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Intestinal Absorption of Amino Acid Derivatives: Structural Requirements for Membrane Hydrolysis

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Abstract □ The intestinal absorption of L-lysine-*p*-nitroanilide, L-alanine-*p*-nitroanilide, and glycine-*p*-nitroanilide was studied in perfused rat intestine in the presence of a variety of potential competitive inhibitors. The results indicate that the hydrolysis site(s) show side-chain specificity, and that inhibitors require a free amino group in the α-position and must be in the L-configuration to be effective. Glycyl-L-proline, a peptide transport inhibitor, had no effect on the absorption rate.

Keyphrases □ Absorption, intestinal—amino acid derivatives, structural requirements for membrane hydrolysis, rats □ Hydrolysis, membrane—structural requirements, intestinal absorption of amino acid derivatives, rats □ Amino acid derivatives—intestinal absorption, structural requirements for membrane hydrolysis, rats

Previous reports (1, 2) have demonstrated that intestinal membrane (or brush-border) enzymes may serve as useful prodrug reconversion sites. For example, compounds that are insoluble, unstable, or have other undesirable pharmaceutical properties may be derivatized so as to improve these properties with the regeneration of the active drug occurring just prior to entry into the systemic circulation. Clearly the specificity of the enzymes in the brush-border region sets a boundary for this strategy. In this report the intestinal absorption of L-lysine-, L-alanine-, and glycine-*p*-nitroanilides is studied in the presence of a variety of potential competitive inhibitors to more clearly define the specificities of the surface peptidases.

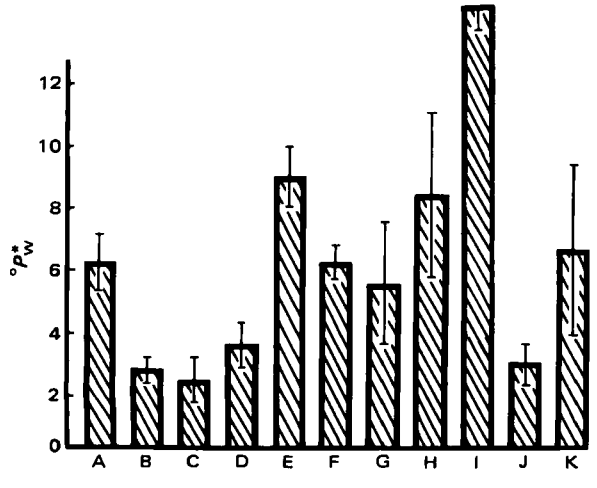

Figure 1—*Intestinal wall permeability (°P^*_w) of L-lysine-p-nitroanilide alone (A) and with L-lysine methyl ester (B), L-arginine-β-naphthylamide (C), L-arginine methyl ester (D), α-N-acetyl-L-lysine methyl ester (E), L-lysine (F), L-phenylalanine methyl ester (G), L-alanine methyl ester (H), glycine methyl ester (I), L-prolylglycine (J), or glycyl-L-proline (K).*

EXPERIMENTAL

Materials—L-lysine-*p*-nitroanilide[1], L-alanine-*p*-nitroanilide[2], and glycine-*p*-nitroanilide[1] were used as received. The inhibitors L-lysine[3], L-lysine methyl ester[2], α-*N*-acetyl-L-lysine methyl ester[3], L-alanine methyl ester[2], L-alanine amide[1], β-alanine methyl ester[1], D-alanine methyl ester[1], L-phenylalanine methyl ester[3], L-phenylalanine amide[1], glycine methyl ester[2], L-arginine methyl ester[1], L-arginine-β-naphthylamide[1], L-prolylglycine[1], and glycyl-L-proline[1] were used as received.

Perfusion Experiments—Rat intestinal perfusion experiments were carried out as previously described (1, 2). Inlet (*Co*) and exit (*Cm*,) concentrations of the perfused segment were measured by determining the *p*-nitroaniline concentration after a 12-hr hydrolysis. The *Cm/Co* ratio was determined using a three-point spectral analysis to account for any background absorbance due to protein in the perfusate. Experiments were carried out with the substrate concentration at 4×10^{-5} *M* and the inhibitor concentration at 4×10^{-3} *M*. Each permeability is the average result from 6–10 rats.

RESULTS AND DISCUSSION

The dimensionless intestinal wall permeability, °P^*_w, was calculated as previously described (3). Tests for significance were done using the two-sample *t* test for samples with unequal variance[4] (95% confidence level). The results are shown in Table I and Figs. 1–3.

L-Lysine methyl ester, L-arginine-β-naphthylamide, and L-arginine

[1] U.S. Biochemical Corp., Cleveland, Ohio
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] MINITAB, University of Pennsylvania, Philadelphia, Pa.

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Table I—Inhibition Results for the Various Substrates [a]

Inhibitor	Substrate		
	L-Lysine-*p*-nitroanilide	L-Alanine-*p*-nitroanilide	Glycine-*p*-nitroanilide
L-Lysine methyl ester	Yes	No	No
L-Arginine-β-naphthylamide	Yes	—	—
L-Arginine methyl ester	Yes	No	—
L-Phenylalanine methyl ester	No	No	—
Glycine methyl ester	No	No	No
L-Lysine	No	—	—
α-*N*-Acetyl-L-lysine methyl ester	No	—	—
L-Alanine methyl ester	No	Yes	—
β-Alanine methyl ester	—	No	—
D-Alanine methyl ester	—	No	—
L-Alanine amide	—	No	—
L-Phenylalanine amide	—	No	—
L-Prolylglycine	Yes	Yes	Yes
Glycyl-L-proline	No	No	No

[a] Using a *t* test at the 95% confidence level.

methyl ester reduced the permeability of L-lysine-*p*-nitroanilide, while L-phenylalanine methyl ester, glycine methyl ester, *N*-acetyl-L-lysine methyl ester, and L-alanine methyl ester did not. This suggests that the hydrolysis site for lysine-*p*-nitroanilide shows a preference for positively charged side chains (lysine, arginine) and a lower affinity for the nonpolar side chains (alanine, glycine). The fact that *N*-acetyl-L-lysine methyl ester is not an inhibitor while L-lysine methyl ester is a good inhibitor demonstrates the requirement for a free L-amino group.

The results for L-alanine-*p*-nitroanilide indicate that, of the compounds studied, only L-alanine methyl ester is a good competitive inhibitor. Since the methyl esters of lysine, arginine, phenylalanine, and glycine did not show significant inhibition, the site of L-alanine-*p*-nitroanilide hydrolysis must be relatively specific for small nonpolar amino acid side chains. The fact that β-alanine methyl ester and D-alanine methyl ester were not inhibitors suggests that the free amino group must be in the α-position and that the stereochemistry is important. This is

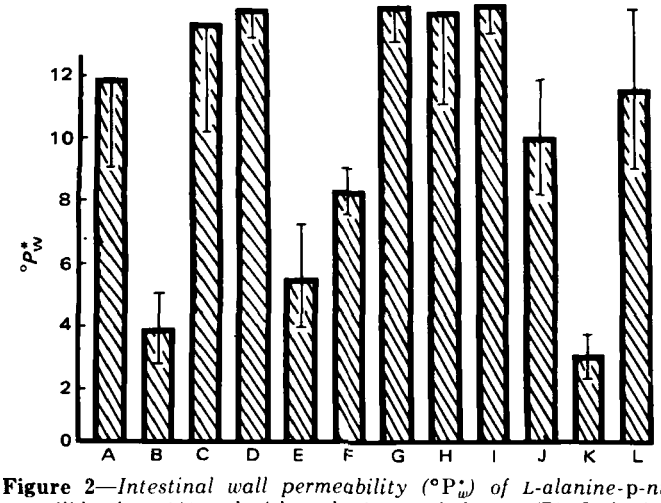

Figure 2—*Intestinal wall permeability ($^{\circ}P_w^*$) of L-alanine-p-nitroanilide alone (A) and with L-alanine methyl ester (B), β-alanine methyl ester (C), D-alanine methyl ester (D), L-lysine methyl ester (E), L-phenylalanine methyl ester (F), L-arginine methyl ester (G), glycine methyl ester (H), L-alanine amide (I), L-phenylalanine amide (J), L-prolylglycine (K), or glycyl-L-proline (L).*

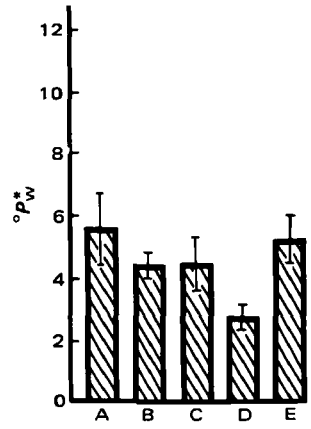

Figure 3—*Intestinal wall permeability ($^{\circ}P_w^*$) of glycine-p-nitroanilide alone (A) and with glycine methyl ester (B), L-lysine methyl ester (C), L-prolylglycine (D), or glycyl-L-proline (E).*

what one would expect for an enzymatic reaction, where the free α-amino group was located in the primary binding site. If the free amino group is located in any other position, the carbonyl carbon that is the subject of attack during hydrolysis would be improperly located with respect to the catalytic groups on the enzyme. The fact that L-alanine amide is not a good competitive inhibitor is somewhat surprising. However, as is the case with α-chymotrypsin substrates (4), it would appear that the amide of alanine has a higher K_m (weaker binding) than the corresponding ester.

The results for glycine-*p*-nitroanilide suggest that the affinity of glycine for the hydrolysis site is weak since glycine methyl ester was not an inhibitor of glycine-*p*-nitroanilide uptake.

The results using the dipeptides glycyl-L-proline and L-prolylglycine show that L-prolylglycine inhibited the absorption of all three amino acid anilides studied. It has been shown that glycyl-L-proline has a high affinity for the intestinal peptide transport process and is not a substrate nor inhibitor of brush-border amino peptidase (5, 6). Since this compound did not inhibit absorption of any of the substrates studied, the result suggests that direct uptake by the peptide transport process does not occur for these compounds. On the other hand, L-prolylglycine appears to be a general amino peptidase inhibitor.

Further studies on the specificity of the intestinal brush-border peptidases in an intestinal perfusion system show that, in addition to the required presence of an α-amino group, the enzymes show specificity for the amino acid side chains as well. The results clearly demonstrate that the absorption mechanism for these compounds is not simple passive diffusion and that the hydrolysis site is probably of the amino peptidase type.

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