The histamine H2-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 non-competitive 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 $1.1 \pm 0.2 \mu M$ and $1.0 \pm 0.0 \mu M$, respectively; both are the first ever described potent and selective inhibitors of the E3 isozyme.

Examination of the H2-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 H2-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 H1-, H2-, and H3-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 H2-receptor activation (2). In the last 20 years, H2-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 ($K_m = 5$–$14 \mu M$) (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 H2-receptor antagonists. The structures of the H2-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.
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 mM 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 H₂-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>
<thead>
<tr>
<th>Compound</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burimamide</td>
<td>102</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Metiamide</td>
<td>110</td>
<td>72</td>
<td>19</td>
</tr>
<tr>
<td>Cimetidine guanidine</td>
<td>111</td>
<td>71</td>
<td>23</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>80</td>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>Tiotidine</td>
<td>43</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Famotidine</td>
<td>134</td>
<td>71</td>
<td>60</td>
</tr>
</tbody>
</table>

**Enzyme activity was measured at 25° in 0.05 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, 500 μM NAD, and Kᵦₐ 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.**

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**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.
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 an 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 + [concentration of glycolaldehyde]/$K_m$ glycolaldehyde).

**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-

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**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. $K_i$ values were determined in vitro on guinea pig right atrium against histamine or dimaprit stimulation.

<table>
<thead>
<tr>
<th>Inhibitor (range)</th>
<th>Varied substrate (glycolaldehyde) range</th>
<th>$K_i$ (mean ± standard error)</th>
<th>In vitro $K_i$ for H$_2$-receptor$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(μM)</td>
<td>(μM)</td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>Burimamide (0–600)</td>
<td>0.1–8</td>
<td>202 ± 46</td>
<td>7.8</td>
</tr>
<tr>
<td>Metiamide (0–600)</td>
<td>0.1–8</td>
<td>122 ± 23</td>
<td>0.92</td>
</tr>
<tr>
<td>Cimetidine guanidine</td>
<td>0.03–2</td>
<td>76 ± 8</td>
<td>16</td>
</tr>
<tr>
<td>(0–600)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cimetidine (0–10)</td>
<td>0.03–2</td>
<td>1.1 ± 0.2</td>
<td>0.79</td>
</tr>
<tr>
<td>Tiotidine (0–12)</td>
<td>0.05–1.5</td>
<td>1.0 ± 0.0</td>
<td>0.01$^b$</td>
</tr>
<tr>
<td>Famotidine (0–5000)</td>
<td>0.05–1</td>
<td>3400.0 ± 630</td>
<td></td>
</tr>
</tbody>
</table>

$^*$ Brimblecombe et al. (16).
$^b$ Yellin et al. (17).
$^c$ Ganellin et al. (4.)
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 cyanothiazone group (cimetidine) by a thiourea group (metiamide). Replacement of the methyl cyanoguanidino group of tiotidine by a sulfonlamido 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 cyanoguanidino 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; cyanoguanidino 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, $\gamma$-guanidinobutyraldehyde (8), substrates resembling the methyl cyanoguanidino-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.

### 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 $\sim 1$ $\mu$m (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 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 H2-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, H2-receptor, $K_i = 0.063 \mu M$) resulted in more potent antagonists of the H2-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 H2-receptor antagonists bound solely to the E3-coenzyme binary complex (20). The slope effect with varied NHD+ shows that the H2 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 $K_{\text{intercept}}$ values in Table 3 is from the E3-NH2I complex. Despite these differences, the $K_{\text{slope}}$ values in Table 2 and values calculated from the $K_{\text{intercept}}$ in Table 3 are similar and possibly identical within the experimental error of the procedure employed. The dissociation constants for the E1 binary complex (represented by the $K_{\text{slope}}$ values, Table 3), except for burimamide, were also similar to those of E3-coenzymeI 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 E1 binary complex, the dissociation constant (Table 3, $K_{\text{slope}}$) 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.

H2-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 ~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.

Acknowledgments

We thank SmithKline Beecham Pharmaceuticals for supplying the burimamide, metiamide, and cimetidine guanidine and Dr. T. O. Yellin for the gift of the tiotidine maleate.

References