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THE PROPERTIES OF Na^+-DEPENDENT AND Na^+-INDEPENDENT LYSINE UPTAKE BY ISOLATED INTESTINAL EPITHELIAL CELLS

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SUMMARY

1. The 5-min uptake of 1 mM leucine by epithelial cells isolated from rat intestine was reduced 75% in the absence of extracellular Na^+ and leucine did not attain intracellular concentrations greater than unity. In contrast, the uptake of 1 mM lysine was reduced only 32% and lysine reached an intracellular concentration of 1.54 mM, showing that a large component of the concentrative uptake of lysine is Na^+ independent.

2. The Na^+-independent component of lysine uptake attained levels significantly greater than unity after 5 min, but the total contribution of Na^+-independent lysine uptake decreased from 82 to 62% as the incubation time was increased from 1 to 10 min.

3. The Na^+-independent component of lysine uptake was significantly inhibited by dinitrophenol after 5 and 10 min, but not after 2 min. Ouabain was non-inhibitory.

4. At pH 9.0, Na^+-dependent lysine uptake increased while Na^+-independent lysine uptake decreased. At pH 3.5 lysine uptake in the presence and absence of Na^+ was reduced to the same level.

5. Dinitrophenol, anaerobiosis and ouabain specifically decreased the Na^+-dependent component of lysine uptake after 2 min. Ethacrynic acid, *N*-ethylmaleimide, ATP and arginine inhibited both the Na^+-dependent and Na^+-independent components of lysine uptake.

6. The isotonic replacement of K^+, Ca^{2+} and Mg^{2+} by choline produced similar changes in the 2-min uptake of lysine in the presence or absence of Na^+.

INTRODUCTION

While at least four distinct structurally specific carrier sites are currently believed to mediate the initial binding of various classes of amino acids to the mammalian intestinal membrane[1–3], the energy required for active transport is believed to be supplied by a Na^+-dependent process generally common to all of these amino acid transport systems[4,5]. However, there appear to be major quantitative differences in the Na^+ dependence of these amino acid transport systems. The transport of the

basic amino acids appears to be the least Na^+-dependent[4]. Although lysine transport was decreased in the absence of extracellular Na^+, active Na^+-independent lysine transport has been demonstrated in the epithelial cells of toad bladder[6], rat kidney cortex slices[7], and intact rabbit ileum[8].

Our interest in lysine transport stems from studies involving interactions between basic and neutral amino acid uptake in isolated intestinal epithelial cells[9,10]. Previous studies[9] have shown the stimulation of lysine uptake by intracellular leucine or alanine to be independent of extracellular Na^+, with uptake values greater than unity obtained for lysine.

The presence of these Na^+-independent transport pathways in mammalian intestine capable of mediating the active uptake of lysine stimulated our interest in determining whether these processes were related. The main purpose of this study was to determine the properties of the Na^+-dependent and the Na^+-independent components of lysine uptake in isolated intestinal epithelial cells and thereby establish a basis of comparison for subsequent studies[11] aimed at evaluating the possible involvement of a heteroexchange[9], or electrical potential gradient across the epithelial cell membrane[8] as the mediating factor energizing Na^+-independent lysine transport.

METHODS AND MATERIALS

Wistar strain, male rats weighing 180–260 g were used as a source of the intestinal 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 previously described in detail[9,12].

The standard incubation medium employed to measure lysine or leucine uptake by the cells 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 cpm/ml) and non-radioactive amino acid to the desired final concentration. A Krebs–Tris choline medium prepared by isotonically replacing the NaCl of the Krebs–Tris medium with choline chloride was used to study amino acid uptake in a Na^+-free system. The Na^+ concentration of the incubation media was determined by direct analysis in a Coleman flame photometer. After incubation of Krebs–Tris choline-washed cells in the Krebs–Tris choline medium, the average Na^+ concentration of the medium in 100 representative experiments was 2.9 ± 0.2 mM, confirming that the medium was essentially free of Na^+ during incubation. The replacement of other cations of the incubation medium was also carried out with isotonic choline chloride. The isotonicity of the choline chloride was confirmed with a freezing-point osmometer (Advanced Instruments, Inc.). In the studies at pH 9.0, the $CaCl_2$ and $MgSO_4$ of all the incubation media were replaced by choline chloride. In the low pH studies, varying proportions of isotonic Tris and citric acid were used to achieve and maintain the desired pH.

In all the experiments in which Na^+-deficient media were used, all the cells were initially washed once at 4 °C with 20 vol. of the Krebs–Tris choline medium. To measure amino acid uptake, 0.3 ml of the cells representing an average of 5.42 mg protein were added to 5 ml of the oxygenated Krebs–Tris amino acid medium described above. An aliquot of the reaction mixture was taken immediately after the cells were dispersed in the medium for a determination of the initial counts. The reaction

mixture was then incubated with shaking for the desired time period at 37 °C, 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 $275 \times g$ for 2 min. The cells were then washed and centrifuged 3 additional times with 5 ml of cold Krebs–Tris. The final pellet was diluted to 1 ml with saline and mixed, and aliquots were taken for the determination of protein[13] and radioactivity[14]. The uptake of lysine or leucine by the cells was determined as previously described[9,12] and expressed as the concentration of the amino acid in the cell water.

The uniformly labeled L-[^{14}C]lysine and L-[^{14}C]leucine used in these studies were obtained from Amersham/Searle and had a specific activity of 336 Ci/mole and 331 Ci/mole, respectively.

RESULTS

The results presented in Table I illustrate some of the fundamental differences in the energy requirement of the 5-min concentrative uptake of 1 mM leucine and 1 mM lysine by epithelial cells isolated from rat intestine. In the absence of extracellular Na^+, the uptake of leucine was decreased by 75% and the amino acid did not attain intracellular concentrations greater than that initially present in the incubation medium. In contrast, although decreased by about 32%, lysine uptake in the absence of extracellular Na^+ reached an intracellular concentration of 1.54 mM confirming that a large component of the intestinal uptake of lysine is Na^+ independent and concentrative[8,9]. In the absence of extracellular K^+, the decrease in leucine uptake was 2.6 times as great as the decrease in lysine uptake. The K^+ dependence ex-

TABLE I

COMPARATIVE Na^+, K^+, DINITROPHENOL AND OUABAIN SENSITIVITY OF THE UPTAKE OF 1 mM L-LEUCINE AND 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER A 5-MIN INCUBATION

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of either Krebs–Tris, Krebs–Tris with choline chloride isotonically replacing NaCl (no Na^+ experiments) or Krebs–Tris with choline chloride isotonically replacing KCl (no K^+ experiments). The cells were then incubated for 5 min at 37 °C in a Krebs–Tris medium modified as indicated containing either 1 mM L-leucine or 1 mM L-lysine. Choline chloride was used as an isotonic replacement for the Na^+ or K^+ removed for the incubation medium. Each value represents the mean ±1 S.E. from at least 8 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 amino acid uptake.

Medium alteration	*Leucine uptake*			*Lysine uptake*		
	mM/5 min	*%inhibition*	*P*	*mM/5 min*	*%inhibition*	*P*
None	2.92±0.13	—	—	2.26±0.11	—	—
No Na^+	0.73±0.11	75.0	<0.001	1.54±0.07	31.9	<0.001
No K^+	1.20±0.13	58.9	<0.001	1.75±0.10	22.6	<0.001
0.4 mM dinitrophenol	0.49±0.08	83.2	<0.001	1.23±0.04	45.6	<0.001
0.5 mM ouabain	0.89±0.09	69.5	<0.001	1.78±0.10	21.3	<0.02

hibited by isolated intestinal epithelial cells has been previously attributed to a partial loss of intracellular K^+ (ref. 12) which is needed in amounts sufficient to satisfy the requirements for ATPase or other metabolic reactions[15] involved in energizing amino acid uptake. Dinitrophenol and ouabain inhibited leucine uptake by 70–83% thereby preventing concentrative accumulation. In contrast, lysine uptake was reduced only 21–46% by these inhibitors and the resultant concentrations of intracellular lysine were significantly greater than unity.

TABLE II

THE EFFECT OF TIME ON THE Na^+-DEPENDENT AND Na^+-INDEPENDENT UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of a Krebs–Tris medium in which choline chloride isotonically replaced NaCl (Krebs–Tris–choline medium). The cells were then incubated at 37 °C for the indicated times in either the Krebs–Tris medium (with Na^+) or the Krebs–Tris–choline medium (without Na^+) both containing 1 mM L-lysine. Each value represents the mean ± S.E. from 8 individual experiments. Probability values were obtained and interpreted as indicated for Table I.

Time (min)	*Lysine uptake (mM)*			*% Na^+ independent*
	With Na^+	*Without Na^+*	*P*	
0.5	0.46 ± 0.03	0.41 ± 0.04	—	91.3
1	0.71 ± 0.05	0.58 ± 0.03	< 0.01	81.7
2	1.19 ± 0.07	0.88 ± 0.05	< 0.001	73.9
5	2.03 ± 0.11	1.41 ± 0.07	< 0.001	69.4
10	3.12 ± 0.15	1.93 ± 0.11	< 0.001	61.9

Table II shows the effect of the time of incubation on the uptake of 1 mM lysine in the presence or absence of extracellular Na^+. As the incubation time increased from 0.5–10 min, the component of lysine uptake that was Na^+ insensitive decreased from 91 to 62%. Lysine uptake was assumed to be essentially at initial velocity after 2 min on the basis of 5-min linear correlation coefficients of greater than 0.98, both in the presence and absence of Na^+. The uptake of mucosal lysine by intact rabbit ileum has been reported to be a linear function of time for at least 1.25 min[8]. When the lysine-uptake values shown in Table II were plotted as a function of time between 0.5 and 2 min, two straight lines were obtained, both of which intercepted the ordinate at 0.21 mM lysine. A very rapid surface binding of lysine to the cells would be expected to show this type of time curve[8]. After correcting the values shown in Table II for the 0.21 mM time-independent lysine uptake, Na^+-independent lysine uptake was still found to decrease from 80% at 0.5 min to 59% at 10 min.

Na^+-independent lysine uptake was found to be significantly greater than unity after 5 and 10 min (Table II). In order to determine whether these values represent active accumulation of lysine, or whether they are the result of nonspecific factors (*e.g.* surface binding), the effect of dinitrophenol and ouabain on the Na^+-independent uptake of lysine at 5 and 10 min was investigated (Table III). Dinitrophenol significantly lowered lysine uptake to values approximating unity. The inhibitory effect

TABLE III

THE EFFECT OF DINITROPHENOL AND OUABAIN ON THE Na^+-INDEPENDENT UPTAKE OF 1 mM LYSINE AFTER 5 AND 10 MIN

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of a Krebs–Tris medium in which choline chloride isotonically replaced NaCl (Krebs–Tris–choline medium). The cells were then incubated at 37 °C for 5 or 10 min in the Krebs–Tris–choline medium, either with or without dinitrophenol or ouabain. Each value represents the mean ±S.E. from at least 14 individual experiments. Probability values were obtained and interpreted as indicated for Table I.

Inhibitor	*Lysine uptake (mM)*	*% inhibition*	*P*
5 min			
None	1.46±0.07	—	—
0.4 mM dinitrophenol	1.20±0.06	17.8	<0.01
0.5 mM ouabain	1.40±0.06	4.1	—
10 min			
None	1.89±0.14	—	—
0.4 mM dinitrophenol	1.17±0.05	38.1	<0.001
0.5 mM ouabain	1.76±0.14	6.9	—

TABLE IV

THE EFFECT OF HIGH pH (9) ON THE Na^+-DEPENDENT AND Na^+-INDEPENDENT UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER A 2-MIN OR 5-MIN INCUBATION

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of a Krebs–Tris medium in which choline chloride isotonically replaced NaCl (Krebs–Tris–choline medium). The cells were then incubated at the indicated pH at 37 °C for 2 or 5 min in either the Krebs–Tris medium (with Na^+) or the Krebs–Tris–choline medium (without Na^+) both containing 1 mM L-lysine. The $CaCl_2$ and $MgSO_4$ of all the incubation media were isotonically replaced by choline chloride. Each value represents the mean ±S.E. from 8 individual experiments for the 2-min determinations and 10 individual experiments for 5-min determinations. Probability values were obtained and interpreted as indicated for Table I.

pH	*Lysine uptake*						*% Na^+ independent*
	With Na^+			*Without Na^+*			
	mM	*% pH 9 change*	*P*	*mM*	*% pH 9 change*	*P*	
2 min							
7.4	1.32±0.10	—	—	0.92±0.06	—	—	69.7
9.0	1.60±0.08	+21.2	<0.02	0.78±0.07	−15.2	<0.05	48.7
5 min							
7.4	2.09±0.17	—	—	1.53±0.15	—	—	73.2
9.0	2.37±0.21	+13.3	—	1.37±0.05	−10.5	—	57.8

of dinitrophenol after 5 min was not mediated by a change in the cell volume. Ouabain had no effect on Na^+-independent lysine uptake.

Table IV shows the effect of raising the pH of the incubation media from 7.4 to 9.0 on the Na^+ dependence of the uptake of 1 mM lysine at 2 and 5 min. Since Ca^{2+} and Mg^{2+} precipitate from the medium at pH values above 8.0, choline chloride was used as an isotonic replacement for these cations in all of these experiments. Omission of Ca^{2+} and Mg^{2+} from the pH 7.4 incubation medium results in only a small decrease in lysine uptake (see Table VII). In the presence of Na^+, lysine uptake at pH 9.0 was 13–21% higher than that found at pH 7.4. In agreement with previous findings[12], the 2-min uptake of 1 mM leucine determined concurrently under the same pH conditions was reduced 48% at pH 9.0. In the absence of Na^+, the uptake of lysine at pH 9.0 was 10–15% less than the uptake at pH 7.4. The net result of these changes was that lysine uptake was much more dependent on Na^+ at pH 9.0 than at pH 7.4. Since the protonated amino group described by pK_2 would be expected to be approximately one-half dissociated at pH 9.0, these results suggest that Na^+-independent lysine uptake is dependent on the net positive charge on lysine.

TABLE V

THE EFFECT OF LOW pH (3.5) ON THE Na^+-DEPENDENT AND Na^+-INDEPENDENT UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER A 2-MIN INCUBATION

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of a Krebs–Tris medium in which choline chloride isotonically replaced NaCl (Krebs–Tris–choline medium). The cells were then incubated at the indicated pH 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. Isotonic Tris–citrate was used to achieve and maintain the desired pH in these experiments. Each value represents the mean ± S.E. from 12 individual experiments. Probability values were obtained and interpreted as indicated for Table I.

pH	*Lysine uptake*						*% Na^+ independent*
	With Na^+			*Without Na^+*			
	mM/2 min	*% pH 3.5 inhibition*	*P*	*mM/2 min*	*% pH 3.5 inhibition*	*P*	
7.4	1.29 ± 0.07	—	—	1.00 ± 0.04	—	—	77.5
3.5	0.44 ± 0.04	65.9	< 0.001	0.53 ± 0.04	47.0	< 0.001	100

When the pH of the incubation media was lowered from 7.4 to 3.5, the uptake of 1 mM lysine after 2 min was reduced by 66% in the medium with Na^+ and by 47% in the medium without Na^+ (Table V). The residual lysine uptake found at pH 3.5 was completely independent of Na^+. These results are in general agreement with the findings that Na^+-dependent amino acid transport pathways in both mammalian intestine[4] and Ehrlich cells[16], are inhibited much more by low pH than are Na^+-independent amino acid transport pathways.

The effect of various inhibitors on the Na^+-dependent and Na^+-independent uptake of 1 mM lysine after 2 min is shown in Table VI. Inhibitors of energy metab-

TABLE VI

THE EFFECT OF INHIBITORS ON THE Na^+-DEPENDENT AND Na^+-INDEPENDENT UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER A 2-min INCUBATION

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of a Krebs–Tris medium in which choline chloride isotonically replaced NaCl (Krebs–Tris–choline medium). The cells were 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 and the various inhibitors at the indicated final concentrations. Each value represents the mean ± S.E. from at least 10 individual experiments using the medium with Na^+ and 18 individual experiments using the medium without Na^+. The % inhibition values were obtained by comparing the mean lysine uptake in the presence of the inhibitor to the mean lysine uptake from an identical number of experiments run concurrently without the inhibitor. Probability values were obtained and interpreted as indicated for Table I.

Inhibitor	*Lysine uptake*					
	With Na^+			*Without Na^+*		
	mM/2 min	*%inhibition*	*P*	*mM/2 min*	*%inhibition*	*P*
None	1.42 ± 0.04	—	—	1.03 ± 0.04	—	—
0.4 mM dinitrophenol	1.07 ± 0.06	27.9	< 0.001	0.95 ± 0.05	7.8	—
Anaerobiosis	1.14 ± 0.08	16.2	< 0.01	1.04 ± 0.03	—	—
1 mM NaF	1.34 ± 0.07	5.7	—			
0.5 mM ouabain	1.21 ± 0.08	18.2	< 0.001	0.98 ± 0.04	4.9	—
5 mM ethacrynic acid	1.07 ± 0.12	24.6	< 0.001	0.83 ± 0.04	19.4	< 0.001
0.4 mM *N*-ethyl-malcimide	0.87 ± 0.06	38.6	< 0.001	0.85 ± 0.02	17.5	< 0.001
2 mM ATP	0.81 ± 0.04	43.0	< 0.001	0.59 ± 0.02	42.7	< 0.001
5 mM L-arginine	0.47 ± 0.05	66.5	< 0.001	0.42 ± 0.03	59.2	< 0.001
4 °C	0.19 ± 0.03	83.8	< 0.001			

olism such as dinitrophenol and anaerobiosis reduced lysine uptake in the presence of Na^+ to values approximating that found in the absence of Na^+. These inhibitors did not significantly lower Na^+-independent lysine uptake after 2 min. Since dinitrophenol did not produce an increase in cell volume after the 2-min incubation, the lack of a dinitrophenol inhibition could not be attributed to the use of improper cell volumes during the calculation of lysine uptake. Dinitrophenol did significantly inhibit Na^+-independent lysine uptake after 5 and 10 min (Table III). NaF was not inhibitory either after 2 or 5 min, indicating that the energy required for lysine uptake is not immediately dependent on glycolysis[17,18]. Ouabain inhibited lysine uptake only in the presence of Na^+ as would be expected on the basis of an effect of ouabain on a (Na^+, K^+)-dependent ATPase. Surprisingly, ethacrynic acid, which is also an inhibitor of (Na^+, K^+)-dependent ATPase[19] and presumably inhibits intestinal transport[20] by increasing the intracellular Na^+ concentration[21], inhibited lysine uptake both in the presence and absence of Na^+. After 5 min the inhibition of Na^+-independent lysine uptake by ethacrynic acid increased to 31%. The sulfhydryl inhibitor, *N*-ethylmaleimide, reduced lysine uptake 39% in the presence of Na^+ and 18% in the absence of Na^+. *N*-ethylmaleimide has also been reported to inhibit lysine

accumulation by rat kidney cortex slices[22]. ATP, which has been shown to be a potent inhibitor of amino acid uptake by intestinal cells[23], lowered Na^+-dependent and Na^+-independent lysine uptake by 43%. Arginine inhibited both Na^+-dependent and Na^+-independent lysine uptake by about the same magnitude. Lysine uptake was virtually eliminated at 4 °C.

Isotonic replacement of the K^+, Ca^{2+} and Mg^{2+} of the incubation medium with choline chloride produced similar changes in the 2-min uptake of 1 mM lysine in the presence or absence of Na^+ (Table VII). The absence of K^+ did not significantly decrease lysine uptake after 2 min, but did inhibit lysine uptake after 5 min (Table I). The inhibition of leucine uptake in a K^+-deficient medium has also been shown to increase as a function of incubation time (22.3% after 2 min, 58.9% after 5 min (Table I) and 76.5% after 15 min[12]). While neither the absence of Ca^{2+} or Mg^{2+} significantly lowered lysine uptake, the absence of both Ca^{2+} and Mg^{2+} resulted in a small but significant inhibition of lysine uptake both in the presence and absence of Na^+. This inhibition was not influenced by K^+.

TABLE VII

THE EFFECT OF CATION OMISSION FROM THE INCUBATION MEDIUM ON THE UPTAKE OF 1 mM L-LYSINE BY ISOLATED INTESTINAL EPITHELIAL CELLS AFTER A 2-MIN INCUBATION

Isolated intestinal epithelial cells were washed once at 4 °C with 20 vol. of an isotonic Tris–choline chloride medium (21 parts Tris and 109 parts choline chloride). The cells were then incubated at 37 °C for 2 min in the Krebs–Tris medium containing 1 mM L-lysine with choline chloride isotonically replacing the indicated cation. Each value represents the mean ± S.E. from at least 10 individual experiments. Probability values were obtained and interpreted as indicated for Table I.

Cation omitted	*Lysine uptake*					
	With Na^+			*Without Na^+*		
	mM/2 min	*%decrease*	*P*	*mM/2 min*	*%decrease*	*P*
None	1.29 ± 0.06	—	—	0.96 ± 0.04	—	—
K^+	1.20 ± 0.10	7.0	—	0.91 ± 0.09	5.2	—
Ca^{2+}	1.17 ± 0.06	9.3	—	0.89 ± 0.06	7.3	—
Mg^{2+}	1.22 ± 0.07	5.4	—	0.93 ± 0.06	3.1	—
Ca^{2+}, Mg^{2+}	1.13 ± 0.06	12.4	< 0.05	0.84 ± 0.04	12.5	< 0.05
K^+, Ca^{2+}, Mg^{2+}	1.09 ± 0.09	15.5	< 0.05	0.85 ± 0.06	11.5	< 0.02

DISCUSSION

The active transport of basic amino acids across intact intestine was first demonstrated by Hagihara *et al.*[24]. Since then relatively few studies pertaining to the effects of Na^+ or metabolic inhibitors on this process have been reported. Finch and Hird[25] showed that the uptake of 10 mM of all the amino acids tested, except for lysine and arginine, was inhibited by dinitrophenol. The same investigators were, however, unable to demonstrate active uptake of lysine or arginine at this relatively

high concentration[26]. Robinson and Felber[27] reported that the active accumulation of arginine by intestinal slices after 60 min was inhibited but not eliminated by dinitrophenol, and was prevented by *N*-ethylmaleimide or the absence of Na^+. Rosenberg *et al.*[28] demonstrated that active lysine accumulation by everted intestinal sacs was lowered, but not eliminated, in a low Na^+ medium (*i.e.* 25 mM) or in the presence of ouabain. However, this effect did not appear to be specific for lysine since methionine exhibited the same properties. The first detailed demonstration of the relative Na^+ insensitivity of intestinal lysine transport was given by Munck and Schultz[8]. These investigators showed that the Na^+ dependence of lysine influx is small compared to that of neutral amino acids and that intracellular lysine concentrations exceeding unity could be demonstrated in the absence of Na^+. In contrast, the transmural transport of lysine was shown to exhibit a high Na^+ dependence. These findings are similar to studies carried out in this laboratory using intestinal sacs in which lysine intracellular accumulation was found to be concentrative and relatively Na^+ insensitive, while the transmural transport of leucine and lysine were found to be equally Na^+ dependent (unpublished results).

The results of the present study have demonstrated that approximately 75% of the initial uptake of lysine by isolated intestinal cells is Na^+ independent. Approximately 68% of the influx of 1 mM lysine into intact rabbit ileum was also reported to be Na^+ independent[8]. It was also shown that the Na^+-independent uptake of lysine results in a concentrative accumulation after 5- and 10-min incubations. The main evidence in support of this contention was the finding of intracellular lysine concentrations significantly exceeding unity, even after corrections for possible surface binding were made. Lysine uptake values are based on cell volumes calculated from dry wt per protein ratios[12]. During the time studies, 80% of these ratios were found to lie within a range that would yield a deviation of no more than $\pm 10\%$ from the mean lysine concentration of the cell calculated on the basis of 1·83 mg dry wt per mg protein[9,12]. It is, therefore, unlikely that the concentrative Na^+-independent uptake of lysine, especially after 10 min, is due to an error in the estimation of the cell volume. The dinitrophenol inhibition of Na^+-independent lysine uptake to values approximating unity is also consistent with this process being concentrative. Dinitrophenol would not be expected to inhibit a concentrative lysine uptake due to surface binding, diffusion or the use of improper cell volumes. It is also unlikely that dinitrophenol inhibits lysine uptake by impairing its cellular utilization since it has been shown that lysine is not essentially metabolized by the small intestine even after incubation periods as long as 1 h[8,12]. This finding of a concentrative Na^+-independent lysine uptake confirms similar results obtained using intact intestine in a system uncomplicated by the presence of non-absorptive tissue and corrections for extracellular space[8].

The Na^+-dependent component of lysine uptake showed the same general properties as that of the Na^+-dependent uptake of other amino acids. These properties included an effect of Na^+ on the K_m rather than the V of lysine uptake, and an inhibition at low pH[4]. In addition, the Na^+-dependent influx and accumulation of lysine was inhibited by dinitrophenol and ouabain. An inhibition of Na^+-dependent leucine influx by dinitrophenol and ouabain was also observed using cells prewashed in Krebs–Tris Na^+ or Krebs–Tris choline (unpublished results). In contrast, an inhibition of Na^+-dependent amino acid influx by dinitrophenol and ouabain has not

been observed in intact intestine depleted of Na^+ by preincubation in a Na^+-free medium[21]. These results suggest that in the isolated cell system these inhibitors can elicit an almost immediate equilibration of Na^+ between the extracellular medium and the cell interior. A unique feature of Na^+-dependent lysine uptake was the tendency toward greater activity exhibited at pH 9.0. A similar pH effect has been found for lysine accumulation by rat kidney cortex slices[22]. This increased uptake cannot be readily explained on the assumption that lysine partially assumes the characteristics of neutral amino acid uptake at this high pH, since leucine uptake was inhibited about 50% under the same pH conditions. The omission of Ca^{2+} and Mg^{2+} from these incubation media would also be expected to decrease lysine uptake by a neutral amino acid pathway[12]. These results indicate that optimal Na^+-dependent lysine uptake is possible for time periods as long as 5 min at a relatively high pH.

The major component of lysine influx proved to be independent of extracellular Na^+, and insensitive to metabolic inhibitors such as dinitrophenol, anaerobiosis and ouabain. Only after increased incubation times was it possible to show an inhibition of Na^+-independent lysine uptake by dinitrophenol or anaerobiosis, while ouabain remained non-inhibitory. The relative contribution of Na^+-independent lysine uptake also decreased as a function of incubation time even when no inhibitor was present. This decrease was still evident after correction for surface binding, but may be partially due to lysine entry by diffusion at the short time incubations. These findings suggest a relatively slow dissipation of the processes involved in energizing Na^+-independent lysine uptake. Na^+-independent lysine influx was reduced by ethacrynic acid, *N*-ethylmaleimide, ATP, arginine and the absence of Ca^{2+} and Mg^{2+}. Correction of lysine uptake by the estimated contribution of surface binding resulted in small quantitative changes in the absolute inhibitions produced, but did not influence the relative inhibition in this or the subsequent study[11]. Na^+-independent lysine influx was slightly reduced at pH 9.0, differentiating this pathway further from the Na^+-dependent influx which was increased at this pH. At pH 3.5 lysine influx was halved but was entirely Na^+ independent. Although no apparent unity of action can be derived from the effects of these diverse inhibitors, metabolites and incubation conditions, these results establish a characteristic pattern of response for Na^+-independent lysine uptake that can be used as a basis for subsequent comparative studies involving other Na^+-independent lysine uptake processes. Studies designed to determine whether the Na^+-independent component of lysine uptake can be attributed either to a Na^+-independent exchange transport[9], or to a flow of a cation down an electropotential gradient[8] will be the subject of the following paper[11].

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