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STIMULATION OF BASIC AMINO ACID UPTAKE BY CERTAIN NEUTRAL AMINO ACIDS IN ISOLATED INTESTINAL EPITHELIAL CELLS

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SUMMARY

1. Using everted intestinal sacs from the rat the transport of 1 mM lysine from the mucosal to the serosal medium was stimulated by the presence of 1 mM leucine, 1 mM methionine, or 1 mM alanine in the mucosal medium. Under the same conditions the intracellular accumulation of lysine was not increased.

2. The uptake of 1 mM lysine and 1 mM arginine by isolated intestinal cells was stimulated by 1 mM leucine, 1 mM methionine, 1 mM alanine, or 1 mM phenylalanine; inhibited by 1 mM isoleucine or 1 mM tryptophan; and unaffected by 1 mM valine, 1 mM histidine, or 1 mM glycine.

3. The stimulation of lysine uptake by leucine in the cells was present after a 1-min incubation and optimum after 2 min. The concurrent inhibition of 1 mM leucine uptake by 1 mM lysine was also maximal after 2 min.

4. Preincubation of the cells in 1 mM lysine, 1 mM arginine, or 1 mM leucine followed by incubation in lysine resulted in an increased uptake of lysine as compared to cells preincubated in Krebs–Tris alone.

5. The stimulation of 1 mM lysine uptake by leucine-loaded cells was not decreased by the exclusion of extracellular Na^+.

6. The stimulation of lysine uptake by leucine in the cells is attributed to the action of two transport systems. The first mediates an energy-dependent, Na^+-dependent, active uptake of leucine and the other mediates a Na^+-independent hetero-exchange between intracellular leucine and extracellular lysine resulting in a stimulation of lysine uptake at the expense of intracellular leucine.

INTRODUCTION

Numerous studies on the stimulation of basic amino acid transport by various neutral amino acids in intact mammalian intestine have been previously reported[1–5]. While these studies are unanimous in finding an increase in the transmural transport of lysine or arginine across the intact intestine, contradictory results have been reported with respect to the intracellular levels of the stimulated basic amino acids. ROBINSON AND FELBER[2] reported an increase in the concentration of arginine and,

to a lesser extent, lysine in intestinal segments in the presence of L-methionine, L-leucine, and DL-norleucine. MUNCK AND SCHULTZ[5] reported that during the stimulation of the transmural transport of lysine by leucine, the mucosal-to-cell flux was not increased and the steady-state intracellular lysine concentration did not increase. However, tissue preincubated with leucine did show an increase in the mucosal-to-cell lysine flux. These authors attributed the stimulatory effect of leucine on lysine transport as primarily due to an increase in the unidirectional flux of lysine out of the cell across the serosal and/or lateral membranes. Recent studies have shown that isolated epithelial cells from both rat[6] and chicken[7,8] intestine can be used to study the properties of amino acid and sugar transport. Based on the explanation of the stimulation of lysine transmural transport by leucine proposed by MUNCK and SCHULTZ[5], the uptake of lysine should not be stimulated by leucine in the isolated cell system.

The main purposes of this study were to first determine whether the uptake of lysine is stimulated by leucine or other neutral amino acids in isolated intestinal epithelial cells, and then, if such a stimulation were found, to investigate the properties of the stimulation in order to determine the mechanism mediating this interaction.

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 tissue handling and manipulations involved in the preparation of the everted sacs have been previously described[9,10]. The methodology used to prepare the isolated intestinal epithelial cells has been described in detail[6]. The only modification employed was the filtering of the cell suspension through a single layer of gauze prior to the final collection step.

The standard incubation medium employed to measure amino acid transport in intact intestine and amino acid uptake in the cells 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 KH_2PO_4, and radioactive (10000–15000 counts/min per ml) and nonradioactive amino acids to the desired final concentration. In addition, the medium used in the sac studies contained 8000–12000 counts/min per ml of [*Me*-^{3}H]inulin. Sac systems containing lysine only were designated controls while sac systems containing lysine and an additional amino acid were called experimentals. In the sac studies the serosal compartment was filled with 1 ml of the incubation medium without the amino acids or the inulin. The methodology used for the calculation of the serosal appearance[1] and the intracellular accumulation[1,11] of the amino acid after incubation in the sac system has been previously described.

In the preincubation experiments all the sacs were filled with the oxygenated Krebs–Tris medium. The sacs then were placed either in a Krebs–Tris medium containing no additions (controls) or in a Krebs–Tris medium containing nonradioactive 1 mM L-leucine (experimentals). The preincubation was carried out at 37° for 15–20 min in order to saturate the leucine uptake sites in the intestine[12].

The sacs then were opened and quickly rinsed in saline, and the same intestinal segments were used to prepare another series of sacs all of which were incubated in the Krebs–Tris medium containing 1 mM L-lysine and inulin.

In a typical experiment utilizing the isolated epithelial cells to measure amino acid uptake, 0.3 ml of the cells representing an average of 6.20 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 [13] and radioactivity[14].

The uptake of the amino acids by the cells was expressed as the intracellular accumulation of the amino acids in the cell water[6]. This parameter was calculated with the use of the following relationship: mM amino acid (cellular water) = total amino acid taken up by the cells/(cell wet weight) (0.8). The cell wet weight was calculated as follows: (total mg protein in cells) (mg dry wt./mg protein) (5 mg wet weight/mg dry wt). This conversion was based on the assumptions that the dry weight of a cell is 20% of its wet weight and that the density of the cell is about 1 g/ml. The mg dry weight/mg protein in the cells was determined in 32 individual experiments and found to be 1.85 ± 0.04 (mean ± S.E.). This value is in excellent agreement with the dry weight/protein ratio of 1.83 ± 0.07 previously reported[6]. The dry weight of the cells was determined by heating aliquots of the cells at 90° for 12–15 h on preweighed planchets and subtracting from this value the dry weight of an equivalent volume of isotonic saline dried in the same manner. The value for the cell water obtained as described above was within 8% of the value for the cell water calculated from the equilibrating distribution of 1 mM leucine in the presence of 0.4 mM dinitrophenol as described by KIMMICH[7].

The counting rate of the radioactive amino acids in the original medium was mathematically treated so that 10000 counts/min per ml were equivalent to the initial concentration of the amino acids. With the use of the same proportionality factors employed in converting the medium, the number of counts of the radioactive amino acids in the serosal medium, intact intestine, and the cells was converted to a 10000 count equivalent. This manipulation permitted the conversion of counts to μmoles amino acid from identical experiments having somewhat different initial counting rates.

The sodium concentration of the incubation media used in sodium replacement studies was determined by direct analysis in a Coleman flame photometer.

The source and specific activity of the radioactive compounds used in this study were: L-[^{14}C]lysine (312 mC/mmole), and L-[^{14}C]leucine (334 mC/mmole) from Amersham/Searle and L-[4,5-^{3}H]leucine (58.2 mC/mmole), and [*Me*-^{3}H]inulin (431 mC/mmole) from New England Nuclear.

TABLE I

THE EFFECT OF VARIOUS AMINO ACIDS ADDED TO THE MUCOSAL MEDIUM ON THE TRANSPORT OF 1 mM L-LYSINE IN INTACT INTESTINE

Six control and three experimental sacs were made alternately from the intestine of a rat and incubated for 30 min at 37° in a Krebs–Tris medium containing 1 mM L-lysine in the mucosal medium only. The experimental medium contained, in addition, 1 mM of the various amino acids in the mucosal medium only. Each value represents the mean determination derived from the number of rats shown in parentheses ± S.E. A paired-difference t test was used to obtain the probability values. A P of 0.05 or less was considered significant, and only these probability levels are shown. Percent of control values were obtained by dividing the average experimental values by the average control values.

Amino acid (1 *mM*)	*Serosal appearance* (*μmoles lysine/500 mg per 30 min*)			*Intracellular accumulation* (*mM lysine/30 min*)		
	Experimental	*Control*	% *Control*	*Experimental*	*Control*	% *Control*
L-Leucine (7)	0.822 ± 0.088	0.536 ± 0.063	153	2.603 ± 0.235	2.483 ± 0.212	105
	$P<0.001$					
L-Methionine (8)	0.976 ± 0.120	0.677 ± 0.070	144	2.757 ± 0.250	2.479 ± 0.258	111
	$P<0.01$			$P<0.05$		
L-Alanine (6)	0.822 ± 0.146	0.601 ± 0.098	137	2.590 ± 0.218	2.369 ± 0.188	109
	$P<0.02$					
L-Valine (8)	0.815 ± 0.090	0.747 ± 0.076	109	2.707 ± 0.171	2.712 ± 0.163	100
L-Isoleucine (11)	0.522 ± 0.036	0.514 ± 0.028	102	2.048 ± 0.088	2.154 ± 0.080	95
L-Tryptophan (6)	0.459 ± 0.075	0.568 ± 0.051	81	2.042 ± 0.070	2.287 ± 0.126	89
	$P<0.02$			$P<0.05$		
L-Arginine monohydrochloride (6)	0.375 ± 0.053	0.621 ± 0.062	60	1.484 ± 0.157	2.578 ± 0.223	58
	$P<0.01$			$P<0.01$		
L-Ornithine monohydrochloride (7)	0.411 ± 0.052	0.575 ± 0.058	71	1.667 ± 0.175	2.372 ± 0.079	70
	$P<0.01$			$P<0.02$		

RESULTS

Table I shows the specificity of the stimulation of 1 mM lysine transport by 1 mM of the indicated amino acids in everted intestinal sacs. Of the neutral amino acids tested, only L-leucine, L-methionine, or L-alanine produced a significant stimulation of lysine serosal appearance, a parameter which measures the transport of lysine from the mucosal to the serosal medium. In agreement with the findings of MUNCK AND SCHULTZ[5] this increase in lysine transmural transport was not generally associated with an increase in the steady-state intracellular accumulation of lysine. Other neutral amino acids such as valine and isoleucine, generally accepted as sharing the same transport pathway as leucine, methionine, and alanine, did not stimulate lysine transport. Arginine and ornithine caused a significant inhibition of both lysine serosal appearance and intracellular accumulation as would be expected on the basis of their transport by the basic amino acid pathway[15]. Tryptophan was also inhibitory, supporting the finding by MUNCK[16] that this neutral amino acid also utilizes the basic amino acid transport pathway.

Table II (top) records the effect of time on the stimulation of 1 mM lysine serosal appearance and intracellular accumulation by 1 mM leucine. The serosal appearance of lysine was significantly stimulated at incubation times as short as

TABLE II

TIME SEQUENCE OF THE STIMULATION OF 1 mM L-LYSINE TRANSPORT BY 1 mM L-LEUCINE IN INTACT INTESTINE AND IN INTESTINE PREINCUBATED WITH 1 mM L-LEUCINE

In the incubation studies, the control sacs were incubated for the indicated time periods at 37° in a Krebs–Tris medium containing 1 mM L-lysine in the mucosal medium and the experimental sacs were incubated in a Krebs–Tris medium containing both 1 mM L-lysine and 1 mM L-leucine in the mucosal medium. Each value was obtained by averaging the means from at least six rats yielding six control and three experimental sacs each. In the preincubation studies, the experimental sacs were preincubated for 15–20 min in a Krebs–Tris medium containing 1 mM L-leucine in the mucosal medium while the control sacs were preincubated in the Krebs–Tris medium alone. The sacs were then opened and washed, and the same tissue used to prepare another series of nine sacs, all of which were incubated for the indicated time periods in a Krebs–Tris medium containing 1 mM L-lysine in the mucosal medium. Each value was obtained by averaging the means from eight rats ± S.E. Probability values and percent of control values were obtained as described in Table I.

Time (min)	*Serosal appearance (μmoles lysine/500 mg tissue)*			*Intracellular accumulation (mM lysine)*		
	Experimental	*Control*	*% Control*	*Experimental*	*Control*	*% Control*
1				0.314 ± 0.013	0.359 ± 0.029	88
2				0.673 ± 0.081	0.709 ± 0.080	95
3	0.060 ± 0.007	0.056 ± 0.007	107	0.914 ± 0.115	0.848 ± 0.079	108
5	0.100 ± 0.010	0.081 ± 0.007	124	1.425 ± 0.113	1.342 ± 0.069	106
	$P<0.05$					
10	0.302 ± 0.030	0.207 ± 0.013	146	2.486 ± 0.305	2.274 ± 0.160	109
	$P<0.01$					
30	0.822 ± 0.094	0.600 ± 0.096	137	2.553 ± 0.216	2.407 ± 0.139	106
	$P<0.01$					
Preincubated intestine						
3	0.056 ± 0.011	0.067 ± 0.011	84	0.815 ± 0.033	0.607 ± 0.068	134
				$P<0.05$		
5	0.100 ± 0.024	0.074 ± 0.009	135	1.784 ± 0.200	1.316 ± 0.180	136
	$P<0.05$			$P<0.001$		
10	0.254 ± 0.023	0.181 ± 0.019	140	2.693 ± 0.277	2.111 ± 0.192	128
	$P<0.05$			$P<0.02$		
30	0.761 ± 0.103	0.460 ± 0.077	165	3.097 ± 0.655	2.536 ± 0.457	122
	$P<0.001$			$P<0.05$		

5 min with optimum stimulation evident after 10 min. The serosal appearance of lysine was a linear function of time through the first 30 min as evidenced by correlation coefficients for both the experimental and control values of greater than 0.99. In contrast to the stimulation of lysine serosal appearance, the intracellular accumulation of lysine was not increased by the presence of 1 mM leucine. The intracellular accumulation of lysine was a linear function of time through the first 5 min with correlation coefficients of greater than 0.98 for the experimentals and greater than 0.99 for the controls. If one assumes that these correlations permit lysine accumulation at 5 min or less to represent an approximation of the mucosal-to-cell flux, then it must be concluded that the increase in the transmural transport of lysine is not mediated by an increase in the mucosal-to-cell flux.

In order to determine whether the presence of leucine in the mucosal medium was a prerequisite for the stimulation of lysine transport, the sacs were pre-

incubated for 15 min with Krebs–Tris (controls) or Krebs–Tris containing 1 mM leucine (experimentals). Lysine transport in the absence of mucosal leucine was then measured in the sacs as a function of time (Table II, bottom). The stimulation of lysine serosal appearance after leucine preincubation was analogous to the stimulation noted when leucine was initially present in the mucosal medium. However, leucine preincubation resulted in a significant stimulation of lysine intracellular accumulation at time periods as short as 3 min indicating that the mucosal-to-cell flux of lysine was increased under these conditions.

Because of the contradictory nature of these results and the possibility that the stimulation of lysine transport by the neutral amino acids may be mediated by transport processes present at the serosal aspect of intact intestine[5], a study of this stimulation was initiated using isolated intestinal epithelial cells[6]. Since this intestinal preparation contains only the medium and the cell as transport compartments, it should be possible to obtain unequivocal data pertaining to increases in the intracellular accumulation and/or the medium-to-epithelial cell flux of lysine in the presence of neutral amino acids. Table III shows the effects of various amino acids at 1 mM and 10 mM concentrations on the uptake of 1 mM lysine by isolated epithelial cells after 15 min. The cells actively accumulated 1 mM lysine with steady-state cell water/medium ratios of 3 or more usually observed. At concen-

TABLE III

THE EFFECT OF VARIOUS AMINO ACIDS ON THE UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER 15 min INCUBATION

Isolated intestinal cells were incubated for 15 min at 37° in a Krebs–Tris medium containing 1 mM L-lysine and 1 mM or 10 mM of the indicated amino acids. Each value represents the mean ± S.E. from the number of individual experiments shown in parentheses. The percent of control values were obtained by dividing the average uptake of lysine in the presence of the additional amino acid by the average uptake of lysine in the absence of the additional amino acid. A paired-difference t test was used to obtain the probability values and a P of 0.05 or less was considered significant.

Amino acid added	*Lysine uptake (mM lysine/15 min)*					
	1 mM lysine + 1 mM amino acid	% *Control*	*P*	*1 mM lysine + 10 mM amino acid*	% *Control*	*P*
None (12)	2.942 ± 0.215			2.942 ± 0.215		
L-Leucine (12)	4.222 ± 0.480	144	<0.01	1.535 ± 0.149	52	<0.001
L-Valine (12)	2.640 ± 0.194	90		2.278 ± 0.150	77	<0.001
L-Methionine (12)	3.948 ± 0.290	134	<0.01	1.444 ± 0.089	49	<0.001
L-Isoleucine (12)	1.898 ± 0.171	65	<0.001	1.370 ± 0.157	47	<0.001
L-Arginine (12)	1.411 ± 0.105	48	<0.001	0.602 ± 0.056	21	<0.001
None (10)	3.888 ± 0.332			3.888 ± 0.332		
L-Histidine (10)	3.796 ± 0.442	98		1.953 ± 0.363	50	<0.001
L-Tryptophan (10)	3.123 ± 0.210	80	<0.05	1.977 ± 0.171	51	<0.001
D-Methionine (10)	3.574 ± 0.397	92		3.108 ± 0.296	80	<0.02
D-Leucine (10)	3.708 ± 0.238	95		3.648 ± 0.275	94	
None (10)	3.415 ± 0.233			3.415 ± 0.233		
L-Alanine (10)	6.967 ± 0.724	204	<0.001	6.475 ± 0.741	190	<0.001
L-Phenylalanine (10)	6.380 ± 0.830	187	<0.01	4.434 ± 0.378	130	<0.01
L-Proline (10)	2.985 ± 0.305	87		2.978 ± 0.402	87	
Glycine (10)	3.556 ± 0.436	104		3.967 ± 0.534	116	

TABLE IV

THE UPTAKE OF 1 mM L-LEUCINE AND 1 mM L-LYSINE WHEN PRESENT ALONE OR CONCURRENTLY BY ISOLATED EPITHELIAL CELLS AFTER 15 min

Isolated intestinal cells were incubated 15 min at 37° in a Krebs–Tris medium containing 1 mM L-[^{14}C]lysine and/or 1 mM L-[^{3}H]leucine as indicated. Each value represents the mean ± S.E. from 12 individual experiments. Percent of control and probability values were obtained as described in Table III.

Amino acid	*Amino acid uptake*					
	Leucine (mM/15 min)	*% Control*	*P*	*Lysine (mM/15 min)*	*% Control*	*P*
1 mM L-Leucine	2.575 ± 0.133					
1 mM L-Lysine				2.876 ± 0.191		
1 mM L-Leucine + 1 mM L-Lysine	1.917 ± 0.115	74	<0.001	3.777 ± 0.300	131	<0.01

trations as low as 1 mM, arginine inhibited lysine uptake by 52 % indicating that lysine uptake was mediated by a basic amino acid transport system[1]. The dichotomy of action of the neutral amino acids on 1 mM lysine transport observed in intact tissue (Table I) was again observed in the isolated cells. At 1 mM, L-leucine, L-methionine, and especially L-alanine and L-phenylalanine significantly increased the uptake of 1 mM lysine by the cells. The activation of lysine uptake by these amino acids appears to be isomerically specific since 1 mM D-leucine or D-methionine was without effect. Lysine uptake was not significantly altered by the presence of 1 mM L-valine, L-histidine, L-proline, or glycine but was inhibited by the presence of 1 mM L-isoleucine or L-tryptophan. Of the amino acids that stimulated lysine uptake at 1 mM only L-alanine was as effective at 10 mM. Ten mM L-leucine or L-methionine inhibited 1 mM lysine uptake. A similar concentration-dependent diphasic action of leucine and methionine on lysine transport in intact intestine has been reported [1,5,17]. All the other amino acids tested at 10 mM inhibited lysine uptake with the exception of D-leucine, L-proline, and glycine. The activation and inhibition of arginine uptake produced by the amino acids were completely analogous to that noted with lysine indicating that the interactions involved are not specific for lysine but probably pertain to all amino acids utilizing the basic amino acid transport pathway.

Table IV illustrates that the leucine stimulation of lysine uptake is accompanied by a concurrent inhibition of leucine uptake by lysine. Similar findings have been reported with other amino acid pairs in Ehrlich ascites cells[18] and Sarcoma 37 cells[19].

The stimulatory neutral amino acids can increase the steady-state levels of the basic amino acids in the cells by either increasing their influx or decreasing their efflux. In order to distinguish between these two possibilities the stimulation of 1 mM lysine uptake by 1 mM leucine as a function of time was investigated (Table V). An increase of lysine uptake at a time when the efflux of lysine out of the cell is minimal (*i.e.*, when uptake is at initial velocity) would be evidence in favor of an increased influx. The results show that leucine activated lysine uptake after an incubation period as short as 1 min. Additional evidence in favor of an

TABLE V

TIME SEQUENCE OF THE STIMULATION OF 1 mM L-LYSINE UPTAKE BY 1 mM L-LEUCINE IN ISOLATED EPITHELIAL CELLS

Isolated intestinal epithelial cells were incubated at 37° for the indicated times in a Krebs–Tris medium containing either 1 mM L-lysine or 1 mM L-lysine + 1 mM L-leucine. Each value represents the mean ± S.E. from 8 individual experiments. Percent of control and probability values were obtained as described in Table III.

Time (min)	*Lysine uptake (mM)*		
	1 mM lysine	*1 mM lysine + 1 mM leucine*	*% Control*
1	0.914 ± 0.074	1.068 ± 0.087	117
		$P < 0.02$	
2	1.184 ± 0.067	1.639 ± 0.112	139
		$P < 0.001$	
3	1.575 ± 0.087	2.117 ± 0.127	134
		$P < 0.01$	
5	2.167 ± 0.153	2.861 ± 0.187	132
		$P < 0.01$	
10	2.859 ± 0.197	4.147 ± 0.331	145
		$P < 0.01$	
15	3.089 ± 0.155	4.032 ± 0.203	131
		$P < 0.01$	

increased lysine influx comes from the finding that maximum activation of lysine uptake was evident after 2 min, *i.e.*, the magnitude of the stimulation of lysine uptake in the presence of leucine did not increase significantly after 2 min. It would be expected that if a decreased efflux mediated the increased lysine uptake, the stimulation would increase as the intracellular lysine concentration increased and become maximum when the steady-state is reached after 10 min.

Previous studies have indicated that leucine uptake by the cells at incubation periods below 5 min represents leucine influx[6]. Table VI shows the pattern of the inhibition of 1 mM leucine influx by 1 mM lysine through the first 3 min of in-

TABLE VI

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

Isolated intestinal epithelial cells were incubated at 37° for the indicated time in a Krebs–Tris medium containing either 1 mM L-leucine or 1 mM L-leucine + 1 mM L-lysine. Each value represents the mean ± S.E. from 8 individual experiments. Percent of control and probability values were obtained as described in Table III.

Time (min)	*Leucine uptake (mM)*		
	1 mM leucine	*1 mM leucine + 1 mM lysine*	*% Control*
1	1.273 ± 0.157	1.144 ± 0.127	90
2	1.964 ± 0.280	1.537 ± 0.294	78
		$P < 0.01$	
3	2.421 ± 0.211	1.858 ± 0.208	77
		$P < 0.02$	

TABLE VII

THE UPTAKE OF 1 mM L-LYSINE OR 1 mM L-LEUCINE BY ISOLATED CELLS FOLLOWING A 10-min PREINCUBATION IN KREBS–TRIS WITH AND WITHOUT EITHER 1 mM L-LEUCINE, 1 mM L-LYSINE OR 1 mM L-ARGININE

Isolated epithelial cells were preincubated for 10 min in either Krebs–Tris, Krebs–Tris–1 mM L-lysine, Krebs–Tris–1 mM L-arginine or Krebs–Tris–1 mM L-leucine. The cells were then washed once in cold 4° Krebs–Tris and reincubated for 5 min in either Krebs–Tris–1 mM L-lysine or Krebs–Tris–1 mM L-leucine. Each value represents the mean ± S.E. from the number of individual experiments (n) indicated. The percent of control and probability values were obtained as described in Table III.

Krebs–Tris preincubation media	n	*Krebs–Tris incubation media*	*Lysine or leucine uptake*		P
			mM/5 min	*% Control*	
Control (no addition)	8	+ 1 mM L-lysine	1.455 ± 0.102		
+ 1 mM L-leucine	8	+ 1 mM L-lysine	2.021 ± 0.121	139	<0.001
Control (no addition)	12	+ 1 mM L-lysine	1.636 ± 0.089		
+ 1 mM L-lysine	12	+ 1 mM L-lysine	2.845 ± 0.127	174	<0.001
+ 1 mM L-arginine	12	+ 1 mM L-lysine	2.247 ± 0.098	137	<0.001
Control (no addition)	10	+ 1 mM L-leucine	1.764 ± 0.098		
+ 1 mM L-leucine	10	+ 1 mM L-leucine	2.124 ± 0.082	120	<0.001
+ 1 mM L-lysine	10	+ 1 mM L-leucine	1.744 ± 0.054	99	

cubation. The inhibition by lysine was found to be maximum after 2 min. Although the steady-state intracellular levels of lysine and leucine were about equal, the influx of leucine at 1, 2 and 3 min was significantly higher than that attained by lysine at the corresponding times.

Table VII shows that preincubation of the cells for 10 min in a Krebs–Tris medium containing 1 mM leucine followed by a 5-min incubation of the cells in Krebs–Tris–1 mM lysine without extracellular leucine resulted in a 38.9 % increase in lysine uptake compared to cells preincubated in Krebs–Tris alone. This stimulation is similar in magnitude to that produced by 1 mM leucine when present in the incubation medium (see Table III) indicating that intracellular leucine rather than extracellular leucine mediates the stimulation of lysine uptake. Not only was the uptake of lysine activated by intracellular leucine but, in contrast to findings with intact intestine[5,17], intracellular lysine or arginine also stimulated lysine uptake. These findings demonstrate that the basic amino acids exhibit a tendency to undergo exchange transport. The uptake of extracellular leucine was increased about 20 % after preincubation with leucine but was not increased after preincubation with lysine.

Previous studies have shown that the active accumulation of 1 mM L-leucine by the cells is Na^+-dependent[6]. In contrast, the percent stimulation of the uptake of 1 mM lysine by leucine-preincubated cells (mM cellular leucine/10 min = 2.178 + 0.141, $n = 4$) was not decreased when Na^+ was excluded from the incubation media (Table VIII). In the absence of Na^+, the uptake of lysine by leucine-preincubated cells reached values significantly greater than 1 mM ($P < 0.001$) indicating that the uptake was concentrative. The low Na^+ concentration used in the preincubation

TABLE VIII

EFFECT OF Na^+ ON THE UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS PREINCUBATED IN A KREBS–TRIS MEDIUM WITHOUT OR WITH 1 mM L-LEUCINE

The desired Na^+ concentrations were attained by the isotonic substitution of the 118 mM NaCl of the Krebs–Tris media with choline chloride. Isolated intestinal epithelial cells were preincubated for 10 min in a 30 mM Na^+–Krebs–Tris medium without or with 1 mM L-leucine. The cells were then washed once in 4° choline chloride Krebs–Tris and reincubated for 5 min in a normal or a Na^+-free Krebs–Tris medium, both containing 1 mM L-lysine. Each value represents the mean ± S.E. from 10 individual experiments.

Krebs–Tris preincubation media	*Krebs–Tris incubation media*	*Final Na^+ incubation media (mM)*	*Lysine uptake*		*P*
			mM/5 min	*% Leucine stimulation*	
30 mM Na^+	118 mM Na^+; 1 mM lysine	109.5 ± 1.7	1.586 ± 0.094		
30 mM Na^+; 1 mM leucine	118 mM Na^+; 1 mM lysine	109.2 ± 2.1	2.183 ± 0.154	38	<0.01
30 mM Na^+	No Na^+; 1 mM lysine	2.6 ± 0.2	1.086 ± 0.058		
30 mM Na^+; 1 mM leucine	No Na^+; 1 mM lysine	2.6 ± 0.3	1.731 ± 0.095	59	<0.001

media minimized the final Na^+ concentration in the Na^+-free incubation media but still permitted leucine uptake to reach about 70 % of that attained in 118 mM Na^+ (ref. 6).

DISCUSSION

This study has shown that the stimulation of concentrative basic amino acid transport by certain neutral amino acids noted in intact intestine can also be shown in isolated intestinal epithelial cells, a finding which is incompatible with the explanation of this interaction proposed by MUNCK AND SCHULTZ[5] for intact intestine. The similarities in the stimulation or inhibition of lysine transport exhibited by amino acids in intact intestine (Table I) and isolated cells (Table III) are evidence against the possibility that lysine uptake by the cells represents a nonphysiological uptake system produced by modification of the transport systems during cell preparation.

It appears that the stimulatory action of the neutral amino acids on basic amino acid transport can best be explained on the basis of an exchange transport between intracellular neutral amino acid and extracellular basic amino acid similar to that proposed to explain a similar interaction between amino acids in Ehrlich ascites cells[20,21]. Two transport systems appear to play a role in this interaction. The first involves the Na^+-dependent, energy-dependent, concentrative, and structurally specific neutral amino acid transport system described so extensively in intact intestine. The operation of this transport system results in the rapid accumulation of relatively high concentrations of an amino acid such as leucine. The second transport system mediates a Na^+-independent hetero-exchange between intracellular leucine and extracellular amino acids such as lysine resulting in a concurrent net increase of lysine uptake and net decrease of leucine uptake. The

exchange transport system is driven by the cellular-to-medium concentration gradient of leucine established by the first transport system. The main experimental evidence in favor of this explanation can be outlined as follows:

(a) The uptake of leucine by the cells resulted in active accumulation within 1 min at which time the stimulation of lysine uptake was also noted.

(b) The magnitudes of the concurrent increase in lysine uptake and decrease in leucine uptake showed similar time responses.

(c) Leucine appeared to mediate the increase of lysine uptake by increasing lysine influx rather than by decreasing lysine efflux.

(d) In the absence of extracellular leucine, lysine uptake was optimally stimulated by intracellular leucine and this stimulation was independent of the extracellular Na^+ concentration.

(e) Both lysine and leucine showed a tendency to participate in exchange transport.

Certain predictions can be made concerning the properties of the exchange transport system. If the driving force for the exchange transport system is derived from the concentration gradient of the neutral amino acids, then the higher this gradient becomes, the greater is the potential for stimulation. Alanine was found to produce a stimulation of lysine uptake about 2.5 times greater than that produced by leucine (Table III). After 2 min the intracellular concentration of 1 mM alanine was found to be approx. 2 times greater than that of 1 mM leucine (unpublished results). The exchange transport system is assumed to require no additional energy for its operation except that utilized to create and maintain the necessary intracellular concentration of the neutral amino acids. It is therefore not surprising that the operation of the exchange system does not require the presence of Na^+, which is generally believed to be necessary for the energy processes involved in the active uptake of amino acids. The exchange transport of methionine in Ehrlich ascites cells has also been reported to be independent of extracellular Na^+ (ref. 22). MUNCK AND SCHULTZ[5] have shown that the stimulation of lysine influx after leucine preincubation occurred in the absence of Na^+, suggesting that an exchange transport system may also mediate the stimulation of lysine transport in intact intestine.

The present study has shown that the inhibition of 1 mM leucine uptake by 1 mM lysine is the result of the concurrent stimulation of lysine uptake by leucine. Generalizing on this finding, 1 mM lysine would be expected to inhibit the uptake of the stimulatory neutral amino acids but not necessarily inhibit the uptake of the non-stimulatory amino acids. It has been shown previously that the intracellular accumulation of 1 mM valine is not inhibited by 1 mM lysine in intact intestine[1]. Studies have recently been initiated to test this generalization further using other amino acids. Preliminary results indicate that the cellular uptake of 1 mM of the amino acids that stimulate lysine uptake (*i.e.*, methionine, alanine, or phenylalanine) is inhibited by 1 mM lysine, while the cellular uptake of non-stimulatory amino acids such as isoleucine or valine is not inhibited.

One of the most interesting characteristics of this interaction concerns the specificity of the neutral amino acids able to produce a stimulation of lysine and arginine uptake. Stimulation appears to be a specific property of L-leucine, L-methionine, L-alanine, or L-phenylalanine. Valine, isoleucine, histidine, and other

neutral amino acids generally accepted as sharing the same neutral amino acid transport pathway as the stimulatory amino acids did not stimulate basic amino acid transport. This dichotomy in the ability of the neutral amino acids to stimulate basic amino acid transport must therefore be a reflection of their differential capacity to participate in the exchange transport system. The finding that the cells accumulate 1 mM valine or 1 mM histidine to higher levels than 1 mM methionine (unpublished results) indicates that the inability of valine or histidine to stimulate lysine uptake is not related to an inability to drive the exchange transport, but rather appears to be due to a poor affinity for the hetero-exhange transport system.

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