Hsu, l. C., A. Yoshida, and T. Mohandas. Chromosomal assignment of the gene for human aldehyde dehydrogenase-1 and aldehyde dehydrogenase-2. *Am. J. Hum. Genet.* **38:**641–648 (1986).
11. McPherson, J. D., J. D. Wasmuth, G. Kurys, and R. Pietruszko. Human aldehyde dehydrogenase: chromosomal assignment of the gene for the isozyme that metabolizes γ-aminobutyraldehyde. *Hum. Genet.* **93:**211–212 (1994).
12. Ambroziak, W., and R. Pietruszko. Human aldehyde dehydrogenase: activity with aldehyde metabolites of monoamines, diamines and polyamines. *J. Biol. Chem.* **266:**13011–13018 (1991).
13. Chern, M.-K., and R. Pietruszko. Human aldehyde dehydrogenase E3 isozyme is a betaine aldehyde dehydrogenase. *Biochem. Biophys. Res. Commun.* **213:**561–568 (1995).
14. Goa, J. A micro biuret method for protein determination. Determination of total protein in cerebrospinal fluid. *Scand. J. Clin. Lab. Invest.* **5:**218–222 (1953).
15. Linewaever, H., and D. Burk. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56:**658–667 (1934).
16. Brimblecombe, R. W., W. A. M. Duncan, G. J. Durant, J. C. Emmett, C. R. Ganellin, G. B. Leslie, and M. E. Parsons. Characterization and development of cimetidine as a histamine H_2-receptor antagonist. *Gastroenterology* **74:**339–347 (1978).
17. Yellin, T. O., S. H. Buck, D. J. Gilman, D. F. Jones, and J. M. Wardleworth. ICI 125,211: a new gastric antisecretory agent acting on histamine H_2-receptors. *Life Sci.* **25:** 2001–2009 (1979).
18. Takeda, M., T. Takagi, Y. Yoshima, and H. Maeno. Effect of a new potent H_2-blocker, 3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N_2-sulfamoylpropionamidine (YM-11170), on gastric secretion, ulcer formation and weight of male accessory sex organs in rats. Arzneimittelforschung **32:**734–737 (1982).
19. Leurs, R., M. J. Smit, and H. Timmerman. Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther.* **66:**413–463 (1995).
20. Cleland, W. W. Steady state kinetics, in *The Enzymes* (Student Edition) (Boyer, P. D., ed.). Vol. II. Academic Press, New York, 1–65 (1971).
21. Stone, C. L., T. D. Hurley, C. F. Peggs, N. Y. Kedishvili, G. J. Davis, H. R. Thomasson, T. K. Li, and W. F. Bosron. Cimetidine inhibition of human gastric and liver alcohol dehydrogenase isoenzymes. Identification of inhibitor complexes by kinetics and molecular modeling. *Biochemistry* **34:** 4008–4014 (1995).

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