BBA 75810

A BASIS FOR THE DIFFERENCE IN THE INHIBITION OF THE UPTAKE OF VARIOUS NEUTRAL AMINO ACIDS BY LYSINE IN INTESTINAL EPITHELIAL CELLS

SHELDON REISER AND PHILIP A. CHRISTIANSEN

Gastrointestinal Research Laboratory, Departments of Medicine and Biochemistry, Indiana University School of Medicine and Veterans Administration Hospital, Indianapolis, Ind. (U.S.A.)

(Received November 15th, 1971)

SUMMARY

1. 1 mM lysine inhibited the uptake of 1 mM alanine, leucine, methionine, phenylalanine, threonine and histidine but not 1 mM valine, isoleucine, tryptophan and proline by epithelial cells isolated from rat intestine.

2. A stimulation of lysine uptake by leucine or alanine was always accompanied by an inhibition of leucine or alanine uptake by lysine at amino acid concentrations between 1 and 10 mM.

3. The lysine-induced increase of the movement of alanine out of alanine-loaded cells was independent of extracellular Na^+.

4. In the absence of extracellular Na^+, 1 mM alanine uptake was not inhibited by 1 mM lysine but was inhibited by 1 mM leucine.

5. The inhibition of 1 mM alanine uptake by lysine was essentially maximal at lysine concentrations as low as 1 mM.

6. Lysine was a partially competitive inhibitor of alanine uptake.

7. These results show a definite correlation between the properties of the lysine inhibition of neutral amino acid uptake and the stimulation of lysine uptake by neutral amino acids and are generally compatible with the operation of a hetero-exchange transport system between intracellular neutral amino acid and extracellular ysine.

INTRODUCTION

The ability of basic amino acids such as lysine and arginine to inhibit the transport of various neutral amino acids across mammalian intestine is well documented[1–6]. This inhibition has been previously attributed to allosteric interactions between the neutral and basic amino acid binding sites[1] or to an overlapping in the transport specificities of certain neutral and basic amino acids such as leucine and lysine[4]. However, one of the characteristics of this inhibition appears to be a difference in the ability of the basic amino acid to inhibit the transport of neutral amino acids having very similar transport specificities. In this regard it has been shown that lysine and arginine inhibit methionine uptake in rat intestine while not significantly

decreasing isoleucine uptake[3]. Similarly, the transport of leucine[4,6] but not valine[7] has been reported to be inhibited by lysine. Recently it has been shown that leucine and methionine, but not valine and isoleucine, stimulate the transport of lysine across intact intestine[4] and stimulate the uptake of lysine by isolated intestinal epithelial cells[6]. This similarity in the dichotomy between the neutral amino acids whose transport is inhibited by lysine and the neutral amino acids producing a stimulation of lysine transport suggest that these two interactions may be related. The purpose of this study was to examine the properties of the lysine inhibition of neutral amino acid uptake in order to determine whether this inhibition is a result of a concurrent stimulation of lysine uptake by neutral amino acids which has been attributed to a hetero-exchange transport system[6].

MATERIALS AND METHODS

Wistar strain, male rats weighing 180–260 g were used as a source of both the everted intestinal sacs and the isolated epithelial cells. The animals were fed on a standard diet and watered *ad libitum* but deprived of food 4–8 h prior to sacrifice. The methodology used to prepare the isolated intestinal epithelial cells has been described in detail[8]. The only modification employed was the filtering of the cell suspension through a single layer of gauze prior to the final collection step. The tissue handling and manipulations involved in the preparation of the everted sacs have also been previously described[9,10].

The standard incubation medium employed to measure amino acid uptake by the cells and amino acid transport in intact intestine was 5 ml of 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 KH_2PO_4, and radioactive (10000–15000 counts/min per ml) and non-radioactive amino acids to the desired final concentration. In addition, the medium used in the sac studies contained 8000–12000 counts/min per ml of methoxy [^{3}H]inulin. The serosal compartment of each sac was filled with 1 ml of the identical incubation medium bathing the mucosal aspect of the sac. The methodology used for the calculation of the intracellular accumulation[7,11] and the medium translocation[11] of the amino acid after incubation in the sac system has been previously described.

To measure amino acid uptake by the isolated epithelial cells, 0.3 ml of the cells representing an average of 6.05 mg protein were added to 5 ml of the oxygenated Krebs–Tris amino acid medium without inulin described above. An aliquot of the incubation medium was taken after the cells were dispersed in the medium for a determination of the initial counts in the reaction mixture. The reaction mixture was then incubated with shaking for the desired time period at 37° after which the reaction was terminated by pouring the contents of the reaction mixture into a graduated centrifuge tube in an ice bath and the cells centrifuged in the cold at 275 × *g* for 2 min. The cells were then washed and centrifuged 3 additional times with 5 ml cold Krebs–Tris. The final pellet was diluted to 1 ml with cold saline and mixed, and aliquots were taken for the determination of protein[12] and radioactivity[13]. The uptake of the amino acids by the cells was determined as previously described[6,8] and expressed as the concentration of the amino acids in the cell water.

In the studies using Na^+-free incubation media, choline chloride was used as

an isotonic replacement for the NaCl of the Krebs–Ringer Tris buffer. The Na^+ concentration of the incubation media was determined by direct analysis in a Coleman flame photometer.

In the kinetic studies the 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 slopes of the lines was that of Jervis and Smyth[14].

The source and specific activity of the radioactive compounds used in this study were: L-[^{14}C]alanine (156 mC/mmole), L-[^{14}C]leucine (334 mC/mmole), L-[^{14}C]phenylalanine (447 mC/mmole), L-[^{14}C]valine (260 mC/mmole), L-[^{14}C]isoleucine (312 mC/mmole), L-[^{14}C]histidine (310 mC/mmole), L-[^{14}C]threonine (208 mC/mmole), L-[^{14}C]tryptophan (312 mC/mmole), L-[^{14}C]proline (265 mC/mmole) and L-[^{14}C]lysine (312 mC/mmole), all from Amersham Searle and L-[^{14}C]methionine (210 mC/mmole) and methoxy-[^{3}H]inulin (431 mC/mmole) from New England Nuclear.

RESULTS

Table I illustrates the lack of uniformity in the inhibition of 1 mM neutral amino acid uptake by the cells in the presence of 1 mM lysine. Alanine, leucine, methionine, phenylalanine, threonine and histidine all were significantly inhibited by lysine while valine, isoleucine, tryptophan and proline were not. These amino acids are generally accepted as being transported by a pathway having a high affinity for neutral amino acids with lipophilic side chains. Proline, in addition, also appears to have affinity for an imino acid-glycine preferring pathway[15,16]. Of the amino acids inhibited by lysine, all except histidine have been previously shown to stimulate lysine uptake[6]. On the basis of 8 individual experiments, 1 mM threonine was found to significantly ($P < 0.05$) stimulate 1 mM lysine uptake by 14.7% (2.20 ± 0.07 mM lysine per 5 min, in the presence of 1 mM threonine 2.52 ± 0.08 mM lysine per 5 min). In

TABLE I

THE EFFECT OF 1 mM L-LYSINE ON THE UPTAKE OF VARIOUS NEUTRAL AMINO ACIDS BY ISOLATED INTESTINAL EPITHELIAL CELLS

Isolated intestinal epithelial cells were incubated for 5 min at 37° in a Krebs–Tris medium containing 1 mM of the indicated neutral amino acids without or with 1 mM L-lysine. Each value represents the mean ± 1 S.E. from the number of individual experiments shown in parentheses. A paired-difference *t* test was used to obtain the probability values and a *P* of 0.05 or less was interpreted as indicating a significant inhibition by lysine.

Neutral amino acid (1 mM)	*Amino acid uptake (mM/5 min)*		*% Lysine inhibition*	*P*
	No lysine	*With 1 mM lysine*		
L-Alanine (20)	5.69 ± 0.26	3.74 ± 0.21	34.3	<0.001
L-Leucine (23)	3.21 ± 0.20	1.99 ± 0.10	38.0	<0.001
L-Methionine (22)	1.70 ± 0.12	1.31 ± 0.08	23.0	<0.01
L-Phenylalanine (20)	3.49 ± 0.19	2.20 ± 0.15	37.0	<0.001
L-Threonine (14)	2.92 ± 0.11	2.47 ± 0.10	15.4	<0.01
L-Valine (22)	2.46 ± 0.18	2.32 ± 0.22	5.7	
L-Isoleucine (22)	1.87 ± 0.12	1.74 ± 0.09	7.0	
L-Histidine (22)	3.44 ± 0.20	2.79 ± 0.14	18.9	<0.001
L-Tryptophan (14)	5.71 ± 0.29	5.65 ± 0.35	1.1	
L-Proline (14)	1.60 ± 0.08	1.60 ± 0.09	0.6	

contrast, the amino acids which did not stimulate 1 mM lysine uptake such as valine, isoleucine, tryptophan and proline[6] were not inhibited by 1 mM lysine.

Since the stimulation of lysine uptake by leucine has been shown to be dependent on the leucine concentration[6], the validity of the interrelationship between concurrent stimulation and inhibition was tested by varying the concentrations of lysine and leucine over the range 1–10 mM. The resultant uptake values are shown in Table II. At all concentrations at which leucine stimulated lysine uptake, the uptake of leucine was inhibited by lysine. At the concentrations at which leucine either inhibited or had no effect on lysine uptake, the uptake of leucine was not significantly inhibited by lysine.

TABLE II

THE INTERRELATIONSHIPS BETWEEN LYSINE AND LEUCINE UPTAKE BY ISOLATED INTESTINAL EPITHELIAL CELLS AS A FUNCTION OF THE RELATIVE CONCENTRATIONS OF THE AMINO ACIDS

Isolated intestinal cells were incubated for 5 min at 37° in a Krebs–Tris medium containing L-lysine and L-leucine at the indicated concentrations. Each value represents the mean amino acid uptake ± 1 S.E. from at least 6 individual experiments. The percent of control was obtained by dividing the average uptake of lysine in the presence of leucine by the average uptake of lysine alone or by dividing the average uptake of leucine in the presence of lysine by the average uptake of leucine alone. A paired-difference t test was used to obtain the probability values and a P of 0.05 or less was considered significant. All percent of control values significantly different from the control are italicized.

Lysine concn. (mM)	*Leucine concn. (mM)*	*Lysine uptake*		*Leucine uptake*	
		mM per 5 min	*% Control*	*mM per 5 min*	*% Control*
1	1	4.01 ± 0.23	*144*	1.99 ± 0.10	*62*
1	5	2.60 ± 0.18	94	7.79 ± 0.75	102
1	10	1.33 ± 0.12	*48*	12.37 ± 0.82	95
5	1	12.89 ± 0.67	*140*	1.67 ± 0.08	*52*
5	5	11.07 ± 0.94	95	7.78 ± 0.72	93
5	10	8.92 ± 0.37	*77*	11.74 ± 0.58	90
10	1	17.94 ± 1.09	*136*	1.70 ± 0.11	*53*
10	5	14.21 ± 0.99	*121*	5.79 ± 0.24	*76*
10	10	13.07 ± 0.96	*112*	11.00 ± 1.08	*85*

Table III illustrates the interrelationships between lysine and alanine uptake by the cells as a function of the relative concentrations of the amino acids over the concentration range 1–10 mM. In contrast to leucine, alanine stimulated lysine uptake at all concentrations tested and was inhibited by lysine at the corresponding concentrations. On the basis of these results, it appears that the interactions between leucine and lysine are more complex than those between alanine and lysine and that the characteristics of the inhibition of neutral amino acid uptake due to stimulation of lysine uptake could best be interpreted using alanine instead of leucine.

Table IV shows the comparison between the lysine and leucine inhibition of alanine uptake in the presence of extracellular Na^+ (lysine uptake stimulated by alanine) and in the absence of extracellular Na^+ (lysine uptake not stimulated by alanine). Leucine significantly inhibited alanine uptake in the presence or absence of extracellular Na^+ while the lysine inhibition of alanine uptake was evident only in the presence of extracellular Na^+. These results indicate that the Na^+ dependency

TABLE III

THE INTERRELATIONSHIPS BETWEEN LYSINE AND ALANINE UPTAKE BY ISOLATED INTESTINAL EPITHELIAL CELLS AS A FUNCTION OF THE RELATIVE CONCENTRATIONS OF THE AMINO ACIDS

Isolated intestinal cells were incubated for 2 or 5 min at 37° in a Krebs–Tris medium containing L-lysine and L-alanine at the indicated concentrations. Each value represents the mean amino acid uptake ± 1 S.E. from at least 6 individual experiments. Percent of control and probability values were obtained and expressed as described in Table II.

Lysine concn. (*mM*)	*Alanine concn.* (*mM*)	*Lysine uptake*		*Alanine uptake*	
		mM per 5 min	*% Control*	*mM per 2 min*	*% Control*
1	1	4.78 ± 0.28	*182*	2.26 ± 0.12	*64*
1	5	5.29 ± 0.35	*191*	8.44 ± 0.38	*74*
1	10	3.49 ± 0.24	*159*	12.61 ± 0.44	*82*
5	1	8.19 ± 0.41	*134*	2.14 ± 0.11	*60*
5	5	10.32 ± 0.93	*176*	6.68 ± 0.30	*72*
5	10	13.18 ± 1.25	*142*	9.90 ± 0.44	*73*
10	1	11.78 ± 0.61	*125*	1.89 ± 0.11	*53*
10	5	15.66 ± 1.43	*134*	5.88 ± 0.65	*65*
10	10	15.59 ± 1.31	*133*	10.43 ± 0.77	*85*

TABLE IV

COMPARISON OF THE LYSINE AND LEUCINE INHIBITION OF 1 mM ALANINE UPTAKE BY ISOLATED INTESTINAL EPITHELIAL CELLS IN THE PRESENCE AND ABSENCE OF EXTRACELLULAR SODIUM

Isolated intestinal epithelial cells were washed once with 20 vol. of a 4° Krebs–Tris–choline medium prepared by the isotonic replacement of the NaCl of the Krebs–Ringer–Tris buffer with choline chloride. The cells were then incubated for 2 min at 37° in either the normal Krebs–Tris medium or the Krebs–Tris–choline medium containing 1 mM of the amino acids indicated. Each value represents the mean alanine uptake ± 1 S.E. from 16 individual experiments. The probability values were obtained and interpreted as described in Table I.

Amino acid (*1 mM*)	*Extracellular Na^+* (*mM*)	*Alanine uptake* (*mM per 2 min*)	% *Inhibition*	*P*
Alanine	112.2 ± 2.9	3.17 ± 0.23		
Alanine *plus* lysine	112.5 ± 3.0	2.38 ± 0.16	24.9	<0.001
Alanine *plus* leucine	111.8 ± 3.1	1.49 ± 0.06	53.0	<0.001
Alanine	1.9 ± 0.2	0.77 ± 0.04		
Alanine *plus* lysine	1.9 ± 0.1	0.76 ± 0.03	1.3	
Alanine *plus* leucine	2.0 ± 0.2	0.53 ± 0.02	31.2	<0.001

of the lysine inhibition of neutral amino acid uptake corresponds to the Na^+ dependency of the stimulation of lysine uptake by the neutral amino acids.

Table V shows that 1 mM extracellular lysine produced a significant increase in the net movement of alanine out of alanine-loaded cells. Increasing the lysine concentration from 1 mM to 10 mM did not significantly increase the magnitude of this alanine movement. The lysine-induced increase in the movement of alanine out of the cells appears to be related to the lysine inhibition of alanine uptake since 1 mM lysine neither inhibited the uptake of 1 mM valine nor influenced the movement of valine out of valine-loaded cells. The movement of lysine out of lysine-loaded cells was not influenced by the presence of 1 mM alanine in the extracellular medium.

TABLE V

THE EFFECT OF Na^+ ON THE LYSINE-INDUCED ACCELERATION OF ALANINE MOVEMENT OUT OF INTESTINAL EPITHELIAL CELLS PRELOADED WITH ALANINE

Isolated epithelial cells were preincubated for 5 min at 37° in the normal Krebs–Tris medium containing 1 mM L-alanine. The cells then were washed once in a 4° Krebs–Tris–choline medium prepared by the isotonic replacement of the NaCl of the Krebs–Ringer–Tris buffer with choline chloride. The 0 time cells were washed two additional times with cold Krebs–Tris and the alanine concentration in the cells determined as described in Results. The remaining cells were then incubated for 2 min at 37° in the indicated Krebs–Tris media without or with 1 mM lysine. Each value represents the mean cellular alanine concentration ± 1 S.E. from 6 individual experiments. The probability values were determined and interpreted as described in Table I.

Incubation time (min)	*Incubation medium*	*Lysine (1 mM)*	*Extracellular Na^+ (mM)*	*Cellular alanine*		*P*
				mM	*% Decrease due to lysine*	
0				5.35 ± 0.33		
2	Krebs–Tris	−	117.5 ± 1.7	1.99 ± 0.17		
2	Krebs–Tris	+	115.5 ± 0.7	1.49 ± 0.08	25.1	$P < 0.01$
2	Krebs–Tris–choline	−	4.4 ± 0.3	1.83 ± 0.22		
2	Krebs–Tris–choline	+	4.2 ± 0.4	1.29 ± 0.09	29.5	$P < 0.0$

The lysine-induced movement of alanine out of the cells was of similar magnitude in the presence or absence of extracellular Na^+ (Table V). The magnitude of the stimulation of the uptake of 1 mM extracellular lysine by cells preloaded with alanine was also found to be independent of extracellular Na^+. These findings are consistent with the operation of the hetero-exchange between intracellular alanine or leucine and extracellular lysine independent of the presence of extracellular Na^+ (ref. 6).

The effect of time on the inhibition of 1 mM alanine uptake by 1 mM lysine is shown in Table VI. The inhibition was evident after a 1 min incubation and reached

TABLE VI

TIME SEQUENCE OF THE INHIBITION OF 1 mM L-ALANINE UPTAKE BY 1 mM L-LYSINE IN ISOLATED INTESTINAL EPITHELIAL CELLS

Isolated intestinal epithelial cells were incubated at 37° for the indicated time in a Krebs–Tris medium containing either 1 mM alanine or 1 mM alanine and 1 mM lysine. Each value represents the mean alanine uptake ± 1 S.E. from 8 individual experiments. Probability values were determined and interpreted as described in Table I.

Time (min)	*Alanine uptake (mM)*		*% Inhibition*
	1 mM alanine	*1 mM alanine and 1 mM lysine*	
1	2.39 ± 0.12	1.78 ± 0.16	25.5
	$P < 0.001$		
2	4.00 ± 0.36	2.41 ± 0.15	39.7
	$P < 0.001$		
3	4.53 ± 0.17	2.72 ± 0.15	40.0
	$P < 0.001$		
5	5.50 ± 0.25	3.51 ± 0.19	36.2
	$P < 0.001$		
10	5.81 ± 0.26	3.60 ± 0.21	38.0
	$P < 0.001$		

a maximum of about 40 % after 2 min from which it did not significantly vary over the course of the study. The stimulation of lysine uptake by alanine was also present after 1 min and maximum after 2 min. A similar time response in the interactions between leucine and lysine has been previously reported[6] indicating a general relationship exists between the stimulatory and inhibitory patterns in the amino acid pairs. It is also apparent from the data in Table VI that alanine uptake after 2 min is no longer at initial velocity.

Fig. 1 shows the effect of varying the lysine concentration over the range 0–25 mM on the uptake of 1 mM alanine. At a concentration as low as 0.5 mM, lysine produced a significant ($P < 0.05$) 23 % inhibition of alanine uptake. Increasing the lysine concentration from 1–25 mM did not significantly increase the magnitude of the inhibition which had reached a maximum level of about 40 %. Lysine can therefore be classified as a partial inhibitor of alanine uptake. The low lysine concentration producing maximum inhibition suggests that lysine has a high affinity for the transport system mediating the interaction between lysine and alanine.

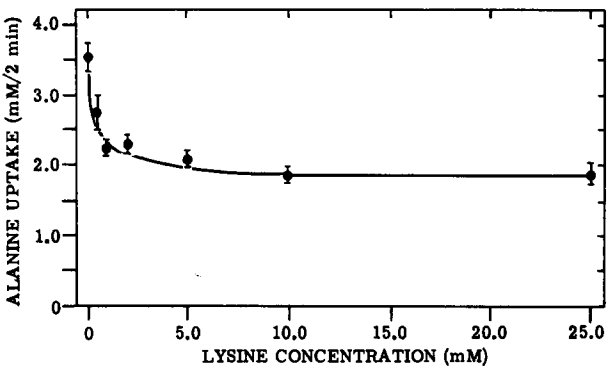

Fig. 1. The effect of lysine concentration over the range 0–25 mM on the uptake of 1 mM alanine by isolated intestinal epithelial cells. The cells were incubated for 2 min at 37° in a Krebs–Tris medium containing 1 mM alanine and the indicated concentration of lysine. Each point represents the mean of 12 individual experiments with the vertical lines representing ± 1 S.E.

In order to determine whether the inhibition of alanine uptake by lysine is reflected in a change in the apparent affinity of alanine for its binding site on a membrane component, a study of the kinetic characteristics of the lysine inhibition of alanine uptake was undertaken. Table VII shows the effect of alanine concentration over the range 0.5–10 mM on the uptake of alanine in the absence and presence of 3 mM lysine. Lysine significantly decreased alanine uptake at each alanine concentration tested. In general, as the concentration of alanine increased, the magnitude of the lysine inhibition decreased suggesting competitive inhibition. Fig. 2 presents the data of Table VII plotted according to Lineweaver and Burk[17]. The equation of the line representing alanine uptake in the absence of lysine was $y = 0.274x + 0.044$. The intercept on the y axis, $1/V_{max}$, was 0.044 with 95 % confidence limits of ± 0.018. The intercept on the x axis, $-1/K_m$, was −0.161 with 95 % confidence limits of ± 0.063. The equation of the line and x and y intercepts ± the 95 % confidence limits for alanine uptake in the presence of 3 mM lysine were $y = 0.426x + 0.038$,

TABLE VII

EFFECT OF L-ALANINE CONCENTRATION ON THE INHIBITION OF L-ALANINE UPTAKE BY ISOLATED INTESTINAL EPITHELIAL CELLS IN THE ABSENCE AND PRESENCE OF 3 mM L-LYSINE

The cells were incubated for 2 min at 37° in a Krebs–Tris medium containing L-alanine at the initial concentrations indicated in the absence and presence of 3 mM L-lysine. Each value represents the mean from 16 individual experiments ± 1 S.E. The probability values were obtained as described in Table I.

Alanine concn. (mM)	*Alanine uptake (mM per 2 min)*		*% Inhibition*
	No lysine	*3 mM lysine*	
0.5	1.73 ± 0.08	1.17 ± 0.07	32.4
		$P < 0.001$	
1.0	3.22 ± 0.14	2.30 ± 0.09	28.6
		$P < 0.001$	
2.0	5.90 ± 0.35	4.31 ± 0.19	27.0
		$P < 0.001$	
5.0	10.14 ± 0.46	7.81 ± 0.37	23.0
		$P < 0.001$	
10.0	14.40 ± 0.55	11.25 ± 0.43	21.9
		$P < 0.001$	

$y = 0.038 \pm 0.035$ and $x = -0.089 \pm 0.069$, respectively. These lines yielding the same y intercepts but different x intercepts are characteristic of competitive inhibition. The lysine inhibition of alanine uptake can therefore be classified as partially competitive[18]. The arginine inhibition of cycloleucine transport[1] and the arginine inhibition of methionine and citrulline uptake[3] have also been reported to be partially competitive. Competitive inhibition was also indicated when the experiments were repeated using 1 mM lysine instead of 3 mM lysine and when the data were plotted

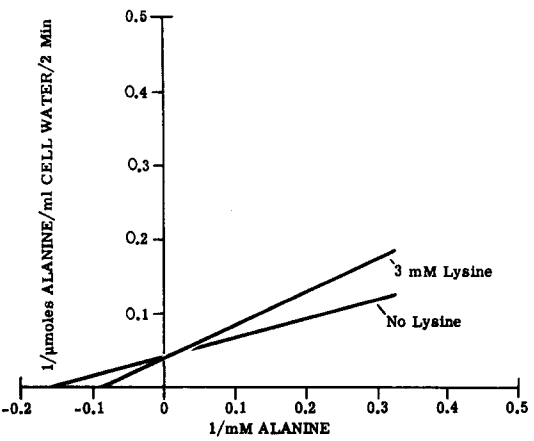

Fig. 2. Lineweaver–Burk plot of the data shown in Table IV. The equations of these regression lines with the 95% confidence limits of their intercepts are as follows: alanine uptake in the absence of lysine: equation to line, $y = 0.274\ x + 0.044$; intercept on y axis $(1/V) = 0.044 \pm 0.018$, intercept on x axis $(-1/K_m) = -0.161 \pm 0.063$. Alanine uptake in the presence of 3 mM lysine: equation to line, $y = 0.426\ x + 0.038$; intercept on y axis $= 0.038 \pm 0.035$, intercept on x axis $= -0.089 \pm 0.069$.

as the velocity as the ordinate against the velocity divided by the initial substrate concentration as the abscissa[19,20].

A lysine inhibition of neutral amino acid transport having some of the same characteristics as that described using the isolated epithelial cells also appears to be operating in intact intestine (Table VIII). The mucosal-to-cell flux as measured by the intracellular accumulation at 5 min[6,11] and the transmural transport as measured by the medium translocation at 30 min[7,9] was determined for 1 mM valine and 1 mM leucine as a function of lysine concentration over the range 1–50 mM. Valine, a neutral amino acid which has been previously shown not to stimulate lysine transport in intact intestine[6], was not inhibited by lysine concentrations as high as 50 mM. In contrast, lysine significantly inhibited leucine transport at concentrations as low as 1 mM. Leucine has previously been shown to stimulate lysine transport in intact intestine[4,6]. As with the cells, increasing the lysine concentration above 5 mM did not appreciably increase the magnitude of the inhibition of leucine transport. In similar studies utilizing other neutral amino acids, the transport of isoleucine was not inhibited by lysine while the transport of methionine and alanine exhibited inhibitions similar to that of leucine.

DISCUSSION

The major purpose of this study was to determine whether the inhibition of the uptake of certain neutral amino acids by lysine was a result of the concurrent ability of these neutral amino acids to stimulate lysine uptake. Such information would clarify one of the aspects of the complex interaction between neutral and basic amino acid transport[3,4,6,7]. In addition, such a correlation would lend indirect support to the suggestion that these processes are the result of a hetero-exchange transport system which mediates the concurrent stimulation of the uptake of extracellular lysine at the expense of intracellular neutral amino acid[6]. We believe that these results show a definite correlation between the properties of the lysine inhibition of neutral amino acid uptake and those of the stimulation of lysine uptake by neutral amino acids. In addition, many of the properties of the inhibition are compatible with the operation of a hetero-exchange transport system.

If the lysine inhibition of neutral amino acid uptake is the result of the concurrent stimulation of lysine uptake by certain neutral amino acids, then certain predictions can be made about the properties of this inhibition. It would be expected that the uptake of all the neutral amino acids that stimulate lysine uptake (*i.e.* alanine, leucine, methionine, phenylalanine and threonine) would be inhibited by lysine. In these studies, 1 mM lysine was seen to inhibit the uptake of all the stimulatory neutral amino acids. In contrast, lysine would not be expected to necessarily inhibit the uptake of the neutral amino acids that did not stimulate lysine (*i.e.* valine, isoleucine, tryptophan, proline and histidine). With the exception of histidine, lysine did not inhibit the uptake of the nonstimulatory neutral amino acids. The differences in the sensitivity of the neutral amino acids to lysine inhibition do not appear to be related to differences in the affinities of the neutral amino acids for their binding site since the apparent K_m values of leucine, valine, methionine and isoleucine have been shown to be virtually identical[21–23]. This dichotomy is also not an artifact

TABLE VIII

COMPARATIVE EFFECT OF LYSINE ON THE INTRACELLULAR ACCUMULATION AND MEDIUM TRANSLOCATION OF 1 mM VALINE AND 1 mM LEUCINE IN EVERTED INTESTINAL SACS

Everted intestinal sacs from the rat were incubated for 5 and 30 min at 37° in a Krebs–Tris medium containing 1 mM L-valine or 1 mM L-leucine in the presence of the indicated concentrations of L-lysine. Each value represents the mean determination from 6 rats ± 1 S.E. Percent of control values were obtained by dividing the average transport values in the presence of lysine by the average transport values found in identical sac systems run concurrently in the absence of lysine. A paired-difference t test was used to obtain the probability levels. A P of 0.05 or less was considered significant.

Lysine (mM)	*Intracellular accumulation*				*Medium translocation*			
	mM valine per 5 min		*mM leucine per 5 min*		*μmoles valine per 500 mg per 30 min*		*μmoles leucine per 500 mg per 30 min*	
	With lysine	*% Control*	*With lysine*	*% Control*	*With lysine*	*% Control*	*With lysine*	*% Control*
1	3.00 ± 0.11	104.2	2.17 ± 0.13	83.8	0.85 ± 0.12	98.8	0.89 ± 0.09	80.2
			$P < 0.05$				$P < 0.05$	
5	2.85 ± 0.16	96.0	1.77 ± 0.11	69.7	0.83 ± 0.07	94.3	0.64 ± 0.03	62.7
			$P < 0.01$				$P < 0.05$	
10	2.88 ± 0.08	92.3	1.53 ± 0.11	64.3	0.88 ± 0.19	106.0	0.70 ± 0.15	60.9
			$P < 0.01$				$P < 0.02$	
25	2.67 ± 0.21	87.3	1.59 ± 0.21	66.8	0.78 ± 0.14	89.7	0.53 ± 0.09	49.1
			$P < 0.02$				$P < 0.05$	
50	2.72 ± 0.20	90.4	1.59 ± 0.11	66.3	0.76 ± 0.10	91.6	0.52 ± 0.06	52.0
			$P < 0.001$				$P < 0.05$	

of the cell preparation since lysine was shown to inhibit leucine but not valine transport in everted intestinal sacs. Previous studies have shown similar examples of this dichotomy in intact intestine, *e.g.* lysine inhibited methionine[3] and leucine[4] transport but not isoleucine[3] and valine[7] transport. A basis for the inhibition of histidine uptake by lysine may be found from the recent work of Chez *et al.*[5] who reported a similar inhibition in intact rabbit ileum. These authors reported that the dissociation constants that characterize the binding of Na^+ to the lysine–carrier and histidine–carrier binary complexes are of the same order of magnitude and higher than the corresponding dissociation complexes for other neutral amino acids[23]. These results suggest that histidine is transported, at least in part, in the cationic form and that the lysine inhibition of histidine uptake may be the result of a partial affinity of histidine for the basic amino acid transport pathway.

It would also be predicted that when a neutral amino acid stimulates lysine uptake, an inhibition of the uptake of the neutral amino acid should always be evident. The concentration-dependent stimulation of lysine uptake by leucine presented a further test of the interrelationships between stimulation and inhibition. As expected on the basis of a concerted process, at all amino acid concentrations resulting in a stimulation of lysine uptake by leucine there was always an inhibition of leucine uptake by lysine. A similar relationship existed between lysine stimulation by alanine and alanine inhibition by lysine.

If the lysine inhibition of neutral amino acid uptake is dependent on the concurrent stimulation of lysine uptake then lysine should not inhibit neutral amino acid uptake when the exchange transport is not operating. In the absence of extracellular Na^+, 1 mM alanine does not stimulate the uptake of 1 mM lysine, presumably because the alanine can not reach the intracellular concentration needed to energize the hetero-exchange. Similarly, the inhibition of 1 mM alanine uptake by 1 mM lysine, but not by 1 mM leucine, was prevented by the absence of extracellular Na^+. This finding also differentiates the lysine inhibition of alanine uptake from that of leucine which is probably the result of a competition for the formation of the binary complex with the neutral amino acid carrier site[23].

The stimulation of lysine uptake by cells preloaded with leucine[6] or alanine (unpublished result) has been shown to be independent of extracellular Na^+. Assuming that the lysine-induced increase in the movement of alanine out of cells preloaded with alanine is a reflection of the lysine inhibition of alanine uptake, then a corresponding Na^+-insensitive lysine inhibition of alanine uptake occurs during the Na^+-insensitive stimulation of lysine uptake by alanine-loaded cells. These findings of an increase in the net movement of alanine out of the cells in the presence of extracellular lysine represents the only direct evidence for the operation of the hetero-exchange transport system as previously visualized[6]. However, the limitations inherent in the use of the isolated cell method makes it extremely difficult to interpret these results as they pertain to the mechanism or kinetics of the hetero-exchange in a more definitive manner. Some of these experimental difficulties include an inability to accurately measure the very rapid unidirectional movement (*i.e.* efflux) of alanine out of the alanine-loaded cells and the inability to separately evaluate the contribution of the brush border membrane and the other epithelial cell membranes to this process. The characteristics of the increased net movement of alanine out of the cells do not appear to be consistent with a competitive type of exchange diffusion since increasing

the lysine concentration from 1 to 10 mM did not significantly increase the movement of alanine out of the cells and the reverse increase in the net movement of lysine out of lysine-loaded cells was not affected by 1 mM alanine.

The affinity of lysine for the exchange transport system appears to be very high as seen from the half-maximal inhibition of alanine uptake produced by about 0.5 mM lysine. In previous studies[7] it was found that the apparent K_m for lysine uptake in intact rat intestine was 3.8 mM. The apparent K_m for lysine uptake in isolated cells was found to be 1.1 mM (unpublished results). These values indicate that the affinity of lysine for the exchange carrier is at least as great as for the basic amino acid uptake carrier. In Ehrlich cells it has been reported that the concentration at which ethionine and methionine saturate an exchange process is less than that at which they saturate the transport process[24]. On the basis of our results, it is not possible to establish whether lysine uptake by the exchange process is mediated by an independent carrier system or by a modification of the basic amino acid carrier mediating normal lysine uptake.

The classification of lysine as a partial competitive inhibitor of alanine uptake can be explained on the basis of the action of the two transport systems believed to be required for the operation of the exchange. The first transport system mediates the active uptake of alanine by the structurally specific, Na^+-dependent neutral amino acid transport system. The failure of lysine to generally inhibit all neutral amino acids utilizing this pathway as well as the failure of lysine to influence alanine uptake in the absence of Na^+ argues against a lysine inhibition of alanine uptake due to an interaction with this transport system. The second transport system mediates a Na^+-independent, hetero-exchange between a portion of the intracellular alanine and extracellular lysine which results in a concurrent increase in the movement of lysine into the cell and alanine out of the cell. As a result of the operation of these transport systems, it would appear that the neutral amino acid transport system requires a higher concentration of alanine for saturation in the presence of extracellular lysine than in its absence. It has been shown (unpublished results) that alanine, like leucine[6], can participate in exchange transport as the stimulated as well as the stimulating amino acid. A competition between extracellular lysine and extracellular alanine for the same site on the exchange carrier would also be compatible with partial competitive inhibition.

An alternate explanation of the inhibition of neutral amino acid transport by basic amino acids has been proposed based on an allosteric interaction between separate neutral and basic amino acid binding sites on a polyfunctional carrier[1]. In pursuing this line of reasoning, the position at which Na^+ is bound to the carrier may alternately be occupied by the distal cationic group of lysine[25], or the presence of lysine at its binding site may decrease the affinity of Na^+ for its binding site on the carrier[23]. As a result of the partial decrease in the affinity of Na^+ for its binding site, the formation of the ternary carrier–alanine–Na^+ complex would be limited resulting in a decrease in the affinity of alanine for the carrier[23]. Consistent with this explanation are the findings that lysine is a partially competitive inhibitor of alanine uptake, that lysine does not inhibit alanine uptake in the absence of Na^+, and that the affinity of lysine for the inhibitory process is quite high and about equal to the apparent K_m for lysine uptake by the basic amino acid transport system. However, the above explanation is not compatible with the observed differences in the lysine inhibition

of neutral amino acids having similar Na^+ requirements (*i.e.* valine, leucine, isoleucine and methionine), and with the finding that lysine increases alanine movement out of cells even in the absence of extracellular Na^+.

ACKNOWLEDGMENTS

We wish to thank Loutitia Ferdinandus and William Heaton for their very valuable technical assistance and the Indiana University Medical Center Computer Center (Public Health Service Research Grant FROO-162) for its assistance in calculating the kinetic data. This investigation was supported in part by Public Health Service Grant AM 5223 from National Institutes of Health.

REFERENCES

1 F. Alvarado, *Science*, 151 (1966) 1010.
2 B. G. Munck, *Biochim. Biophys. Acta*, 120 (1966) 282.
3 J. W. L. Robinson, *Eur. J. Biochem.*, 7 (1968) 78.
4 B. G. Munck and S. G. Schultz, *Biochim. Biophys. Acta*, 183 (1969) 182.
5 R. A. Chez, C. K. Strecker, P. F. Curran and S. G. Schultz, *Biochim. Biophys. Acta*, 233 (1971) 222.
6 S. Reiser and P. A. Christiansen, *Biochim. Biophys. Acta*, 241 (1971) 102.
7 S. Reiser and P. A. Christiansen, *Biochim. Biophys. Acta*, 183 (1969) 611.
8 S. Reiser and P. A. Christiansen, *Biochim. Biophys. Acta*, 225 (1971) 123.
9 S. Reiser and P. A. Christiansen, *Am. J. Physiol.*, 208 (1965) 914.
10 S. Reiser and P. A. Christiansen, *Am. J. Physiol.*, 212 (1967) 1297.
11 S. Reiser and P. A. Christiansen, *Am. J. Physiol.*, 216 (1969) 915.
12 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
13 M. S. Patterson and R. C. Green, *Anal. Chem.*, 37 (1965) 854.
14 E. J. Jervis and D. H. Smyth, *J. Physiol.*, 149 (1959) 433.
15 H. Newey and D. H. Smyth, *J. Physiol.*, 170 (1964) 328.
16 B. G. Munck, *Biochim. Biophys. Acta*, 120 (1966) 97.
17 H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, 56 (1934) 658.
18 M. Dixon and E. C. Webb, *Enzymes*, Academic Press, New York, 1964, p. 320.
19 K. B. Augustinsson, *Acta Physiol. Scand.*, 15 Suppl. (1948) 52.
20 B. H. J. Hofstee, *Nature*, 184 (1959) 1296.
21 L. R. Finch and F. J. R. Hird, *Biochim. Biophys. Acta*, 43 (1960) 278.
22 D. M. Matthews and L. Laster, *Am. J. Physiol.*, 208 (1965) 593.
23 S. G. Schultz and P. F. Curran, *Physiol. Rev.*, 50 (1970) 637.
24 M. L. Belkhode and P. G. Scholefield, *Biochim. Biophys. Acta*, 173 (1969) 290.
25 E. L. Thomas and H. N. Christensen, *J. Biol. Chem.*, 246 (1971) 1682.