BBA 76073

INFLUX OF NEUTRAL AMINO ACIDS ACROSS THE BRUSH BORDER OF RABBIT ILEUM

STEREOSPECIFICITY AND THE ROLES OF THE α-AMINO AND α-CARBOXYLATE GROUPS

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(Received May 2nd, 1972)

SUMMARY

The influxes of the L- and D-stereoisomers of alanine, valine, serine, leucine, histidine, phenylalanine and tryptophan across the brush border of rabbit ileum and the roles of the α-carboxylate and α-amino groups in the influx process have been examined. Our results indicate that:

1. The interactions between neutral amino acids and the influx mechanism(s) involve the α-amino and α-carboxylate groups as well as the side chain.

2. The requirement for both the α-amino and α-carboxylate groups indicate that their interactions with the influx mechanism are cooperative rather than independent.

3. With the exception of D-valine, the influxes of all of the D-amino acids examined display saturation kinetics, are Na^+-dependent and are subject to classical competitive inhibition by their L-enantiomorphs. The influx of D-valine appears to be largely attributable to simple diffusion, although a small mediated component characterized by a high K_t cannot be excluded.

4. When D-amino acids interact with their influx mechanisms, the neutral side chain is displaced by 180 degrees from the "natural" position, and the ability of the D-amino acids to interact with their influx mechanisms appears to depend upon the ability of the displaced side chain to engage in interactions with neighboring groups.

INTRODUCTION

Current notions regarding the selectivity of amino acid transport mechanisms in mammalian small intestine are based largely on results obtained from studies of transmural transport across *in vivo* or *in vitro* preparations or accumulation of amino acids by isolated segments of small intestine. As pointed out previously, these results do not reflect the properties of a single barrier and, therefore, do not provide justifiable grounds for speculations regarding the molecular properties of amino acid transport

mechanisms[1]. Previous studies from this laboratory dealing with the influxes of lysine[2] and the dicarboxylic amino acids[3] across the brush border of rabbit ileum serve to illustrate the fact that properties of brush border transport mechanisms cannot be deduced from the results of studies of transmural transport or tissue accumulation.

The purpose of the present investigation is to examine the extent to which the brush border carrier mechanisms* for neutral amino acids exhibit stereospecificity and to define the importance of α-amino acid and α-carboxylate groups in these transport processes. We will show that both the α-amino and α-carboxylate groups appear to be essential for interaction with the transport mechanism and, thus, that their roles are cooperative rather than independent. In addition, although the L-enantiomorph is preferred for all amino acids tested, stereospecificity appears to decrease with increasing reactivity of the side chain.

MATERIALS AND METHODS

Male and female white rabbits that had been maintained on normal food intake were killed by intravenous injection of pentobarbital. The distal ileum was excised, opened along the mesenteric border and rinsed free of intestinal contents. Unidirectional influxes of solutes from the mucosal solution across the brush border into the absorptive epithelium were determined as described previously[1].

Unless otherwise indicated, the mucosal solution contained (mM); NaCl, 140; $KHCO_3$, 10; K_2HPO_4, 1.2; KH_2PO_4, 0.2; $CaCl_2$, 1.2; and $MgCl_2$, 1.2. The gas mixture employed for stirring and aeration was 95 % O_2–5 % CO_2 and the pH at 37 °C was 7.2–7.4. Na^+-free solutions were prepared by replacing NaCl with choline chloride. In all studies of amino acid influx *vs* concentration, mannitol was added to the mucosal solutions to avoid effects that could result from differences in osmolarity. Thus, in studies of amino acid influx in the presence of 2.5, 5.0, 10 and 20 mM amino acid, the osmolarity of all solutions was 325 ± 3 mosM. Similarly, in all studies of the effect of one amino acid (or amino acid analogue) upon the influx of another, the appropriate concentration of mannitol was included in the control solution. Phenethylamine, phenylacetic acid and hydrocinnamic acid were stored in concentrated stock solutions that were neutralized with HCl or NaOH. When these salts were employed in concentrations greater than 5 mM, appropriate adjustments were made in the Na^+ or Cl^- concentration of the standard electrolyte solution to maintain constant osmolarity and a final Na^+ concentration of 140 mM.

All chemicals were reagent grade. [^{3}H]inulin and ^{14}C-labeled D- and L-stereoisomers of alanine, leucine, valine, phenylalanine and tryptophan were obtained from New England Nuclear Corporation (Boston, Mass.). The sources of other labeled compounds were: L-serine, Calatomic; D-serine, ICN; phenylacetic acid and hydrocinnamic acid, Tracerlab; and, phenethylamine, New England Nuclear Corporation.

* As will be discussed below, more than one carrier mechanism may be implicated in the influxes of the amino acids examined in this study. This possibility does not affect the interpretation of the present data since, in all instances, comparisons are made between the L- and D-forms of the same amino acid.

RESULTS AND DISCUSSION

Stereospecificity

Kinetics of L- *and* D-*amino acid influxes.* The influxes of the L- and D-stereoisomers of alanine, valine, serine, leucine, phenylalanine, tryptophan and histidine are given as functions of amino acid concentration in the mucosal solution in Figs 1–3; each point is the average of 6–8 determinations. Lineweaver–Burk (double reciprocal) plots of these data were used to estimate the maximal influx (J^{lm}) and the concentration of amino acid needed to elicit a half-maximal influx (K_t). These parameters are given in Table I and the curves shown in Figs 1–3 are the hyperbolae corresponding to these values. The good agreement between these curves and the experimental

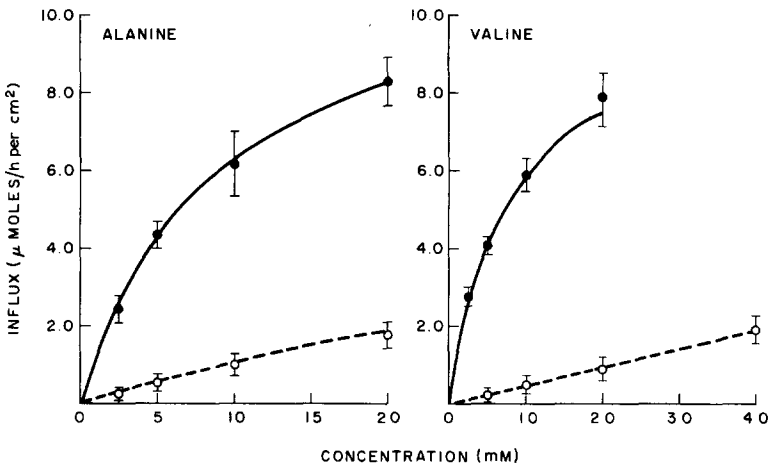

Fig. 1. Influxes of L- (●) and D- (○) alanine and valine across the brush border of rabbit ileum as functions of the amino acid concentration in the mucosal solution. In each instance, influxes of the L- and D-enantiomorphs were determined on adjacent areas of tissue from the same animals. The curves were reconstructed from the kinetic parameters given in Table I.

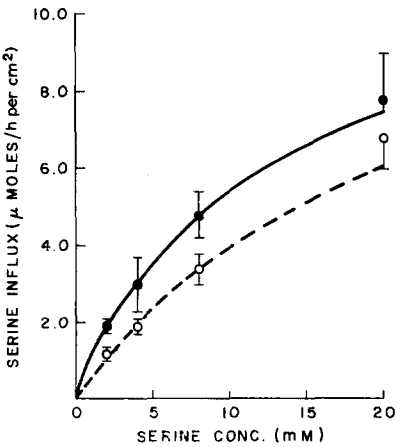

Fig. 2. Influxes of L- (●) and D- (○) serine across the brush border of rabbit ileum. See legend to Fig. 1 for additional detail.

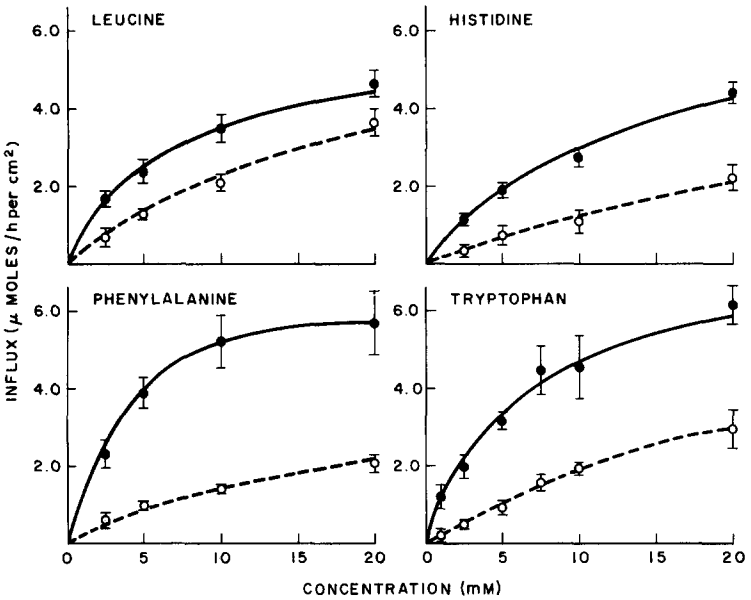

Fig. 3. Influxes of L- (●) and D- (○) enantiomorphs of leucine, histidine, phenylalanine and tryptophan across the brush border of rabbit ileum. See legend to Fig. 1 for additional detail.

TABLE I

KINETIC PARAMETERS OF AMINO ACID INFLUX

J^{lm} expressed in μmoles/h per cm²; K_t expressed in mM.

Amino acid	L-*form*		D-*form*	
	J^{lm}	K_t	J^{lm}	K_t
Alanine	12	9	12	100
Valine	10	7	∞	∞
Serine	12	12	12	20
Leucine	6	6	8	24
Phenylalanine	6	3.5	5	25
Tryptophan	8	6	8	32
Histidine	7	15	7	50

data suggests that the influxes of each of the amino acids tested, with the exception of D-valine, can be adequately described by a saturable process that conforms to Michaelis–Menten kinetics. In all instances, with the exception of D-valine, there is good agreement between the maximal influxes of the L- and D-enantiomorphs, and in all cases the K_t of the D-stereoisomer is significantly greater than that of the corresponding L-amino acid.

Influx of D-valine is a linear function of concentration up to 40 mM; no evidence for saturation is discernible. The permeability coefficient for D-valine calculated from the line illustrated in Fig. 1 is 0.05 cm/h. This value is in good agreement with the permeability coefficient of arabinose, 0.04 cm/h, a molecule that appears to cross

the brush border by simple diffusion[4]. These observations suggest that the influx of D-valine across the brush border can be attributed predominantly to simple diffusion, a conclusion that is corroborated by data that will be presented below. These considerations further suggest that a diffusional component probably contributes significantly to the influx of D-alanine. However, because of the very large K_t for D-alanine influx, we are unable to dissect the curve shown in Fig. 1 into a saturable component and a linear component*.

Finally, examination of Table I indicates that: (a) stereospecificity for serine, leucine, phenylalanine and tryptophan is not as marked as that for alanine and valine; and (b) the amino acids seem to comprise two groups with respect to maximal influxes. The first group includes alanine, serine and valine, with maximal influxes between 10 and 12 μmoles/h per cm^2. The second group includes the remaining, larger amino acids, with significantly lower maximal influxes between 5 and 8 μmoles/h per cm^2**.

Interactions between L- *and* D-*amino acids.* The effects of D-amino acids on the influxes of their L-enantiomorphs and the effects of L-amino acids on the influxes of the D-amino acids are given respectively in Tables II and III. These data are the results

TABLE II

EFFECT OF D-AMINO ACIDS ON INFLUX OF L-AMINO ACIDS

Amino acid	*Concn (mM)*		*Relative influx (r)*		n*
	L-*form*	D-*form*	*Observed*	*Predicted*	
Alanine	1	10	1.0 ± 0.1	0.9	5
Valine	0.5	20	0.9 ± 0.1	1	8
Leucine	1	10	0.7 ± 0.1	0.7	5
Phenylalanine	1	10	0.7 ± 0.1	0.8	5

* n designates number of paired influx determinations.

TABLE III

EFFECT OF L-AMINO ACIDS ON INFLUX OF D-AMINO ACIDS

Amino acid	*Concn (mM)*		*Relative influx (r)*		n*
	D-*form*	L-*form*	*Observed*	*Predicted*	
Alanine	10	10	0.6 ± 0.1	0.5	5
Valine	1	20	0.8 ± 0.1	1	8
Leucine	1	10	0.3 ± 0.1	0.4	5
Phenylalanine	5	5	0.6 ± 0.1	0.5	5

* n designates the number of paired influx determinations.

* An estimate of the permeability coefficient for the diffusional component of D-alanine influx on the basis of that estimated for D-valine is not possible because the diffusional pathways are unknown. Thus, if both D-valine and D-alanine traverse the luminal surface exclusively *via* aqueous channels or pores, the diffusional component of D-alanine influx should exceed that of D-valine influx. On the other hand, the lipid solubility of valine is approximately three times greater than that of alanine (ref. 5, Table VII); thus, the permeability coefficient for D-valine influx could exceed that for D-alanine if diffusion through the membrane matrix is a significant factor.

** This difference cannot be attributed to animal variation. Studies of alanine and phenylalanine influxes using adjacent areas of tissue from the same rabbits corroborate the finding that the maximal influx of alanine is approximately twice that of phenylalanine.

of paired experiments in which the influx of each amino acid was determined in the presence and absence of the other on adjacent areas of tissue from the same animals. Tables II and III give the concentrations employed and the ratios of the influxes in the presence of inhibitor to those in the absence of inhibitor.

D-Alanine and D-valine did not significantly inhibit the influxes of their L-enantiomorphs whereas D-leucine and D-phenylalanine significantly inhibited the influxes of their L-forms. All of the L-forms with the exception of L-valine, markedly inhibited the influxes of their D-stereoisomers. The influx of D-valine from a solution containing 1 mM D-valine was inhibited by only 20 % by 20 mM L-valine. If the observed interactions are the results of classical competitive inhibition, the relative influxes should be given by

$$r = (K_A + [A])/(K_A + [A] + K_A[I]/K_I) \quad (1)$$

where $[A]$ is the concentration of the amino acid whose influx is being measured, $[I]$ is the concentration of the inhibiting amino acid, and the K values are the appropriate K_t values. The predicted relative influxes given in Tables II and III were derived from Eqn 1 and the data given in Table I. In every instance, there is no significant difference between the observed and predicted relative influxes, thus supporting the kinetic parameters and the notion that the interaction between stereoisomers conforms to strictly competitive inhibition. Two points should be stressed. First, L-alanine significantly inhibited the influx of D-alanine thus supporting the notion that at least part of the influx of the latter is carrier-mediated, albeit with a very large K_t. Second, the observation that L-valine only minimally affected the influx of D-valine supports the previous suggestion that most of the influx of the latter cannot be attributed to a mediated process but rather complies with the properties usually attributed to simple diffusion.

Na^+-dependence of D-*amino acid influx.* The influxes of all naturally occurring L-amino acids are inhibited to varying extents by the removal of Na^+ from the mucosal solution[5]. The relative influxes of some D-amino acids in the absence and presence of Na^+ are given in Table IV. With the exception of D-valine, the influxes of all D-amino acids tested are significantly inhibited by substitution of choline for Na^+ in the mucosal solution. It is of interest that the influx of D-alanine is inhibited

TABLE IV

Na^+ DEPENDENCE OF D-AMINO ACID INFLUXES

J^1_0 designates influx in the absence of Na^+; J^1_{140} designates influx in the presence of 140 mM Na^+. Number of influx determinations in parentheses.

Amino acid	*Concn (mM)*	J^1_0/J^1_{140}
D-Alanine	10	0.37 ± 0.05 (9)
D-Valine	20	1.02 ± 0.08 (15)
D-Serine	5	0.58 ± 0.10 (4)
D-Leucine	10	0.38 ± 0.02 (7)
D-Phenylalanine	10	0.55 ± 0.05 (9)
D-Tryptophan	5	0.55 ± 0.07 (8)
D-Histidine	5	0.48 ± 0.05 (6)

by more than 60 % in the absence of Na^+ so that most of the influx in the presence of Na^+ must be attributed to a Na^+-dependent, carrier-mediated process. In contrast, the influx of D-valine was not significantly affected by complete removal of Na^+ from the mucosal solution; this observation further supports the suggestion that D-valine influx can be attributed almost entirely to Na^+-independent simple diffusion.

Previous studies have shown that removal of Na^+ from the mucosal solution increases the K_t for amino acid influx but does not significantly affect the maximal influx[5]. A Lineweaver–Burk plot of D-phenylalanine influx *vs* the concentration of D-phenylalanine in the mucosal solution in the absence of Na^+ is given in Fig. 4. These data indicate that D-phenylalanine influx in the absence of Na^+ can be adequately described by saturable process having a maximal influx of 7 μmoles/h per

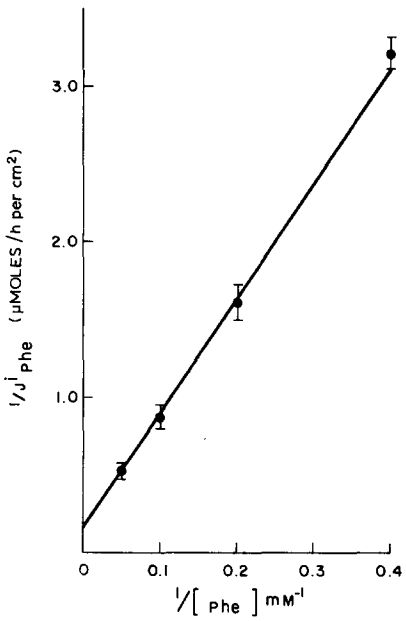

Fig. 4. Lineweaver–Burk plot of the influx of D-phenylalanine across the brush border in the absence of Na^+ as a function of the concentration of D-phenylalanine in the mucosal solution.

cm^2 and a K_t of 50 mM. Similar studies on D-leucine influx in the absence of Na^+ indicate a maximal influx of 8 μmoles/h per cm^2 and a K_t of 67 mM. Thus, for both D-leucine and D-valine, the maximal influx is not significantly affected by removal of Na^+ but the K_t is markedly increased.

A model for the interaction between Na^+ and the influxes of a variety of L-amino acids has been presented in which

$$J_A^i = J_A^{im}[A]/(K_t + [A])$$

and

$$K_t = K_1K_2/(K_2 + [Na^+])$$

where K_1 is the dissociation constant of the binary complex formed by the binding

of amino acid to a membrane component and K_2 is the equilibrium constant for the reaction between Na^+ and the binary complex[6]. Thus,

$$J^i_0/J^i_{140} = (K_{t(140)} + [A])/(K_1 + [A])$$

where J^i_0 and J^i_{140} are the influxes in the absence of Na^+ and in the presence of 140 mM Na^+, respectively, and $K_{t(140)}$ is the K_t in the presence of 140 mM Na^+. Therefore, K_1 can be calculated from the data in Tables I and IV. The calculated values for K_1 for D-leucine and D-phenylalanine are 69 and 54 mM, respectively. These values are in excellent agreement with the K_t values obtained from studies of influx in the absence of Na^+, suggesting that the model derived for L-amino acids may be applicable to the influxes of D-amino acids. Further study is necessary to test this possibility more thoroughly. However, if this is true, the K_2 values for D-leucine and D-phenylalanine can be shown to be 80 mM and 120 mM, respectively. These values are significantly greater than the values of K_2 for the L-enantiomorphs (approx. 20–23 mM)[3,5] suggesting that the D-configuration not only affects the interaction between the amino acid and the binding site on the membrane but also affects the subsequent interaction with Na^+.

The roles of the α-amino and α-carboxyl groups

Influxes of phenylalanine analogues. Previous studies have indicated that the presence of a large lipid-soluble side chain markedly increases the affinity of the interaction between the amino acid and the membrane component, presumably by engaging in hydrophobic bonding[5,7,8]. Thus, in attempting to evaluate the roles of the α-amino and α-carboxyl groups in the influx process, we have chosen to study three analogues of phenylalanine: (a) phenethylamine (β-phenylethylamine) which differs from phenylalanine only by the absence of the α-carboxyl group; (b) hydrocinnamic acid, (β-phenylpropionic acid) which differs from phenylalanine only by the absence of the α-amino group; and (c) phenylacetic acid which is an analogue of phenylglycine and differs from phenylalanine by the absence of the α-amino group and one methyl group in the side chain. In all instances, the presence of the large hydrophobic side chain should maximize the possibility of detecting interactions since two of the three reactive groups are present and presumably could exert their maximal effects. In an analogue of alanine such as ethylamine, not only is the α-carboxyl group absent, but the ability of the CH_3-side chain to engage in hydrophobic bonding is so limited as to prejudice the situation against the detection of interactions with the influx mechanism.

The unidirectional influxes of phenethylamine, hydrocinnamic acid and phenylacetic acid are given as functions of concentration in the mucosal solution in Table V and Fig. 5. In each instance, influx is a linear function of concentration up to 20 mM; no statistically significant evidence for saturation can be discerned. Further, comparison of these data with those in Fig. 3 indicates that the influxes of these three analogues at 20 mM significantly exceed the maximal influx of phenylalanine.

Competitive interactions. The data given in Table V and Fig. 5 do not exclude the possibility that the three analogues tested interact with the phenylalanine influx mechanism; the linear relations observed could be the results of diffusional components *plus* carrier-mediated components with high K_t values. Therefore, studies were performed to determine whether L-phenylalanine influx is affected by the

TABLE V

INFLUX KINETICS OF PHENYLALANINE ANALOGUES

Solute	*Concentration (mM)*:				*n**
	2.5	*5*	*10*	*20*	
Phenethylamine	1.9 ± 0.2	4.1 ± 0.8	7.1 ± 0.7	13.0 ± 1.0	6
Phenylacetic acid	1.2 ± 0.2	2.2 ± 0.4	4.1 ± 0.6	8.4 ± 1.0	6
Hydrocinnamic acid	1.3 ± 0.1	2.6 ± 0.2	4.9 ± 0.3	9.1 ± 0.5	12

* *n* designates the number of influx determinations at each concentration.

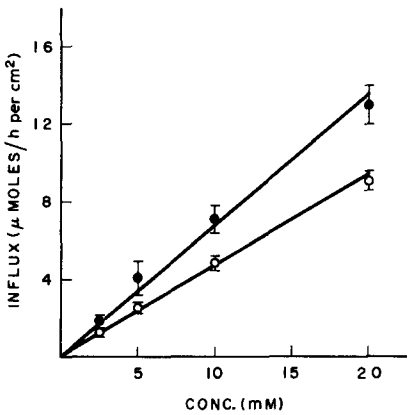

Fig. 5. Influxes of phenethylamine (●) and hydrocinnamic acid (○) across the brush border as a function of their concentrations in the mucosal solution.

presence of these analogues (Table VI) and whether the influxes of phenethylamine, hydrocinnamic acid and phenylacetic acid are affected by the presence of L-phenylalanine (Table VII); in all instances, control influxes and influxes in the presence of the other solutes were obtained using adjacent segments of tissue from the same animals and mannitol was used to maintain equal osmolarities. In no instance was there a statistically significant effect of the analogues on the influx of phenylalanine or an effect of phenylalanine on the influxes of the analogues.

TABLE VI

EFFECT OF ANALOGUES ON PHENYLALANINE INFLUX

Mucosal solution		*Influx (μmoles/h per cm²)*
0.1 mM phenylalanine	+ 20 mM mannitol	0.23 ± 0.02 (10)
	+ 20 mM phenethylamine	0.23 ± 0.03 (10)
	+ 20 mM phenylacetic acid	0.22 ± 0.03 (10)
	+ 20 mM hydrocinnamic acid	0.21 ± 0.02 (10)
0.1 mM phenylalanine	+ 40 mM mannitol	0.16 ± 0.02 (8)
	+ 40 mM phenethylamine	0.16 ± 0.03 (8)
1 mM phenylalanine	+ 40 mM mannitol	1.2 ± 0.1 (4)
	+ 40 mM phenethylamine	1.1 ± 0.1 (4)

TABLE VII

EFFECT OF PHENYLALANINE ON INFLUX OF ANALOGUES

Numbers in parentheses designate number of paired influx determinations.

Mucosal solution		*Influx* (*μmoles/h per cm²*)
1 mM phenethylamine	+ 20 mM mannitol	1.1 ± 0.1 (7)
	+ 20 mM phenylalanine	1.2 ± 0.1 (7)
20 mM phenethylamine	+ 10 mM mannitol	11.9 ± 0.7 (4)
	+ 10 mM phenylalanine	11.2 ± 1.0 (4)
1 mM hydrocinnamic acid	+ 20 mM mannitol	0.62 ± 0.05 (6)
	+ 20 mM phenylalanine	0.60 ± 0.02 (6)
20 mM hydrocinnamic acid	+ 10 mM mannitol	8.9 ± 0.4 (8)
	+ 10 mM phenylalanine	9.0 ± 0.5 (8)
1 mM phenylacetic acid	+ 20 mM mannitol	0.49 ± 0.07 (6)
	+ 20 mM phenylalanine	0.44 ± 0.06 (6)

Partition coefficients. The absence of any evidence for the participation of carrier-mediated processes in the influxes of phenethylamine, hydrocinnamic acid and phenylacetic acid prompted an examination of the lipid-solubility of these compounds. Partition coefficients of [^{14}C]phenylalanine, [^{14}C]phenylacetic acid and [^{14}C]phenethylamine between the buffered electrolyte solution (pH 7.2) and chloroform were determined using a modification of the methods described by Collander[9,10]. The partition coefficients (defined by the equilibrium concentration in chloroform divided by that in the buffer) for phenylalanine, phenylacetic acid and phenethylamine were 0.002 ± 0.001, 0.027 ± 0.010 and 1.5 ± 0.4, respectively. Thus, the removal of the α-amino group from phenylalanine enhances the distribution in chloroform by approximately one order of magnitude, whereas the partition into the chloroform phase of phenethylamine is three orders of magnitude greater than that of phenylalanine. The results qualitatively parallel those reported by Collander[9,10].

CONCLUSIONS

The results of the present investigation provide some insight into possible structural requirements of the mechanisms responsible for the unidirectional influxes of several neutral amino acids across the brush border of rabbit ileum. These results, together with the results of previous studies, indicate that at least three reactive groups are involved in the interaction between a neutral amino acid and the influx mechanism(s), the α-amino group, the α-carboxylate group and the side chain, and provide some information on the relative importance of these interactions.

The requirement for the α-amino and α-carboxylate groups

Previous studies have shown that analogues of neutral amino acids lacking either the α-amino group[11,14] or the α-carboxylate groups[11,15] are not actively transported by everted sac preparations of mammalian small intestine. However, these results have no direct bearing on events at the brush border. Although the demonstration of active transport by the everted intestinal sac generally implicates interaction

with a carrier mechanism, a negative finding does not exclude such interaction. For example: (i) the brush border carrier mechanism may only be capable of facilitated transfer; (ii) transmural transport against a concentration difference may only be demonstrable in the presence of low concentrations of the solute (*e.g.* cationic amino acids[16]) and obscured in the presence of high concentrations; (iii) if the concentration of solute needed to elicit a half-maximal rate of transport is very high, the amount transferred after the usual 1 h incubation may be insignificant; and (iv) interaction with a brush border carrier mechanism may be obscured by intracellular metabolism of the solute (*e.g.* dicarboxylic amino acids[3]). The present results failed to disclose any interaction between phenylalanine analogues lacking either the α-amino group or the α-carboxylate group and the mechanism responsible for phenylalanine influx. These results are at variance with those recently reported by Hajjar and Curran[8]. Using the same methods employed in the present studies, these investigators found that both phenylpropionic acid and phenethylamine inhibited phenylalanine influx. Assuming strictly competitive inhibition the K_t, calculated from their data, for phenethylamine is 35 mM and that for phenylpropionic acid is 132 mM. We are unable to confirm these observations. Neither phenethylamine nor hydrocinnamic acid significantly inhibited phenylalanine influx in the present studies. Further, in view of the findings of Hajjar and Curran[8], we examined the kinetics of the influxes of these analogues as well as the influence of phenylalanine on these influxes. As shown in Table V and Fig. 5, the influxes of phenethylamine, phenylacetic acid and hydrocinnamic acid are linear functions of concentration and display no significant tendency toward saturation. Further, as shown in Table VII, high concentrations of phenylalanine did not significantly affect the influxes of these analogues; this is a particularly sensitive test since the K_t for phenylalanine is much lower than the concentrations used so that any interaction between the influxes of these analogues and the phenylalanine influx mechanism would have been drastically inhibited. In the present studies, all solutions were close to isoosmotic through the addition of mannitol to the control solutions; this procedure was not employed in the studies of Hajjar and Curran[8]. Apart from this difference, we are unable to offer any explanation for these discrepant findings.

All of our data are consistent with the notion that the influxes of phenylacetic acid, hydrocinnamic acid and phenethylamine are entirely the results of simple diffusion and can be attributed to the high lipid solubility of these compounds. This conclusion is supported by the finding that both the permeability coefficient and the partition coefficient of phenethylamine are greater than those of phenylacetic acid or hydrocinnamic acid and is consistent with the data recently reported by Wright and Diamond[17]. We are forced to conclude that both the α-amino group and the α-carboxylate group are essential for interaction with the phenylalanine influx mechanism. It follows that these groups do not interact independently with the influx mechanism but that their effects are cooperative.

The role of the α-side chain

There is an abundant literature indicating that the amino acid transport mechanisms in small intestine, like those in a wide variety of cells, exhibit stereospecific preferences. In most instances, the L-configuration is markedly preferred although it is now well documented that this requirement is not absolute[18,19]. Thus,

the interaction between a neutral amino acid and the carrier mechanism must involve either three points of recognition or the geometric equivalent of one point and a plane[20,21]. Previous studies on this preparation have provided strong evidence that the hydrocarbon side chain of the neutral amino acids markedly influences this interaction and the finding that the affinity of the influx mechanism(s) for a neutral amino acid increases with increasing lipid solubility suggests that hydrophobic bonds are involved[5,7,8].

Oxender[22] has proposed a hypothetical scheme for the interaction of a D-isomer with a binding site that prefers the L-isomer under the conditions that the α-amino and α-carboxylate groups of the two stereoisomers have common bonding points. According to this scheme, the interactions of the L- or D-amino acids with the influx mechanism differ primarily with respect to the mean position of the β-carbon with respect to the α-carbon; in the case of the less preferred D-configuration, the side chain bond is displaced by 180 degrees from the position (with respect to the α-carbon) assumed by the side chain of L-amino acids (Fig. 11, ref. 22). The effect of this displacement on the overall interaction would depend upon steric factors and the ability of the side chain to interact with neighboring groups. Our findings of absolute requirements for the α-amino and α-carboxylate groups suggest that the scheme proposed by Oxender[22] for the Ehrlich tumor cell is applicable to the small intestinal brush border. The finding (Table I) that stereospecificity is least marked for leucine, phenylalanine and tryptophan, amino acids with bulky hydrocarbon side chains, is in complete agreement with Oxender's observations and suggest that the ability of the side chain to engage in hydrophobic bonding enhances the affinity of some D-amino acids.

D-Serine displays a much greater affinity than D-alanine, suggesting that the ability to engage in hydrogen bonding is advantageous compared to the less reactive methyl group. The finding that D-valine has little or no affinity for the influx mechanism is consistent with analogous findings on several enzyme systems[20,23] and suggests that branching of the side chain β to the reactive carboxylate group interferes with binding of the D-stereoisomer[20,24].

Finally, evidence has been presented suggesting that two distinct carrier mechanisms may be responsible for the influxes of L-alanine and L-phenylalanine across the brush border of rabbit ileum[25]. The present observation that the maximal influxes of alanine, serine and valine significantly exceed those of the larger neutral amino acids is consistent with this conclusion. These observations parallel those of Lin *et al.*[11] and Matthews and Laster[26] that the maximum rate of neutral amino acid transport by everted sacs of hamster small intestine decreases with increasing lipid solubility of the side chain, and are consistent with the hypothesis originally proposed by Christensen[27] and his co-workers that the transport of neutral amino acids by a variety of cells is mediated by multiple agencies with overlapping specificities.

In summary, these data are consistent with the notion that (i) for L-amino acids, the interactions with the influx mechanisms involve the α-amino and α-carboxylate groups as well as the side chain; (ii) the absolute requirement for the α-amino and α-carboxylate groups indicate that their effects are cooperative rather than simply additive; (iii) when D-amino acids interact with the influx mechanisms, the side chain is displaced by 180 degrees from the "natural" position; (iv) the ability of the D-amino acids to interact with the influx mechanism appears to depend upon

the ability of the side chain, in this displaced position, to engage in interactions with neighboring groups.

ACKNOWLEDGEMENTS

This investigation was supported by research grants from the U. S. Public Health Service, National Institutes of Health (AM-13744) and the American Heart Association (70-633). Dr Schultz was the recipient of a Research Career Development Award (AM-9013) from the National Institute of Arthritis and Metabolic Diseases.

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