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A CROSS-INHIBITION OF BASIC AMINO ACID TRANSPORT BY NEUTRAL AMINO ACIDS

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SUMMARY

1. At a concentration of 12.5 mM the neutral amino acids inhibited the transport of 1 mM lysine by everted intestinal sacs from the rat in a manner related to their affinity for the neutral amino acid carrier. Leucine and methinine induced a large net flux of lysine from the tissue to the mucosal medium at the lysine steady state while sugars did not, indicating an interaction of the amino acids at the carrier level rather than one due to energy limitations. Leucine and methionine produced a large competitive stimulation of lysine transport, while arginine under the same conditions was inhibitory. This result implies independent carrier systems for the neutral and basic acids. Valine was found to be a partial competitive inhibitor of lysine transport in contrast to arginine which produced total inhibition. The 10–15 % inhibition of valine transport produced by lysine at concentrations between 25 and 100 mM was not found to be competitive. The findings that valine and lysine have the same distribution of transport activity along the intestine and that valine produced a constant inhibition of lysine transport as a function of intestinal location suggests that valine and lysine are not transported by different carrier systems and is consistent with transport by spatially related carrier systems.

2. These results are best explained by an allosteric modification of the basic amino acid site caused by the binding of a neutral amino acid to a structurally specific but closely associated site on a single or polyfunctional carrier.

INTRODUCTION

We have been interested in the nature of the interaction between amino acid and sugar transport[1]. While carrying out experiments pertaining to this problem we had occasion to compare the effects of various sugars and leucine on valine, lysine, and glycine transport. These three amino acids are generally considered to represent three of the four specific pathways of amino acid transport in mammalian intestine[2]. Valine is a representative of the neutral amino acid pathway, lysine a representative of the basic amino acid pathway which probably also includes cystine, and glycine has affinity for a pathway with specificity for imino acids and neutral amino acids without lipophilic side chains. Proline, alanine, and leucine have been reported to have

affinities for both the neutral and imino acid–glycine preferring pathways[3]. It was found during the course of this study that leucine not only was very inhibitory to valine and glycine transport as might be expected but also readily inhibited lysine transport. This unexpected finding[4,5] prompted us to examine in more detail the specificity of the basic amino acid pathways with particular emphasis on the characteristics of the neutral amino acid inhibition and the type of interaction that might exist between the neutral and basic amino acid pathways.

The results indicate that neutral amino acids can inhibit the transport of basic amino acids in a manner generally corresponding to their affinity for the neutral amino acid pathway. The inhibition is partially competitive and is best explained by an allosteric interaction between independent carrier systems or independent uptake sites on a multifunctional carrier.

MATERIALS AND METHODS

Wistar Strain, male rats weighing 180–260 g were used as a source of intestinal tissue. The animals were fed on a standard diet and watered *ad libitum* but deprived of food 12–24 h prior to sacrifice. Tissue handling and manipulation including the preparation of Wilson and Wiseman everted sacs have been previously described[6,7]. The standard incubation medium employed was an oxygenated, Krebs–Ringer Tris buffer, pH 7.4, containing 118 mM NaCl, 25 mM Tris–HCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM K_2HPO_4, tritiated inulin (specific activity 8000–15 000 counts/min per ml) (New England Nuclear), and radioactive [^{14}C]amino acids (specific activity 5000–12 000 counts/min per ml), and nonradioactive amino acids to the desired final concentration. On the basis of previous results 118 mM Na^+ gives optimum amino acid transport[7]. The [^{14}C]lysine and [^{14}C]valine (Amersham/Searle) were uniformly labeled and reported to be at least 98% pure by dilution analysis, paper chromatography, and paper electrophoresis. Paper chromatography of the labeled amino acids in butanol–acetic acid–water was used to check the purity of the amino acids[1]. Chromatography of a mixture of the radioactive and nonradioactive amino acids (1 mM) gave one ninhydrin-positive spot which contained 95% of the total radioactivity applied. In all studies the lysine or valine transport by the experimental sacs (those sacs with the additional amino acid initially present in the incubation medium at the indicated concentration) was compared to the lysine or valine transport of the two control sacs from adjacent portions of the intestine without the amino acid. This procedure not only tended to minimize the variation in amino acid transport due to the sac location in the intestine[8] but also reduced variation due to technical variables. The sacs were incubated at 37° for the time periods indicated for the individual experiments. The sacs were removed from the flasks, and excess incubation medium was removed by touching the sacs on the side of a glass beaker. The sacs were openend; the inside medium was drained and collected, and the empty sacs were blotted and weighed. The residual tissue then was homogenized in 4 times its weight of 5% trichloroacetic acid to make a 20% homogenate. The homogenate was centrifuged, and aliquots of the supernatant, as well as the inside and outside media, were counted in a Tri-Carb liquid scintillation spectrometer (Packard) in a xylene, dioxane, ethanol, naphthalene, 2,5-diphenyloxazole (Packard), 1,4-bis-2-(5-phenyloxazolyl)-benzene (Packard) system. The spectrometer was adjusted to permit less than 0.01% ^{3}H efficiency on the ^{14}C channel and 10%

efficiency of the ^{14}C on the ^{3}H channel. These settings allowed for approx. 60% ^{14}C counting efficiency and approx. 22% ^{3}H counting efficiency. An extracellular fluid space was calculated from the distribution space of [^{3}H]inulin (corrected for 10% ^{14}C contribution) in the residual sac and expressed as percent of tissue wet weight[9].

The experiments which measured changes in the steady state distribution of lysine due to additions of various amino acids and sugars were carried out as follows. After a 90-min incubation of 1 mM lysine the amino acids or sugars were added to the mucosal medium of the experimental sacs to a final concentration of 20 mM. 20 mM mannitol was added to the mucosal medium of the control sacs. The incubation was allowed to proceed 10 min longer. The various parameters of lysine transport were then determined.

In the kinetic studies all regression lines were calculated by the method of least squares. The method used to calculate the confidence limits for the x and y intercepts and the slopes of the lines was that of JERVIS AND SMYTH[10].

Amino acid transport is expressed in the four following forms:

(a) *Intracellular accumulation.* The millimolar concentration of amino acid in the cellular water in a given time period, assuming a water content of approx. 80% of the tissue wet weight[11]. This parameter was calculated on the basis of a modification of a formula used by CRANE AND MANDELSTAM[12] which now takes the following form: mM (cellular water) = (mM homogenate supernatant × homogenate volume) − (extracellular space × tissue wet weight × 0.8 × mM medium)/(1 − extracellular space) × (tissue wet weight × 0.8).

(b) *Medium disappearance.* The amount of lysine disappearing from the 5-ml medium expressed as μmoles per 500 mg tissue wet weight basis after a given time period.

(c) *Medium translocation.* The amount of amino acid (μmoles) translocated from 1 ml of mucosal medium to 1 ml of serosal medium per 500 mg tissue wet weight after a given time period. This parameter was obtained by subtracting the amount of amino acid finally present in 1 ml of mucosal medium from that finally present in 1 ml of serosal medium and equating the results to 500 mg of tissue wet weight.

(d) *Serosal appearance.* The amount of lysine appearing in the serosal media (μmoles) on a 500-mg tissue wet weight basis after a given time period.

The counting rate of each individual experiment was mathematically treated so that 10 000 counts/min per ml were equivalent to the initial concentration of the amino acid. This permitted the conversion of counts to μmoles amino acid from identical experiments having somewhat different initial counting rates. The percent of control values were obtained by dividing the average of the sum of experimental values by the average of the sum of control values. Each rat yielded six control and three experimental sacs. A paired difference t test was used to determine if a significant difference existed between control and experimental values. References to statistical significance pertain to the 5% probability level or lower.

RESULTS

After at least four 10-min incubations with the lysine medium, aliquots of the serosal, mucosal, and tissue homogenate supernatant were subjected to paper chromatographic analysis. The results revealed that all the radioactivity was present in the

ninhydrin spot corresponding to the R_F of the lysine with a recovery of at least 90 % of the counts.

Table I shows the effects of various classes of amino acids present at 12.5 mM on the transport of 1 mM lysine after 10 min. All amino acids were initially present at the same concentration in both the mucosal and serosal media. As might be expected on the basis of competition for the basic amino acid carrier, arginine was the most potent inhibitor of lysine transport. The neutral amino acids, leucine and valine, also markedly inhibited both lysine accumulation and disappearance with leucine being the more inhibitory. Both glycine and proline caused smaller reductions in lysine transport which were generally significant when judged on the basis of most of the transport parameters. The acidic amino acids generally activated lysine transport. In contrast to these results it was found that lysine and arginine concentrations lower than 25 mM did not significantly inhibit the transport of 1 mM valine.

Table II shows the effects of sugars and amino acids on the steady state distribution of lysine. 1 mM lysine was equilibrated for 90 min after which the lysine concentration of the mucosal and serosal media and the tissue water showed no net change. The indicated sugars and amino acids were added to the mucosal medium to a final concentration of 20 mM. 20 mM mannitol was added to the control sacs and the incubation allowed to proceed 10 min longer. If the sugars or amino acids are able to inhibit lysine transport at the carrier level, a net flux of lysine from the tissue to the mucosal fluid will result producing a change from the steady state distribution of the lysine. Galactose and α-methyl-D-glucoside were without effect supporting the conclusion that sugars do not inhibit amino acid transport at the carrier level[1,13]. Of the amino acids tested arginine caused the largest change in the distribution of lysine. Of the neutral amino acids leucine and methionine produced the largest change with valine about one-half as effective. Glutamate and glycine were without consistent effect. Mannitol produced no significant change in the steady state distribution of lysine.

Table III shows the effects of various amino acids added only to the mucosal medium to a final concentration of 1 mM on lysine transport. Lysine was present initially only in the mucosal medium. Arginine produced a large inhibition distinguishing its action on lysine transport from that of the neutral amino acids. Of the latter, leucine and methionine produced a large activation. The activation produced by valine was much smaller and was about the same magnitude as that produced by glycine. Increasing the mucosal leucine concentration to 5 mM negated this stimulation. Glutamate activated lysine transport under these conditions as it did when present in both the mucosal and serosal media (Table I).

Fig. 1 shows the pattern of inhibition of 1 mM lysine medium translocation after 30 min as a function of amino acid concentration over the range 0–100 mM. Glutamic acid produced an activation over the entire concentration range. Glycine produced a significant inhibition of lysine medium translocation at 25 mM and the inhibition further increased at 50 and 100 mM glycine. Valine significantly inhibited lysine medium translocation (27%) at concentrations as low as 5 mM. Above valine concentrations of 25 mM, although the inhibition of lysine transport increased somewhat, the inhibition appeared to be reaching a maximum level, not 100%, indicating a partial inhibition. These findings are in contrast to the total inhibition produced by arginine at a concentration as low as 10 mM.

Fig. 2 presents more conclusive evidence that the valine inhibition of lysine

TABLE I

THE EFFECT OF AMINO ACIDS ON THE TRANSPORT OF L-LYSINE BY EVERTED INTESTINAL SACS

Six control and three experimental sacs were made alternately from the intestine of a rat and incubated for 10 min at 37° in a Krebs–Tris medium containing 1 mM lysine. The experimental medium also contained 12.5 mM of the indicated amino acids. Each value represents the mean determination derived from the number of rats shown in parentheses. *Plus* and *minus* values are one standard error of the mean. A paired difference *t* test was used to obtain the probability levels. A P of 0.05 or less was considered significant. Percent of control values were obtained by dividing the sum of the average experimental values by the sum of the average control values.

Amino acid (12.5 mM)	*Intracellular accumulation (mM intracellular lysine per 10 min)*			*Medium disappearance (μmoles disappearing from mucosal medium per 500 mg per 10 min)*		
	Experimental	*Control*	*% Control*	*Experimental*	*Control*	*% Control*
L-Arginine monohydrochloride (10)	0.52 ± 0.05	3.00 ± 0.16	17.3	0.155 ± 0.045	0.675 ± 0.060	23.0
	$P < 0.001$			$P < 0.001$		
L-Leucine (6)	0.99 ± 0.15	2.98 ± 0.26	33.3	0.215 ± 0.070	0.795 ± 0.110	27.0
	$P < 0.001$			$P < 0.01$		
L-Valine (8)	1.83 ± 0.10	3.00 ± 0.12	61.0	0.245 ± 0.035	0.585 ± 0.075	41.7
	$P < 0.001$			$P < 0.001$		
Glycine (11)	2.87 ± 0.16	3.21 ± 0.13	89.4	0.635 ± 0.050	0.725 ± 0.065	87.6
	$P < 0.05$			$P < 0.05$		
L-Proline (7)	2.45 ± 0.16	2.69 ± 0.23	91.1	0.510 ± 0.085	0.610 ± 0.090	83.6
				$P < 0.05$		
L-Glutamic acid monosodium salt (8)	3.62 ± 0.25	3.23 ± 0.14	112.1	0.790 ± 0.080	0.690 ± 0.112	114.5
	$P < 0.05$			$P < 0.05$		
L-Aspartic acid monopotassium salt (7)	2.88 ± 0.20	2.74 ± 0.17	105.1	0.780 ± 0.085	0.655 ± 0.055	117.3
				$P < 0.05$		

TABLE II

THE EFFECT AFTER 10 min OF SUGARS AND AMINO ACIDS ADDED TO THE MUCOSAL MEDIUM TO A FINAL CONCENTRATION OF 20 mM ON THE INTRACELLULAR ACCUMULATION AND MEDIUM TRANSLOCATILN OF 1 mM LYSINE EQUILIBRATED FOR 90 min

The nine sacs were incubated for 90 min in a Krebs–Tris–1 mM lysine incubation medium. The indicated sugars and amino acids were then added to the mucosal medium of the experimentals to a final concentration of 20 mM while mannitol was added to the mucosal medium of the controls to a final concentration of 20 mM. The incubation was allowed to proceed an additional 10 min after which the intracellular accumulation and medium translocation of lysine was determined. The results are expressed as change from the indicated steady state levels and percent of control. Significant differences are indicated by the probability values. Number of rats shown in parentheses.

Addition (20 mM)	*Intracellular accumulation (mM intracellular lysine)*				*Medium translocation (μmoles lysine per 500 mg)*			
	Steady state control (100 min)	*Change per 10 min*		*% Control*	*Steady state control (100 min)*	*Change per 10 min*		*% Control*
D-Galactose (4)	1.96 ± 0.10	0.01		100.5	0.96 ± 0.17	−0.05		96.4
α-Methyl-D-glucose (3)	2.08 ± 0.10	−0.14		93.3	1.03 ± 0.35	−0.04		95.2
L-Arginine monohydrochloride (4)	2.14 ± 0.12	−0.91		57.5	1.17 ± 0.21	−0.32		72.8
			$P < 0.01$				$P < 0.02$	
L-Leucine (4)	2.01 ± 0.08	−0.62		69.1	0.82 ± 0.23	−0.18		78.8
			$P < 0.01$				$P < 0.05$	
L-Methionine (3)	1.93 ± 0.16	−0.57		70.5	1.03 ± 0.35	−0.19		81.5
			$P < 0.05$				$P < 0.05$	
L-Valine (4)	1.97 ± 0.12	−0.34		82.7	1.15 ± 0.26	−0.15		86.2
			$P < 0.05$					
Glycine (3)	1.91 ± 0.13	−0.22		89.0	0.94 ± 0.28	0.06		106.5
L-Glutamic acid monosodium salt (3)	2.22 ± 0.13	−0.03		98.6	1.15 ± 0.36	0.13		111.9

TABLE III

THE EFFECT OF AMINO ACIDS, ADDED TO THE MUCOSAL MEDIUM ONLY ON THE TRANSPORT OF 1 mM LYSINE INITIALLY PRESENT IN THE MUCOSAL MEDIUM ONLY

Six control and three experimental sacs were incubated for 30 min in a medium containing Krebs–Tris–1 mM lysine at the mucosal aspect. In addition the mucosal medium of the experimentals contained the indicated amino acids to a final concentration of 1 mM. All the serosal media contained only the Krebs–Tris solution. The parameters of lysine transport are expressed as in Table I. Number of rats shown in parentheses.

Amino acid (1 mM)	*Mucosal medium disappearance (μmoles lysine per 500 mg tissue per 30 min)*			*Serosal medium appearance (μmoles lysine per 500 mg per 30 min)*		
	Experimental	*Control*	*% Control*	*Experimental*	*Control*	*% Control*
L-Arginine monohydrochloride (6)	0.628 ± 0.092	1.360 ± 0.150	46.2	0.339 ± 0.049	0.583 ± 0.063	58.1
	$P < 0.001$			$P < 0.01$		
L-Leucine (5)	1.655 ± 0.275	1.270 ± 0.180	130.3	0.790 ± 0.105	0.509 ± 0.060	155.2
	$P < 0.025$			$P < 0.05$		
L-Methionine (5)	1.695 ± 0.285	1.270 ± 0.245	133.5	0.871 ± 0.135	0.569 ± 0.067	153.1
	$P < 0.05$			$P < 0.05$		
L-Valine (5)	1.380 ± 0.160	1.180 ± 0.130	116.9	0.584 ± 0.052	0.540 ± 0.042	108.1
	$P < 0.05$					
Glycine (5)	1.245 ± 0.245	1.255 ± 0.175	99.2	0.446 ± 0.046	0.455 ± 0.053	100.2
L-Glutamic acid monosodium salt (4)	1.535 ± 0.315	1.295 ± 0.205	118.5	0.592 ± 0.086	0.548 ± 0.071	108.0
	$P < 0.05$					

medium translocation is partial, while that of arginine is total. A plot of the reciprocal of the decrease of velocity due to the presence of an inhibitor at various inhibitor concentrations: $1/v_0 - v_i$ (where v_0 = rate of medium translocation of lysine without inhibitor and v_i = rate of medium translocation of lysine at a given concentration of

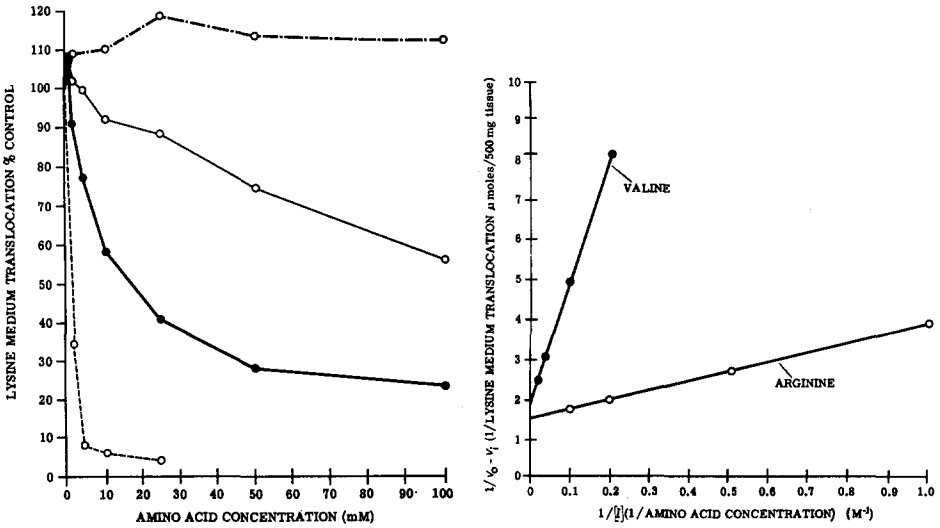

Fig. 1. The effect of glumatic acid (O-·—·-O), glycine (O—O), valine(●—●), and arginine (O-------O) concentration on the medium translocation of 1 mM lysine after 30 min. Each point represents the mean of the average from at least four rats yielding six control and three experimental sacs each.

Fig. 2. Plot to determine the portion of the medium translocation of 1 mM lysine that is subject to inhibition by valine and arginine after 30 min. v_o = rate of concentration difference of lysine without valine or arginine; v_i = rate of concentration difference of lysine at a given concentration of valine or arginine; $[I]$ = concentration of valine or arginine. Each point is the mean from at east four determinations.

inhibitor) plotted against the reciprocal of the inhibitor concentration ($1/[I]$) will yield as the reciprocal of the v intercept the velocity that is subject to inhibition[14]. Taking the reciprocals of these intercepts we find that 0.69 μmole/500 mg per 30 min or about 100% of lysine medium translocation is inhibited by arginine while 0.51 μmole/500 mg per 30 min or about 75% is inhibited by valine. Lysine intracellular accumulation at 5 min gave comparable results with 69% of the lysine accumulation inhibited by arginine and 48% inhibited by valine. The difference in absolute inhibition values is attributed to the contribution of the passive diffusion of lysine to the intracellular accumulation under conditions where a large portion of the active transport is inhibited (*i.e.* valine concentrations as high as 50 mM and arginine concentrations as high as 10 mM).

Table IV shows the effect of lysine concentration over the range 0.5–10 mM on the intracellular accumulation of lysine after 5 min in the presence and absence of 10 10 mM valine. A time curve had shown that 5 min was still on the initial velocity portion of the lysine accumulation curve. The valine inhibition was significant at each lysine concentration tested, but as the concentration of lysine increased the magnitude of the valine inhibition decreased. These results are compatible with competitive

inhibition. Fig. 3 presents the Lineweaver–Burk plot of the values shown in Table IV (ref. 15). The results fell on two straight lines suggesting that lysine accumulation follows apparent Michaelis–Menten enzyme kinetics[16]. The equation of the line representing the intracellular accumulation of lysine in the presence of valine was $y = 0.0669 + 0.4267\ x$. The intercept on the y axis, $1/v_{max}$ (the reciprocal of the maximum velocity), was 0.0669 with 95 % confidence limits of $\pm$ 0.0467. The intercept on the x axis, $1/[S]$ (the negative reciprocal of the apparent Michaelis constant), was —0.1567 with 95 % confidence limits of $\pm$ 0.0521. The equation of the line representing the intracellular accumulation of lysine in the absence of valine was $y = 0.0734 + 0.2790\ x$. The y intercept was 0.0734 with 95 % confidence limits of $\pm$ 0.0427, while the x intercept was —0.2630 with 95 % confidence limits of $\pm$ 0.0512.

TABLE IV

THE EFFECT OF LYSINE CONCENTRATION ON THE INTRACELLULAR ACCUMULATION OF LYSINE IN THE PRESENCE AND ABSENCE OF 10 mM VALINE AFTER 5 min

Six control and three exerimental sacs were incubated for 5 min at 37° in a Krebs–Tris medium containing lysine at the initial concentrations indicated. The experimental medium contained, in addition, 10 mM valine. Each value represents the mean determination from 6 rats $\pm$ S.E. The percent inhibition values were obtained by subtracting the percent control values (see Table I) from 100%.

Lysine concn. (mM)	*Intracellular accumulation (mM lysine per 5 min)*		
	Experimental	*Control*	*% Inhibition*
0.5	1.111 ± 0.060	1.640 ± 0.093	32.3
1.0	1.867 ± 0.115	2.584 ± 0.207	27.8
2.0	3.346 ± 0.128	4.287 ± 0.148	22.0
5.0	7.180 ± 0.584	8.772 ± 0.524	18.1
10.0	11.338 ± 0.755	12.854 ± 0.679	11.8

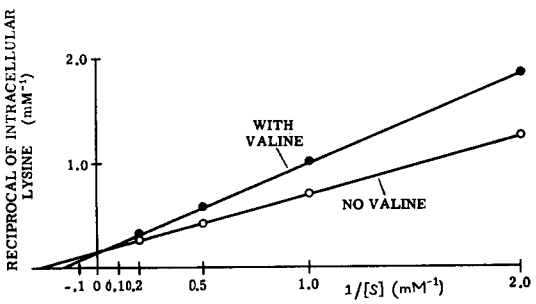

Fig. 3. Lineweaver–Burk plot of the data sown in Table IV. The equations of the regression lines with the 95 % confidence limits of their intercepts are as follows: lysine with 10 mM valine, equation to line, $y = 0.0669 + 0.4267\ x$; intercept on y axis, $1/v = 0.0669 \pm 0.0467$; intercept on x axis, $1/[S] = -0.1567 \pm 0.0521$. Lysine without valine, equation to line, $y = 0.0734 + 0.2790\ x$; intercept on y axis, $1/v = 0.0734 \pm 0.0427$; intercept on x axis, $1/[S] = -0.2630 \pm 0.0512$.

These lines yielding the same y intercepts but different x intercepts are characteristic of a competitive inhibition of lysine intracellular accumulation by valine. The kinetic values from this plot are: v_{max} for lysine accumulation 14.2 mM per 5 min, the

apparent K_m for lysine accumulation 3.8 mM, and the apparent K_m for lysine accumulation in the presence of valine 6.4 mM. The K_i (dissociation constant of the carrier–inhibitor complex) calculated by extrapolation to infinite valine concentration[17] was 5.1 mM. This value is in acceptable agreement with the valine of 7.5 mM for the K_m of valine intracellular accumulation in the rat[1].

Therefore, valine can be classified as a partial competitive inhibitor of lysine transport. One explanation for this kinetic classification is that lysine has more than one pathway of transport, and one of these is totally inhibited by valine in a competitive manner. If this were the case, then according to the criteria suggested by AHMED AND SCHOLEFIELD[18] lysine should be a competitive inhibitor of valine and the K_i of lysine in this system should be approximately the same as its transport K_m. Therefore, the kinetic characteristics of the lysine inhibition of valine transport were investigated. At lysine concentrations below 25 mM, valine transport was not significantly inhibited; at 25 mM, lysine decreased valine intracellular accumulation and medium translocation 12 and 14%, respectively. Increasing the lysine concentration to 50 and 100 mM did not appreciably increase this inhibition which appeared to have attained a maximum level. CHRISTENSEN[19] has reported similar inhibition characteristics (and magnitude) for lysine on phenylalanine uptake by Ehrlich ascites cells.

TABLE V

THE EFFECT OF VALINE CONCENTRATION ON THE INTRACELLULAR ACCUMULATION OF VALINE IN THE PRESENCE AND ABSENCE OF 25 mM LYSINE AFTER 5 min

Six control and three experimental sacs were incubated for 5 min at 37° in a Krebs–Tris medium containing valine at the indicated initial concentrations. The experimental medium contained, in addition, 25 mM lysine. Each value represents the mean determination from 4 rats ± S.E. The percent inhibition values were obtained by subtracting the percent of control values from 100%.

Valine concn. (mM)	*Intracellular accumulation (mM valine per 5 min)*		
	Experimental	*Control*	*% Inhibition*
0.5	1.421 ± 0.085	1.611 ± 0.051	11.8
1.0	2.601 ± 0.094	2.863 ± 0.095	9.2
2.0	4.693 ± 0.229	5.211 ± 0.231	9.9
5.0	9.430 ± 0.438	10.497 ± 0.365	10.2
10.0	13.559 ± 1.250	14.618 ± 1.364	7.3

The effect of valine concentration over the range 0.5–10 mM on the intracellular accumulation of valine in the presence and absence of 25 mM lysine after 5 min is shown in Table V. A Lineweaver–Burk plot of this data resulted in two straight lines with the equations $y = 0.0437 + 0.3323\ x$ (valine accumulation in the presence of lysine) and $y = 0.0429 + 0.2924\ x$ (valine accumulation in the absence of lysine). The x intercepts of these lines with the 80% confidence limits were -0.1315 ± 0.0206 and -0.1467 ± 0.0380, respectively. These identical intercepts as determined at statistically nondiscriminating limits indicate that the lysine inhibition of intracellular valine accumulation cannot be classified as competitive. The apparent K_m for valine accumulation was 6.8 mM and the apparent K_m for valine accumulation in the presence of lysine was 7.6 mM. Assuming these values to be meaningful, the K_i for lysine in this

system based on partial competitive inhibition would be approximately 30 mM or about 8 times as great as the apparent K_m of 3.8 mM found for lysine intracellular accumulation.

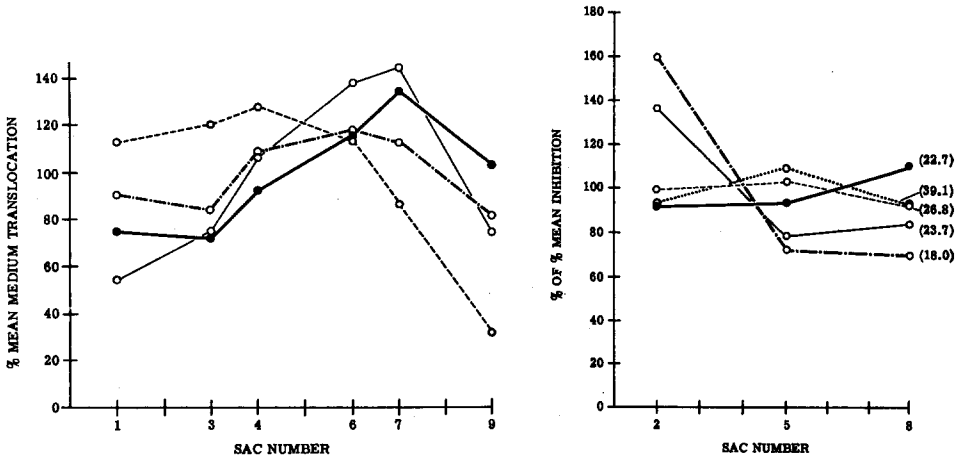

Fig. 4. Pattern of medium translocation of 2 mM galactose (O------O), 1 mM lysine (O-·—·-O), 1 mM valine (●—●), and 3 mM glycine (O—O), after 30 min as a function of sac location along the intestine. The data are expressed as the percent of the mean activity for the whole intestine. Each point is the mean activity from at least twelve rats. Sac 1 is from the proximal jejunum and sac 9 from the terminal ileum.

Fig. 5. Pattern of inhibition of the intracellular accumulation of 1 mM lysine by 25 mM β-methyl-D-glucoside (O-·—·-O), 25 mM galactose (O—O), 0.5 mM arginine (O-------O), 5 mM valine (●—●), and 12.5 mM valine (O·····O) after 10 min as a function of the sac location along the intestine. Each point is the percent of the percent mean inhibition for the whole intestine (values in parentheses) exhibited by the experimental sacs from at least four rats. Sac 2 represents the relative inhibition in the proximal intestine and sac 8 the relative inhibition in the distal intestine.

GEORGE AND BAKER[8] have suggested that if two amino acids show different transport patterns along the small intestine, there must be some difference in the transport system or systems utilized. It might, therefore, be expected that if lysine has more than one pathway of transport and only one of these is also utilized by valine, then the intestinal transport of lysine and valine would probably not show the same distribution of mediating sites in the intestine. Fig. 4 shows the pattern of the medium translocation of valine, lysine, glycine, and galactose as a function of sac location. The amino acids all had their optimum activity in the mid-distal portion of the intestine, while the galactose had its peak activity in the distal jejunum. The pattern of intracellular accumulation of these amino acids and galactose after 10 min showed the same pattern of activity.

If valine and lysine are transported by a common carrier system or a polyfunctional carrier with interacting uptake sites, it would be expected that they will show relatively constant mutual inhibition along the intestine at a constant relative concentration. Fig. 5 shows the distribution of the inhibition of 1 mM lysine intracellular accumulation by 25 mM galactose and α-methyl-D-glucoside, 5 mM and 12.5 mM valine, and 0.5 mM arginine as a function of intestinal location. Arginine, which is generally considered to share the basic amino acid transport pathway with lysine, and

valine produced a relatively constant inhibition along the intestine. The sugars which appear to be transported by separate carrier systems from that of the amino acids[1,13], inhibited primarily in the proximal intestine.

DISCUSSION

The present study has shown that neutral amino acids when present both in the mucosal and serosal media at relatively high concentrations (5 mM and above) can inhibit the transport of lysine. CHRISTENSEN[19] found a similar inhibition of diamino acid uptake by 5 mM and 10 mM neutral amino acids in the Ehrlich ascites cell. There are at least five possible explanations for this inhibition. The merits and drawbacks of each of these explanations will now be dicussed as they relate to the results.

The inhibition may be due to the existence of a common transport carrier for basic and neutral amino acids. This explanation is very unlikely in view of the high affinity of lysine for its carrier ($K_m = 3.8$ mM) and its inability to significantly inhibit valine transport at concentrations below 25 mM. In addition, the inhibition of valine transport at lysine concentrations 25 mM and above was not competitive. If this explanation were valid, the neutral amino acids would produce a total competitive inhibition of basic amino acid transport, such as that produced by arginine, instead of the partial competitive inhibition exhibited by valine. Moreover, the competitive stimulation[20,21] of lysine transport by the neutral amino acids was in contrast to the inhibitory effect of arginine (Table III). The stimulation of basic amino acid transport by certain neutral amino acids has been previously reported[4,5,22] and is generally explained on the basis of two independent carrier systems[23].

The energy required for the simultaneous transport of the neutral and basic amino acids may have become rate limiting. This explanation can be dismissed on the basis of the competitive nature of the neutral amino acid inhibition of basic amino acid transport. Such an inhibition indicates an interaction at the carrier level. In addition, the ability of the neutral amino acids to produce changes from the steady state distribution of lysine (Table II) has been interpreted as an interaction of these amino acids at the carrier level of their transport[13]. The absence of such an effect between the galactose or α-methyl-D-glucoside and the lysine under the same conditions further indicates that energy limitations are not the cause of the interaction between the neutral amino acids and lysine.

The basic amino acids may have two or more distinct transport carriers, and only one of these is inhibited by neutral amino acids in a totally competitive manner. This explanation would be compatible with the partial competitive inhibition of lysine transport by neutral amino acids. Necessarily, it makes the assumption that lysine and valine are transported by a common carrier. However, the characteristics of the lysine inhibition of valine transport fail to meet the criteria proposed for the transport of two amino acids by a common carrier[18]. Lysine was not a competitive inhibitor of valine transport and the K_i of lysine in this sytem differed markedly from that of its K_m. Moreover, if this explanation were valid, it would be expected that the transport of lysine and valine would be mediated by a number of different carriers having a different distribution along the intestine. The results presented in Figs. 4 and 5 are evidence that valine and lysine transport are mediated by carriers having a similar distribution

along the intestine. Finally, no conclusive evidence has yet been presented to indicate that lysine transport occurs by more than one carrier system.

Neutral amino acids may have affinity for an independent neutral amino acid transport carrier as well as an independent basic amino acid transport carrier. Under these conditions valine should be a total competitive inhibitor of lysine transport. Another weakness in this explanation is found in the relationship between the affinities of the neutral amino acids for their carrier site and their ability to inhibit lysine transport (Table I) or produce a net flux of lysine from the tissue to the mucosal medium (Table II). Leucine and methionine have the lowest apparent K_m's for the neutral amino acid carrier followed by valine, proline and glycine[24,25]. The magnitude of their effects follows the same order. There is no reason to assume that the affinity of the neutral amino acids for the neutral carrier site should influence the affinity of the neutral amino acids for the basic amino acid carrier. A total competitive inhibition of valine transport by lysine would also be expected for this explanation to be valid.

We believe that the experimental results are best explained on the basis of an allosteric modification[26,27] of the basic amino acid site caused by the binding of a neutral amino acid to a structurally specific but closely associated site on a single or a polyfunctional carrier[28]. Such a modification may take the form of a change in the conformation or charge of the basic amino acid carrier site. The major supporting evidence for this explanation is the kinetic data which classified valine as a partial competitive inhibitor of lysine transport. This type of competition is explained by a decrease in the affinity of lysine for the basic amino acid site when valine is bound to the neutral amino acid carrier site[17]. In addition, such an allosteric modification would also explain the difference in the characteristics of the arginine and valine inhibition of lysine transport as well as the greater inhibition and net flux of lysine produced by the neutral amino acids with the greater affinity for their carrier site. The 5–20% inhibition of cycloleucine and tyrosine transport by lysine and arginine in hamster intestine has been attributed to an allosteric interaction between separate basic and neutral amino acid binding sites on a polyfunctional carrier[29]. The similar distribution of neutral and basic amino acid transport is consistent with a spatial relationship such as might be expected on the basis of a polyfunctional carrier with independent sites for neutral and basic amino acids. The failure of lysine to produce an inhibition of valine transport comparable to that of valine on lysine transport does not necessarily constitute evidence against the allosteric reaction explanation since the concept of allosteric reaction does not necessarily imply that there would be a reciprocal effect.

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