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EXCHANGE TRANSPORT AND AMINO ACID CHARGE AS THE BASIS FOR Na^+-INDEPENDENT LYSINE UPTAKE BY ISOLATED INTESTINAL EPITHELIAL CELLS

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SUMMARY

1. The properties of the Na^+-independent stimulation of lysine uptake by intracellular alanine in isolated intestinal epithelial cells from the rat were determined and compared to the properties of Na^+-independent lysine uptake previously established in order to ascertain whether these two transport processes were related.

2. Dinitrophenol, anaerobiosis and ouabain did not inhibit the Na^+-independent stimulation of lysine uptake by alanine while ethacrynic acid, *N*-ethylmaleimide ATP, arginine and incubation in the cold produced a wide range of inhibitions. These inhibitors similarly influenced Na^+-independent lysine uptake.

3. Na^+-independent, alanine-stimulated lysine uptake was independent of extracellular K^+ but was decreased 10–17% by the replacement of the Ca^{2+} and/or Mg^{2+} of the incubation medium thus producing a pattern similar to that observed with Na^+-independent lysine uptake.

4. The diverse effects of pH on the stimulation of lysine uptake by alanine-preloaded cells very closely approximated that found for Na^+-independent lysine uptake by non-preloaded cells.

5. These results show that the properties of alanine-stimulated lysine uptake are similar to the properties of the Na^+-independent uptake of this amino acid, suggesting that both systems are mediated by the same or related transport processes.

6. A study of the relationship between the net positive charge of histidine and the magnitude of Na^+-independent histidine uptake revealed that Na^+-independent histidine uptake was increased by 17–24% at pH 4.5 where the cationic form of histidine is the primary molecular species.

7. However, alanine-stimulated histidine uptake showed a 40% increase at pH 4.5.

INTRODUCTION

In the previous report[1] it was shown that the major component of the influx of lysine into isolated intestinal epithelial cells was Na^+ independent and that concentra-

tive intracellular accumulation of lysine occurred in the absence of Na^+. In studies utilizing both intact intestine and isolated intestinal epithelial cells, the transmural transport[2,3], influx[2,3] and steady-state intracellular accumulation[3] of lysine were found to be stimulated by the presence of certain neutral amino acids either in the incubation medium or intracellularly. This stimulation has been attributed to a heteroexchange transport system and has been shown to produce active intracellular accumulation of lysine in the absence of Na^+ (ref. 3). The presence of these two concentrative, Na^+-independent lysine uptake systems in intestinal cells raises the question of whether they are manifestations of related or identical transport processes. The major purposes of this study were to determine the properties of the Na^+-independent stimulation of lysine uptake by intracellular alanine[3,4], compare these properties to that of Na^+-independent lysine uptake established previously[1], and thereby determine whether lysine uptake by these two systems can be attributed to a similar or identical transport mechanism.

Another possible mechanism by which active, Na^+-independent lysine transport can occur is by the flow of a cation down an electrical potential gradient[5]. The interior of the intestinal mucosal cell may be 20–40 mV negative with respect to the incubation media[6–8], a potential difference which could energize the concentrative intracellular accumulation of lysine. A secondary purpose of this study was to assess the importance of the net positive charge of an amino acid on its ability to be transported by a Na^+-independent pathway.

METHODS AND MATERIALS

The techniques used to measure and express amino acid uptake by isolated intestinal epithelial cells in the presence or absence of Na^+ were essentially the same as described in the previous paper[1]. The one major modification used was preincubation of the cells for 5 min in the standard Krebs–Tris incubation medium with or without 1 mM alanine. After washing the cells once in either 20 vol. of Krebs–Tris or Krebs–Tris choline medium at 4 °C, the incubation conditions employed were identical to those reported previously[1].

The mean Na^+ concentration of the Krebs–Tris choline medium from 100 individual experiments after a 2-min incubation of the alanine preloaded, Krebs–Tris choline washed cells was 5.6 ± 0.3 mM.

The uniformly labeled L-[^{14}C]histidine used in these studies was obtained from New England Nuclear and had a specific activity of 297 Ci/mole. The source and specific activity of the uniformly labeled lysine were the same as in the previous study[1].

RESULTS

The intracellular concentration of alanine following 5 min preincubation was determined from a number of representative experiments during this study and found to average 5.36 ± 0.25 mM (mean $\pm$ S.E., $n = 28$). There was no significant difference between the intracellular alanine concentration obtained after washing the cells in either the Na^+-containing or Na^+-free media.

The effects of various inhibitors on the stimulation of 1 mM lysine uptake by

alanine-loaded intestinal epithelial cells in the presence and absence of Na^+ are recorded in Table I. In agreement with previous findings using intracellular leucine[3], the magnitude of the stimulation of lysine uptake by intracellular alanine was independent of extracellular Na^+. Dinitrophenol inhibited alanine-stimulated lysine uptake only in the presence of Na^+. Anaerobiosis and ouabain were not inhibitory. Ethacrynic acid, *N*-ethylmaleimide, ATP and arginine produced a wide range of inhibition both in the presence and absence of Na^+ with inhibition generally greater in the Na^+-containing system.

No stimulation of lysine uptake by alanine was observed at 4 °C. One of the more important properties of neutral amino acid uptake by intestinal cells has been shown to be its essential irreversibility in the cold[9]. On the basis of these findings a stimulation of lysine uptake by intracellular alanine energized by the downhill movement of the neutral amino acid out of the cell[3] would not be expected at 4 °C.

Table I shows that Na^+-independent lysine uptake and alanine-stimulated, Na^+ independent lysine uptake each contribute about 50% of the total lysine uptake in the alanine-stimulated system without Na^+. If these two Na^+-independent pathways are mediated by a similar transport process, then it would be expected that the inhibitors

TABLE I

THE EFFECT OF INHIBITORS ON THE STIMULATION OF THE 2-MIN UPTAKE OF 1 mM L-LYSINE BY L-ALANINE-LOADED ISOLATED INTESTINAL EPITHELIAL CELLS IN THE PRESENCE AND ABSENCE OF EXTRACELLULAR Na^+

With the exception of the control group preincubated in Krebs–Tris for 5 min, all the intestinal epithelial cells were preincubated in Krebs-Tris containing 1 mM L-alanine for 5 min. The cells were then washed once at 4 °C with either 20 vol. of Krebs-Tris (with Na^+) or Krebs-Tris in which choline isotonically replaced NaCl (without Na^+) and incubated for 2 min at 37 °C (unless otherwise indicated) in the corresponding Na^+-containing or Na^+-deficient media each containing 1 mM L-lysine. The inhibitors were added to the incubation media to give the indicated final concentrations. Each value represents the mean ± S.E. from 12 individual experiments. 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 of lysine uptake.

Inhibitors	*Lysine uptake*					
	With Na^+			*Without Na^+*		
	mM/2 min	*% inhibition*	*P*	*mM/2 min*	*% inhibition*	*P*
None; Krebs–Tris preincubated	1.19 ± 0.10	—	—	0.90 ± 0.13	—	—
None; Krebs–Tris 1 mM L-alanine preincubated	2.26 ± 0.09	—	—	1.75 ± 0.08	—	—
0.4 mM dinitrophenol	2.01 ± 0.09	11.1	< 0.01	1.65 ± 0.08	5.7	—
Anaerobiosis	2.21 ± 0.11	2.2	—	1.70 ± 0.07	2.9	—
0.5 mM ouabain	2.06 ± 0.10	8.8	—	1.71 ± 0.06	2.3	—
5 mM ethacrynic acid	1.56 ± 0.10	31.0	< 0.001	1.33 ± 0.11	24.0	< 0.001
0.4 mM *N*-ethylmaleimide	1.25 ± 0.07	44.7	< 0.001	1.35 ± 0.10	22.9	< 0.001
2 mM ATP	0.98 ± 0.08	56.6	< 0.001	0.92 ± 0.11	47.4	< 0.001
5 mM L-arginine	0.52 ± 0.03	77.0	< 0.001	0.69 ± 0.05	60.6	< 0.001
4 °C	0.23 ± 0.02	89.8	< 0.001	0.18 ± 0.02	89.7	< 0.001

would affect these systems in a similar manner. Table II presents the relative percent increase of Na^+-independent lysine uptake by intracellular alanine in the absence or presence of the indicated inhibitors, using the analogous values for Na^+-independent lysine uptake[1] as the base line reference. Despite the wide range of the absolute inhibition of alanine-stimulated lysine uptake (Table I), the relative percent increase due to alanine was generally constant (74.8–98.8%) indicating a similar response to these inhibitors by both Na^+-independent lysine-uptake systems. A similar evaluation of the relative alanine stimulation of lysine uptake in the presence of Na^+ showed a wider range of values, presumably due to the differential action of the inhibitors on the Na^+-dependent component of lysine uptake[1].

TABLE II

THE EFFECT OF INHIBITORS ON THE RELATIVE STIMULATION OF THE Na^+-INDEPENDENT UPTAKE OF 1mM L-LYSINE BY L-ALANINE-LOADED CELLS AFTER A 2-MIN INCUBATION

The relative percent stimulation of lysine uptake by alanine was obtained by dividing the mean Na^+-independent lysine uptake by alanine-loaded cells in the absence and presence of metabolic inhibitors (Table I) by the mean Na^+-independent lysine uptake in the absence and presence of the same inhibitors[1] corrected for the 12.6% decrease in uptake due to the 5-min preincubation.

Inhibitor	*Alanine stimulation of lysine uptake (%)*
None	94.4
0.4 mM dinitrophenol	98.8
Anaerobiosis	88.9
0.5 mM ouabain	98.8
5 mM ethacrynic acid	83.4
0.4 mM *N*-ethylmaleimide	82.4
2 mM ATP	76.9
5 mM L-arginine	88.0

Table III shows the effect of isotonic replacement of K^+, Ca^{2+} and/or Mg^{2+} by choline on the stimulation of lysine uptake by intracellular alanine in the presence and absence of Na^+. In the absence of Na^+, alanine-stimulated lysine uptake was about 15% lower than that found in the presence of Na^+. However, as noted in Table I, the magnitude of the alanine stimulation of lysine uptake was not reduced by the absence of Na^+. The stimulation of lysine uptake by alanine was completely independent of extracellular K^+, both in the presence or absence of Na^+. The small inibition of lysine uptake found in the absence of Mg^{2+} was significant only in the absence of Na^+. The replacement of Ca^{2+} significantly inhibited lysine uptake by 12–13% in the presence and absence of Na^+. The inhibition produced in the absence of both Ca^{2+} and Mg^{2+} appeared to be additive, and independent of the presence or absence of the monovalent cations. When alanine-stimulated lysine uptake in the presence or absence of the cations was compared to non-stimulated lysine uptake under the same experimental conditions, the percent relative alanine stimulation was again found to fall in a narrow range. These results again indicate that both Na^+-independent processes are similarly influenced by experimental variables, but the results are not as de-

TABLE III

THE EFFECT OF CATION OMISSION FROM THE INCUBATION MEDIUM ON THE STIMULATION OF THE UPTAKE OF 1 mM L-LYSINE BY L-ALANINE-LOADED INTESTINAL EPHITHELIAL CELLS

Intestinal epithelial cells were preincubated in Krebs–Tris containing 1 mM L-alanine for 5 min. The cells were then washed once at 4 °C with 20 vol. of an isotonic Tris–HCl–choline medium (21 parts Tris–HCl and 109 parts choline chloride) and incubated for 2 min at 37 °C in the Krebs–Tris medium containing 1 mM L-lysine with choline chloride isotonically replacing the indicated cations. Each value represents the mean ± S.E. from at least 12 individual experiments. Probability values were obtained and interpreted as indicated for Table I. The percent relative alanine stimulations were obtained by dividing the mean uptake in the alanine-loaded cells by the mean lysine uptake in cells not preloaded with alanine but incubated in media with corresponding cation alterations[1] after appropriate correction for the loss of activity due to preincubation.

Cation omitted	*Lysine uptake*							
	With Na^+			*% relative alanine stimulation*	*Without Na^+*			*% relative alanine stimulation*
	mM/2 min	*% Decrease*	*P*		*mM/2 min*	*% Decrease*	*P*	
None	2.09 ± 0.08	—	—	91.7	1.77 ± 0.09	—	—	115.9
K^+	2.10 ± 0.07	—	—	107.9	1.77 ± 0.08	—	—	126.9
Ca^{2+}	1.82 ± 0.06	12.9	< 0.01	83.9	1.56 ± 0.07	11.9	< 0.05	105.3
Mg^{2+}	1.94 ± 0.08	7.2	—	88.3	1.59 ± 0.06	10.2	< 0.05	98.8
Ca^{2+}, Mg^{2+}	1.72 ± 0.08	17.3	< 0.01	81.1	1.46 ± 0.07	17.5	< 0.001	102.8
K^+, Ca^{2+}, Mg^{2+}	1.71 ± 0.09	18.2	< 0.001	85.9	1.50 ± 0.09	15.3	< 0.01	105.5

finitive as those obtained using the inhibitors because of the small absolute inhibitions involved.

The effect of pH and Na^+ on the uptake of 1 mM lysine by cells preincubated without or with 1 mM alanine is presented in Table IV. In the cells preincubated without alanine, lysine uptake showed the same general pattern as reported for cells not previously incubated[1]. The major difference noted was that Na^+-independent lysine uptake was not significantly reduced at pH 9.0. The diverse effects of pH on the absolute stimulation of lysine uptake by alanine-preloaded cells very closely approximated that found in the non-stimulated cells and is reflected in the similar magnitudes of the relative alanine stimulation. Since lysine uptake at pH 3.5 and 5.0 appear to be primarily Na^+ independent, these results are consistent with a similar or common mechanism for both Na^+-independent processes. The increase of Na^+-dependent lysine uptake at pH 9.0 was not reflected in a comparable increase in alanine-stimulated lysine uptake, indicating that these two stimulations are mediated by different processes.

Munck and Schultz[5] have suggested that the Na^+-independent intracellular concentration of lysine may be the result of a downhill flow of a cation into the relatively negative cell interior. The overall decrease in Na^+-independent lysine uptake at pH 9.0 (Table IV, ref. 1), where the positive charge of lysine would be expected to be partially negated, may be interpreted as evidence in favor of such a mechanism. A more dramatic decrease in the magnitude of the Na^+-independent component of

TABLE IV

THE EFFECT OF pH ON THE ABSOLUTE AND RELATIVE STIMULATION OF THE 2-MIN UPTAKE OF 1 mM L-LYSINE BY L-ALANINE-LOADED ISOLATED INTESTINAL EPITHELIAL CELLS IN THE PRESENCE AND ABSENCE OF EXTRACELLULAR Na^+

Isolated intestinal epithelial cells were preincubated for 5 min in either the Krebs–Tris medium or the Krebs-Tris medium containing 1 mM L-alanine. The cells were then washed once at 4 °C with 20 vol. of Krebs–Tris in which choline isotonically replaced NaCl (Krebs–Tris choline medium) and the cells then incubated at 37 °C for 2 min in either the Krebs–Tris medium (with Na^+) or the Krebs–Tris choline medium (without Na^+) both containing 1 mM L-lysine at the indicated pH. The $CaCl_2$ and $MgSO_4$ of all the incubation media were isotonically replaced by choline chloride. The desired pH was achieved and maintained by the use of buffers comprised of varying proportion of isotonic Tris and citric acid. Each value represents the mean ±S.E. from nine individual experiments. Probability values were obtained and interpreted as indicated in Table I.

pH	*Na^+ (118 mM)*	*Lysine uptake*						*Alanine stimulation*
		Krebs–Tris preincubated			*Krebs–Tris 1 mM L-alanine preincubated*			
		mM/2 min	*% pH 7.4 uptake*	*P*	*mM/2 min*	*% pH 7.4 uptake*	*P*	
7.4	+	1.02±0.05	—	—	2.02±0.11	—	—	98.0
7.4	−	0.79±0.04	—	—	1.70±0.11	—	—	115.2
3.5	+	0.39±0.08	38.2	<0.001	0.86±0.09	42.6	<0.001	120.5
3.5	−	0.54±0.08	68.4	<0.05	1.10±0.09	64.7	<0.01	103.7
5.0	+	0.66±0.06	64.7	<0.001	1.43±0.09	70.8	<0.001	116.7
5.0	−	0.61±0.03	77.2	<0.001	1.30±0.09	76.5	<0.001	113.1
9.0	+	1.28±0.06	125.5	<0.01	2.23±0.11	110.4	<0.05	74.2
9.0	−	0.75±0.03	94.9	—	1.66±0.14	97.6	—	121.3

lysine uptake might depend on raising the pH to even higher levels in order to insure the complete negation of the positive charge of the ammonium function of pK_2. The interpretation of these results would be complicated by the extremely high and non-physiological pH required, as well as by the concurrent dissociation of the protonated amino group described by pK_3 above pH 9.5. Histidine appears to be better suited for a study of the effect of molecular charge on uptake. Since the imidazolium group of histidine has a pK of 6.0, histidine would be expected to be primarily in the cationic form at a pH of 4.5. Table V presents the comparison of histidine uptake at pH 7.4 and 4.5 as a function of Na^+. At pH 7.4, histidine uptake was reduced by about 40% in the absence of Na^+. At pH 4.5, Na^+-dependent histidine uptake was lowered by 37% and Na^+-independent histidine uptake was increased by 17%. The net result of these changes was that histidine uptake at pH 4.5 was completely Na^+-independent.

The pattern of alanine-stimulated histidine uptake at pH 7.4 and 4.5 in the presence and absence of Na^+ is shown in Table VI. The main purpose of these experiments was to determine whether the stimulation of Na^+-independent histidine uptake at pH 4.5 could also be explained on the basis of exchange transport. Using the histidine uptake shown in Table V as a base line, alanine preincubation significantly increased histidine uptake under all the experimental conditions employed. These alanine stimulations probably represent minimum values since histidine uptake in Table V was obtained with cells not previously incubated and comparable studies with lysine

TABLE V

THE EFFECT OF LOW pH (4.5) ON THE Na^+-DEPENDENT AND Na^+-INDEPENDENT UPTAKE OF 1 mM L-HISTIDINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER A 2-MIN INCUBATION

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of Krebs–Tris in which choline chloride isotonically replaced NaCl (Krebs–Tris choline medium) and the cells then incubated at 37 °C for 2 min in either the Krebs–Tris medium (with Na^+) or the Krebs–Tris choline medium (without Na^+) both containing 1 mM L-histidine at the indicated pH. The desired pH was achieved and maintained by the use of buffers comprised of varying proportions of isotonic Tris and citric acid. Each value represents the mean ±S.E. from 12 individual experiments. Probability values were obtained and interpreted as indicated for Table I.

pH	*Histidine uptake*						
	With Na^+			*Without Na^+*			*% Na^+ independent*
	mM/2 min	*% pH 4.5 change*	*P*	*mM/2 min*	*% pH 4.5 change*	*P*	
7.4	1.44±0.04	—	—	0.87±0.05	—	—	60.4
4.5	0.91±0.05	−36.8	<0.001	1.02±0.08	+17.2	<0.05	100

TABLE VI

THE EFFECT OF LOW pH (4.5) ON THE STIMULATION OF THE UPTAKE OF 1 mM L-HISTIDINE BY ALANINE-LOADED CELLS AFTER 2-MIN IN THE PRESENCE AND ABSENCE OF EXTRACELLULAR Na^+

Isolated intestinal epithelial cells were preincubated for 5 min in the Krebs–Tris medium containing 1 mM L-alanine. The cells were then washed once at 4 °C with 20 vol. of Krebs–Tris in which choline chloride isotonically replaced NaCl (Krebs–Tris choline medium) and the cells then incubated at 37 °C for 2 min in either the Krebs–Tris medium (with Na^+) or the Krebs–Tris choline medium (without Na^+) both containing 1 mM L-histidine at the indicated pH. The desired pH was achieved and maintained by the use of buffers comprised of varying proportions of isotonic Tris and citric acid. The percent alanine stimulation was obtained by dividing the mean histidine uptake in the alanine-loaded cells by the mean histidine uptake in cells not preloaded with alanine (Table V). Each value represents the mean ±S.E. from 12 individual experiments. Probability values were obtained and interpreted as indicated for Table I. All percent values showing a significant change are italicized.

pH	*Histidine uptake*						
	With Na^+		*% alanine stimulation*	*Without Na^+*		*% alanine stimulation*	*% Na^+ independent*
	mM/2 min	*%pH 4.5 change*		*mM/2 min*	*% pH 4.5 change*		
7.4	1.74±0.08	—	*20.8*	1.14±0.05	—	*31.0*	*65.5*
4.5	1.28±0.05	*−36.4*	*40.7*	1.42±0.08	*+24.6*	*39.2*	100

have shown that preincubation lowers uptake by 10–15% (Table II). At pH 7.4, the alanine stimulation of histidine uptake was greater in the absence of Na^+ than in the presence of Na^+. However, this difference did not essentially change the overall percent Na^+ independence of histidine uptake at pH 7.4 from that found in the nonstimulated system. At pH 4.5, alanine stimulated histidine uptake by about 40% over that found in the nonstimulated system both in the presence and absence of Na^+. As in the nonstimulated system, alanine stimulated histidine uptake at pH 4.5 was completely Na^+ independent. The stimulation of histidine uptake at pH 4.5 was in the opposite direction to the pH effect in the presence of Na^+. The stimulation of histidine uptake at pH 4.5 in the absence of Na^+ appeared to be the additive result of both alanine preloading and the pH. These results indicate that the increase of Na^+-independent histidine uptake at pH 4.5 could be explained on the basis of exchange transport.

DISCUSSION

The ability of an amino acid located on one side of a membrane to stimulate the movement of an amino acid initially located on the other side of the membrane has been demonstrated in ascites cells[10–18], isolated intestinal cells[3], intact intestine[2,3,19–22] brain[23–25], kidney[26,27] and pancreas[28]. This phenomenon has been called exchange diffusion[29] or counterflow[30], although the conditions employed to study the exchange process are usually different from those originally used to describe the term. Most of these studies have employed the same experimental approach as that used in the present study, *i.e.* determining the effect of preloading the cells or tissue with an amino acid on the inward movement of an externally added amino acid under various experimental conditions. Amino acid exchange has been reported to proceed independent of Na^+ (refs 13–15 and 28), K^+ (refs 13, 14 and 18), cellular ATP (ref. 14) and the presence of inhibitors of energy metabolism[10,11,16,20,21]. These properties are similar to those found for the stimulation of lysine uptake by intracellular alanine, further indicating that this process occurs by a heteroexchange transport[3].

In the absence of Na^+, the absolute magnitude of the uptake of lysine by alanine loaded cells was reduced by an average of 17%. This inhibition can be explained on the basis of the contribution of the Na^+-dependent component of lysine uptake on the total lysine uptake. Approximately 25% of the lysine uptake at 2 min was previously shown to be Na^+ dependent[1]. Assuming that 25% of the 1.2 mM lysine uptake found after Krebs–Tris preincubation is due to this Na^+-dependent component and that alanine produces a Na^+-independent stimulation of 90–100%, then the total contribution of Na^+-dependent uptake in the stimulated system would be about 0.3 mM/2.3 mM or 13%. This value is in good agreement with the observed inhibition. The 11% inhibition of the alanine stimulation of lysine uptake by dinitrophenol found only in the presence of Na^+ can be similarly explained. The mechanisms by which ethacrynic acid, *N*-ethylmaleimide and ATP inhibit the alanine stimulation of lysine uptake are not known but are presumed to operate by a direct interaction with the membrane components involved in mediating the movement of alanine out of the cell or the movement of lysine into the cell. The inhibition by arginine is attributed to a competition with lysine for the exchange process for which arginine has been shown to have affinity[3].

The pH studies were designed primarily to determine whether Na^+-independent amino acid uptake could be explained on the basis of the downhill flow of a positively charged amino acid into the comparatively negative cell interior. At pH 9.0, where approximately one-half of the positive charge of lysine would be expected to be neutralized, there was only a 5–15% decrease in uptake. This decrease is much less than would be expected on the basis of uptake primarily governed by the net positive charge. However, the interpretation of these results may be complicated by the presence of areas of lower pH located in the brush border[31]. In the pH range 3.5–5.0, Na^+-independent lysine uptake decreased 23–32%. At these pH values the net positive charge of lysine would not be expected to change. Since Na^+-independent histidine uptake was increased at pH 4.5, the decrease in lysine uptake does not appear to be a nonspecific effect of the low pH on cellular structure and function. Although Na^+-independent uptake of histidine increased 17% as a result of lowering the pH from 7.4 to 4.5 (*i.e.* forming the histidine cation) this increase was less than one-half that produced by alanine preloading. These studies fail to indicate a definitive relationship between the net positive charge on an amino acid and the expected changes in the magnitude of Na^+-independent uptake. On the basis of the different responses of Na^+-independent lysine and histidine uptake to low pH, it appears that the uptake of these cationic amino acids is mediated by different carriers. This finding is consistent with the failure to show significant increases in the cross-inhibition between lysine and histidine in rabbit ileum[31] and Ehrlich cells[32] as the pH is lowered from 7.4 to 5.0.

The final Na^+ concentration of the Krebs–Tris choline incubation medium after lysine uptake by alanine-preloaded cells was about twice as high as the Na^+ concentration of the medium after lysine uptake by cells not previously incubated with alanine (*i.e.* 5.6 mM as compared to 2.9 mM after 2 min). These results suggest that the efflux of alanine out of the cell may be accompanied by a concurrent movement of Na^+ (ref. 33). It may then be possible to explain the stimulation of lysine uptake by alanine on the basis of an increase of the electronegativity of the cell interior due to the accelerated Na^+ efflux, since choline would be not expected to readily penetrate into the cell[34]. However, the finding of appreciably the same magnitude of alanine stimulation of lysine uptake using incubation media containing 118 mM Na^+ is not consistent with this explanation. In addition, if increased cellular electronegativity did mediate alanine-stimulated lysine uptake, it would be expected that all of the neutral amino acids would have appreciably the same capability of stimulating lysine uptake. Previous studies have shown that stimulation is not produced by valine, isoleucine, tryptophan, proline and glycine[3]. The finding that the stimulatory neutral amino acids increase lysine uptake when present in the extracellular medium[3] further argues against an effect due to increased cellular electronegativity, since Rose and Schultz[7] have shown that alanine influx produces a decrease in the cellular negativity with respect to the mucosal solution.

The results of this study have shown that the response of alanine-stimulated lysine uptake and Na^+-independent lysine uptake toward various metabolic inhibitors, changes in pH and cation omission is essentially the same. These findings would be expected if both of these Na^+-independent lysine uptake systems were mediated by similar or identical transport processes. Although support for the hypothesis that Na^+-independent lysine uptake may be mediated by an exchange transport has been obtained circumstantially, it is difficult to explain the similarity in the charac-

teristics of these transport systems to the diverse experimental conditions employed as being fortuitous. In contrast to the comparatively small change in lysine uptake produced by variations in pH, the stimulation of lysine uptake by intracellular alanine was of the magnitude required to account for the observed level of Na^+-independent lysine uptake. The energy for lysine uptake by exchange in the *in vitro* system is a result of the downhill movement of alanine from the cell to the medium[3,4]. The question then arises as to the nature of the substance with which lysine is exchanging in order to energize Na^+-independent lysine uptake in cells not preloaded with neutral amino acids. Sugars such as glucose, fructose, galactose and α-methyl-D-glucoside have been found to be unable to stimulate the uptake of lysine by the cells (unpublished results). It is possible that the endogenous pool of amino acids may represent a potential exchange medium for lysine. In Ehrlich cells the endogenous pool of exchangeable amino acids (*i.e.* valine, isoleucine, leucine, tyrosine and phenylalanine), was found to be at least 2 mM (ref. 14). From the levels of endogenous leucine found in intact rat intestine by Fern *et al.*[35], the free amino acid pool in the epithelial cells may be even greater. If it is assumed that the endogenous stimulatory amino acids act in an additive manner and that they represent a freely exchangeable pool[14], then exchange transport can account for the observed levels of Na^+-independent lysine uptake. A decrease of the intracellular pools of alanine, leucine, methionine and phenylalanine by extracellular lysine would represent direct evidence in favor of this heteroexchange.

Under normal conditions of protein digestion and absorption, the capacity for lysine transport by exchange is potentially very large since a portion of the absorbed stimulatory neutral amino acids may behave as an exchangeable pool, and thus tend to simulate the conditions found in the preloading experiments. The finding that neutral amino acids and lysine have the same distribution of transport activity along the intestine[36] is compatible with such an exchange process.

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