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Inhibition of Intestinal Absorption of L-Phenylalanine *in Vivo* by L-Alanine (39385)

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The active transport of L-amino acids by the mammalian small intestine and the interactions between individual compounds have been studied by several groups of investigators (1-4). Mutual inhibition in the intestinal transport of aromatic amino acids, such as L-tyrosine, L-tryptophan, and L-phenylalanine (L-Phe), in *in vitro* systems occurs when there is a relatively large ratio of the inhibitor to the inhibited amino acid (5, 6). This line of investigation has been conducted also in other laboratories, particularly those interested in the elucidation of the mutual interference between L-Phe and L-alanine (L-Ala) (4), and galactose, L-Phe, and L-Ala (3).

It is of potential clinical interest to search for alternative approaches to the established dietary treatment of phenylketonuria, consisting of a very limited intake of L-Phe, aimed at reducing plasma L-Phe levels. In the present studies we have explored the mode of action and kinetics of L-Ala inhibition on L-Phe intestinal transport in an *in vivo* system using isolated, open segments of the upper jejunum in anesthetized rats. L-Ala was shown to impair a nonspecific amino acid carrier system and substantially reduce L-Phe absorption rates, as well as those of other amino acids, when the L-Ala:amino acid ratio was at least 10:1. The effects on L-Phe transport appeared compatible with a noncompetitive type of inhibition.

Materials and methods. Female Wistar rats, weighing between 200 and 250 g, were used in the experiments. They received a standard pelleted feed (purina Lab Chow, Ralston Purina Co.) and water *ad libitum*. Food was withdrawn the afternoon prior to the morning of the perfusions in order to avoid accumulation of semidigested residues in the upper intestinal tract. The rats were anesthetized with 1.2 g/kg body weight of urethane (Aldrich Chemical Co.) administered intraperitoneally. The abdominal cavity was opened by a midline incision and the small intestine cannulated below the ligament of Treitz. A 20-cm long segment was utilized, avoiding carefully any damage to the mesenteric circulation. The intestinal contents were washed out with two 10-ml portions of warm saline. The proximal junction was attached to a peristaltic pump (Harvard Apparatus, Model 1201), and perfused at a rate of 0.20-0.22 ml/min. The distal end was allowed to drain by gravity into graduated tubes. The solutions perfused were Krebs-Ringer bicarbonate buffers containing variable amounts of the amino acids or other substances tested (Sigma Chemical Co.). Polyethylene glycol, mol wt 3000-3700 (PEG, "Carbowax 4,000," Allied Chemical Co.) was used as a nonabsorbable marker at a concentration of 600 mg/100 ml. Tracer amounts of appropriate ^{14}C- or ^{3}H-labeled amino acids (New England Nuclear Corp.) were added such that the perfusion fluids contained 20,000 to 50,000 dpm/ml, in addition to the unlabeled metabolites. The identity of the radioactive substance was tested in the buffers and in perfusates by thin-layer chromatography of 0.025-ml samples on silica gel plates with butanol:acetic acid:water (60:15:25) as a solvent (7). The solutions were kept isotonic by adjusting the concentration of sodium chloride. The buffers were bubbled with a O_2:CO_2 (95:5) gas mixture (Matheson Gas Products) and maintained at 37° during the experiments. Each rat was perfused with two different buffers containing or omitting L-Ala in order to minimize biological variability. The sequence of the buffers perfused was randomized. Fractions were collected every 15 min for 1 hr, after a 45-min equilibration period. A 30-min wash interval was allowed before collecting samples for a second hour. Actual pumping rates were measured before and after the

completion of the experiment. The intestinal segment was removed immediately after sacrificing the animal by exsanguination and measured on a flat surface to the nearest mm.

All fractions collected were centrifuged at 600 *g* for 10 min to separate debris and cells, and 0.50-ml aliquots of the supernatants were transferred to scintillation vials, mixed with 10 ml of a water-compatible scintillator, and counted with efficiency better than 50% for ^{14}C or 20% for ^{3}H. PEG was assayed by a turbidimetric method (8) and the ratio of concentrations indicated the water flux to and from the intestine. The concentration of the solute was determined by isotope dilution. No detectable chromatographic alteration of the radioactive label was observed after passage through the intestine, determined to take only 9 to 10 min. The rates of absorption for each fraction were calculated according to the following formula (9):

$$\left(S_{bf} - \frac{PEG_{bf}}{PEG_s} \times S_s\right) \times \frac{1000 \times \text{Flow rate (ml/min)}}{\text{Intestinal segment length (cm)}} = \frac{\text{nmole}}{\text{min} \times \text{cm}},$$

where S_{bf} and S_s were the m*M* concentrations of the amino acids in the perfusing buffer and in the sample, and PEG_{bf} and PEG_s were the concentrations of PEG in the buffer and the sample, respectively. Additional extensive details of the perfusion technique and the analytical methods have been published previously (10, 11). The values reported here are means ± SE obtained from perfusions of 4 to 12 rats. Student's *t* test was applied to determine the statistical significance of the difference of means (12).

Results and discussion. The absorption rates of L-Phe, when perfused either at 1 or 2 m*M* concentrations, were affected by the simultaneous presence of L-Ala only when the ratio of L-Ala to L-Phe was 10 to 1 or higher (Fig. 1). These concentrations of L-Phe tested were selected because the carrier system for the intestinal transport of the amino acid is not saturated (9, 10) and because they were low enough to allow the addition of relatively large amounts of possible inhibitors without reducing excessively the sodium concentration. The presence of a 20 to 1 molar ratio of L-Ala to L-Phe decreased L-Phe absorption rates by 27.8% for a 1 m*M* perfusate and by 43.7% in the 2 m*M* L-Phe solution.

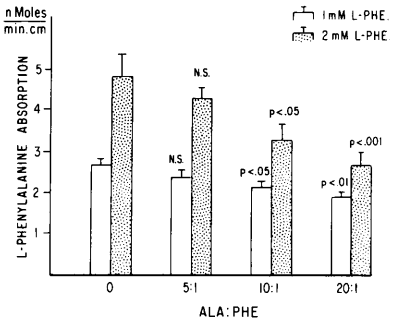

FIG. 1. Inhibition of L-Phe intestinal absorption by L-Ala. The experiments were performed with 1 m*M* (open bars) or 2 m*M* L-Phe (dotted bars) in the presence of no inhibitor, and of L-Ala in various molar ratios, as described in the Materials and Methods section. Each bar represents the mean ± SE of 16 to 48 values, corresponding to 4 to 12 rats perfused. The significance of the differences as compared to the data of the same concentrations without inhibitor was calculated by a nonpaired Student's *t* test (12).

A *V* versus *V/S* plot was drawn from data obtained from L-Phe absorption rates in 0.5 to 3.0 m*M* concentrations in the absence and in the presence of 40 m*M* L-Ala (Fig. 2). It has been shown that the *V* versus *V/S* representation is superior to the *1/V* versus *1/S* Lineweaver-Burk plot, which was included in the illustration for comparative purposes (13–15). These graphs show the rates of absorption *(V)* for different concentrations of the substrate L-Phe *(S)* with or without the simultaneous presence of L-Ala. In order to increase the accuracy of the kinetic analysis, the arithmetic mean between the initial and final concentrations of the perfusates was used for a numerical calculation of V_m and K_t to minimize the error which may arise from consumption of the substrate (16). The K_t derived from the slope of the *V* versus *V/S* plot in the absence of L-Ala was determined to be 3.0 m*M*. The apparent K_t value, in the presence of 40 m*M* L-Ala, was not significantly different, indicating a noncompetitive type of inhibition.

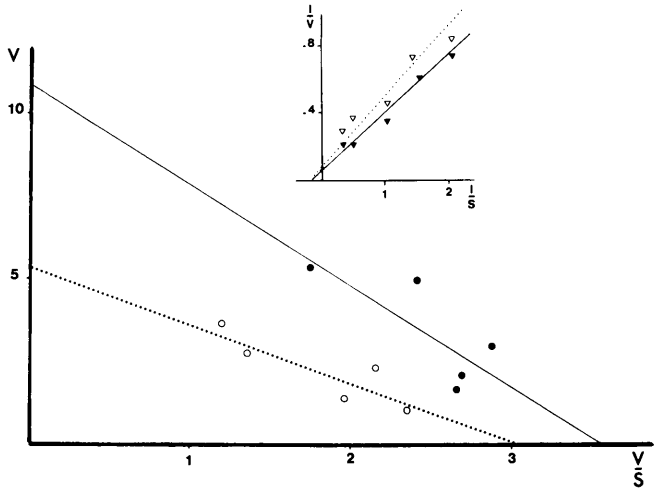

FIG. 2. *V* versus *V/S* plot using the mean intestinal transport rates *(V)* and the concentration of the substrate L-Phe *(S)* in the perfusates. In the insert, the same data were used for the plotting of a Lineweaver–Burk plot. Closed circles or triangles represent points obtained for intestinal transport rates in the absence of L-Ala. Open symbols are values derived from data in the presence of 40 m*M* L-Ala. The regression lines were drawn by the least squares method (12).

The same conclusion could be obtained from the Lineweaver–Burk plot. A statistical numerical estimate of the K_t applying Wilkinson's fine adjustment calculations (13) gave a value of 2.40 m*M* (SE ± 1.35) for buffers without L-Ala, and 1.87 m*M* (SE ± 0.42, $t = 0.39$, N.S.) in the presence of L-Ala, under the conditions stated above. Also, using the same analytical procedure, the V_m for the perfusates containing no L-Ala gave a value of 10.08 nmole/min × cm (SE ± 2.74), while the V_m for the buffer containing 40 m*M* L-Ala yielded a rate of 5.63 (SE ± 0.68, $t = 2.40$, $P < 0.05$). These figures for the intestinal transport affinity constants K_t are somewhat lower than those derived from *in vivo* studies in humans (9), but are similar to those reported for *in vitro* experiments with rabbit intestinal mucosa, which were 2.7 m*M* (2) and 3.5 m*M* (4), respectively. The type of inhibition exerted by L-Ala on L-Phe intestinal transport also was ascertained according to Dixon's graphic procedure (17) for absorption rates of L-Phe at 1 and 2 m*M* concentrations in the presence of increasing levels of L-Ala. A coincidental K_i value of 48 m*M* was obtained.

Our own results agree with previous studies on substrate interrelationships in the active transport of neutral amino acids across the intestinal mucosa (18–21). The L-Phe/L-Ala pair has been extensively explored. Findings indicating that the maximal influx of L-Ala was greater than that of L-Phe suggested to previous investigators that these two amino acids could not be sharing all possible mechanisms of intestinal transport and that L-Ala and L-Phe influxes were mediated by two separate carrier systems which did not differ in their abilities to bind these substances (2, 4, 18). Another important consequence of these studies was the clear discrimination between binding and membrane translocation as two independent processes. These concepts are now confirmed with an *in vivo* experimental model. The similarities between the K_t obtained for L-Phe in the rat *in vivo* system and the *in vitro* rabbit intestinal mucosa, if not an indicator of identity in the binding and transport capacities for that amino acid under different physiological conditions, underscore the comparable order of magnitude for transport constants determined in dissimilar experimental settings.

The effects of a constant 20 to 1 molar ratio of L-Ala to other essential and structurally related amino acids on their intestinal absorption rates are listed in Table I. The L-Ala excess produced significant declines in the rates of intestinal transport for (L-) ly-

TABLE I. INTESTINAL ABSORPTION RATES OF L-AMINO ACIDS IN THE PRESENCE OF A 20:1 MOLAR RATIO FOR L-ALANINE:L-Amino Acid.

Substance	Absorption rates (nmole/min × cm)	
	Without L-Ala	With L-Ala
Lysine[a]	11.76 ± 0.64[b]	6.92 ± 0.36[c]
Histidine	9.16 ± 0.28	4.79 ± 0.45[c]
Methionine	10.23 ± 0.34	8.41 ± 0.60[d]
Arginine	8.10 ± 0.65	9.08 ± 0.35
Leucine	9.38 ± 0.50	9.32 ± 0.94
Isoleucine	8.10 ± 0.65	8.02 ± 0.25
Valine	6.36 ± 0.36	5.71 ± 0.41
Tryptophan	4.10 ± 0.30	3.34 ± 0.42
Tyrosine	5.12 ± 0.48	4.56 ± 0.32
Threonine	7.27 ± 0.57	6.48 ± 0.43

[a] All (L-) amino acids were perfused at a 2 m*M* concentration. L-Alanine (L-Ala) was present at a 40 m*M* concentration.

[b] The absorption rates represent means ± SE; N = 16 or more determinations for each substance tested, in the absence or in the presence of the inhibitor. The significance of the differences was checked by a Student's t test (12).

[c] $P < 0.001$.

[d] $P < 0.05$.

sine, histidine, and methionine. Conversely, 40 m*M* L-Ala had no effect on the absorption of (L-) arginine, leucine, isoleucine, isoleucine, valine, tryptophan, threonine, and tyrosine, when perfused at 2 m*M* levels. These results may indicate that the common transport mechanism for neutral amino acids is also operative for positively charged amino acids and that the proportion of the translocation load for these substances is based toward the common carrier. The heterogeneity in the transport capacity of dibasic amino acids, suggested by the variety of cystinurias and dibasic aminoacidurias described in the literature (22), encourages a similar speculation for neutral amino acids, that is, the coexistence of common and specific carrier systems, either as separate entities or as discrete active sites on the same proteins.

In another series of experiments, the effects of galactose were tested by itself or in conjunction with 40 m*M* L-Ala (Table II). While this amino acid consistently produced a decline in the absorption rates of L-Phe, galactose alone had only minimal consequences. However, the simultaneous perfusion of both substances produced an additive effect and a reduction of the absorption rates to almost half the baseline values. This action could not be attributed to a lower sodium concentration since an equivalent molarity of mannitol had no action on L-Phe absorption rates. In addition, the absolute requirement of the amino group in the alpha position was confirmed, since β-alanine in comparable concentrations had no influence on the transport of L-Phe by the small intestine of the rat.

Since there is general agreement to assume the existence of specific sites or transport systems in addition to common carriers for neutral amino acid translocation (1-6), simultaneous inhibition of both the specific and the nonspecific translocation pathways would be required for a virtual shut off of L-Phe transport. In the experiments reported here, specialized mechanisms appeared responsible for the bulk of intestinal amino acid absorption, since a large molar excess of a potential inhibitor such as L-Ala produced only limited effects.

It is difficult to discriminate under experimental conditions *in vivo* the role of separate carrier systems available for the intestinal transport of L-amino acids. Kinetic studies reveal the physiological options existing at the intestinal mucosa surface. The lack of a competitive type of inhibition for L-Ala over L-Phe can be taken as evidence of the multiplicity of active sites available for the translocation of neutral amino acids. A more effective control of L-Phe absorption may come from the use of selective, specific, antagonists such as β-thienyl-DL-alanine or L-dihydroxyphenylalanine, as we have shown in previous studies (23), in addition

TABLE II. EFFECTS OF VARIOUS SUBSTANCES ON THE INTESTINAL ABSORPTION RATES OF 2 m*M* L-PHE.

Additions	Absorption rates (nmole/min × cm)	Change (%)
None	6.22 ± 0.30[a]	–
40 m*M* L-Ala	4.23 ± 0.43	−32.0[b]
40 m*M* galactose	5.52 ± 0.56	−11.3
40 m*M* L-Ala + 40 m*M* galactose	3.19 ± 0.36	−48.8[c]
80 m*M* mannitol	6.96 ± 0.10	+11.9
40 m*M* β-alanine	6.16 ± 0.32	−1.0

[a] Conditions, number of determinations, and significance, as in Table 1.

[b] $P < 0.01$.

[c] $P < 0.001$.

to noncompetitive agents of low toxicity such as L-Ala. Therefore, unless a considerable reduction of both specific and nonspecific intestinal transport systems can be achieved by the use of appropriate antagonists, the clinical significance of the effects so far described will remain very limited in scope.

Summary. The intestinal transport of L-Phe from 1 and 2 m*M* solutions in the presence of variable concentrations of L-Ala was studied with an *in vivo* technique utilizing 20-cm long jejunal segments in anesthetized rats. The simultaneous perfusion of L-Ala inhibited the absorption of L-Phe when the molar ratio L-Ala:L-Phe was 10:1 or higher. The results suggested a noncompetitive type of inhibition under the experimental conditions described. Other essential amino acids such as L-lysine, L-histidine, and L-methionine at 2 m*M* concentrations were also significantly inhibited by 40 m*M* L-Ala. These data are consistent with the presence of two active transport mechanisms for neutral amino acids in the intestinal mucosa: a common one which can be blocked by large concentrations of L-Ala, and a specific site or carrier which is not affected by this inhibitor.

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