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Lysine Transport across the Small Intestine. Stimulating and Inhibitory Effects of Neutral Amino Acids

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Summary. The ordinary aliphatic, neutral amino acids and phenylalanine have been examined for cis-inhibition of influx of alanine (J^{ala}_{mc}) and lysine (J^{lys}_{mc}) and trans-stimulation of J^{lys}_{mc} across the brush border membrane of rat small intestines; and their effects on the unidirectional mucosa-to-serosa flux (J^{lys}_{ms}) across the short circuited intestine have been studied. The effects of alanine, α-amino-*n*-butyric acid, leucine, and methionine on the steady-state epithelial uptake of lysine $[\text{Lys}]_c$ have also been measured. In addition the trans-effects of alanine and leucine have been examined for sodium-dependence, and alanine was tested as trans-stimulator of influx of galactose across the brush border membrane (J^{gal}_{mc}).

All the neutral amino acids were found to be competitive cis-inhibitors of J^{lys}_{mc}, and all, except isoleucine, were trans-stimulators of J^{lys}_{mc}. The magnitude of the trans-effect was unrelated to the efficiency of the amino acid as cis-inhibitor. As illustrated by alanine, the trans-effects are probably completely sodium-dependent. Alanine was also effective as trans-stimulator of J^{gal}_{mc}. With respect to effects on $[\text{Lys}]_c$ and J^{lys}_{ms} the neutral amino acids fall into two groups: One which reduces $[\text{Lys}]_c$ and stimulates J^{lys}_{ms}, and one which increases $[\text{Lys}]_c$ and relatively inhibits J^{lys}_{ms}. These effects are not correlated with the affinities of the neutral amino acids for the two carriers involved.

It is proposed that the trans-effects on J^{lys}_{mc} are induced by an electrogenic, sodium-coupled efflux of the neutral amino acid across the brush border membrane, that the stimulation of J^{lys}_{ms} is brought about by a selective stimulation (of unknown nature) of efflux of lysine across the basolateral membrane (J^{lys}_{cs}), assisted by competitive inhibition of lysine efflux across the brush border membrane (J^{lys}_{cm}), and that the amino acids which do not stimulate J^{lys}_{cs} increase $[\text{Lys}]_c$ by competitively inhibiting J^{lys}_{cs} and J^{lys}_{cm}.

The inhibitory effect of the neutral amino acids on J^{lys}_{mc} support the view that the carrier of basic amino acids serves as a second carrier of these amino acids.

Key words: Intestinal transport, small intestine, lysine, neutral amino acids.

The interactions between neutral (α-amino-monocarboxylic acids) and basic (diamino acids) during passage of the membranes of intestinal epithelia have been studied by sacs of everted intestine [7–9, 20], by rings of everted intestine [26, 28, 29], by isolated mucosa [16], and by isolated intestinal cells [22–25], by measurements of unidirectional transepithelial fluxes, and by measuring influx across the brush border membrane [16, 15]. In these different inhibitory and stimulating effects of neutral amino acids on the transport of basic amino acids have been observed. The results obtained with different techniques appear contradictory, and although different investigators get the same results when they use identical techniques [12, 28], the conclusions drawn differ markedly [16, 27].

This diversity of opinions invites further study. In addition, further insight into the mechanisms of epithelial transport might be gained if a more unifying set of observations were obtained. Such data would also be appropriate in view of the renewed interest in the question of multiplicity of the transport mechanisms serving intestinal uptake of neutral amino acids [14, 32]. Thus motivated, the relationships between transport by the rat small intestine of lysine and several neutral amino acids have been studied.

Some of the results of the present study have been presented in preliminary communications [12, 13].

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Materials and Methods

Materials

Male albino rats (150–175 g) were used for all the experiments. Before use, the rats were kept with free access to food and water. The rats were anesthetized by intraperitoneal injections of pentobarbital sodium, and the total small intestine was removed, whereupon the rats were killed. The mid 15–20 cm of the total small intestine were used for the measurement of both tissue uptake, transmural fluxes, and influx across the brush border membrane.

^{3}H-methoxy-inulin, ^{3}H-polyethyleneglycol (mol wt 4,000), ^{14}C-alanine, ^{14}C-lysine, ^{14}C-galactose, and ^{3}H-mannitol were purchased from New England Nuclear Co.

All chemicals were analytical grade. L-Amino acids and D-glucose or D-galactose were used throughout.

Methods

In all experiments the tissue was incubated in a Krebs-phosphate buffer whose composition was (in mM): Na, 140; K, 8; Ca, 2.6; Mg, 1; Cl, 140; phosphate, 8; SO_4, 1; pH 7.4. All experiments were made at 37 °C during aeration with pure oxygen. 5 mM glucose was present except when transport of galactose was measured.

Unidirectional transmural fluxes were measured by the Ussing-Zerahn technique used as previously described [11]. In all experiments four segments of tissue were used from each rat using approximately the middle 10 cm of the total small intestine. These four segments were studied simultaneously. J_{ms} and J_{sm} are used as symbols for mucosa to serosa and serosa to mucosa fluxes, respectively.

Steady-state epithelial uptake was measured using the isolated mucosal tissues prepared according to the description of Dickens and Weil-Malherbe [1]. The same part of the intestine was used as for measurements of transmural fluxes. The details were as previously described [11]. Uptake by the ring preparation was measured using 2–4 mm long ring-shaped segments. These rings were handled exactly as was the isolated mucosa.

Influx across the brush border membrane. (J_{mc}) was measured by the method described for rabbit ileum [31], with the modifications previously described [15]. The tissues were first incubated for 30 min (preincubation) under the conditions stated in text and tables. Subsequently, uptake of the appropriate radioactive tracers was accomplished during incubations of 0.4–0.6 min (test incubation) under the conditions specified in text and tables.

Symbols and conventions. In addition to flux symbols defined above, J_{cm} and J_{cs} stand for the flux from inside the epithelial cell to its respective mucosal and serosal sides. $[S]$ with subscript m, c or s indicates the concentration (mM) of substance S in the mucosal, cellular, or serosal compartment, respectively.

The radioactivity of the different samples was measured using Instagel as scintillation fluid.

Evaluation of experimental data. Statistical evaluation of the data is based on the t test [5]. All data are given as means ± 1 SE with the number of observations in brackets. P values by the paired t test below 0.05 are taken as evidence of statistical significance.

Results

Trans-Effects of Neutral Amino Acids on J_{mc}^{lys}

To examine for trans-effects on J_{mc}^{lys}, this flux was measured at 10 mM lysine +5 mM glucose. In paired experiments alternate mucosal areas were pre-incubated at 5 mM glucose and at 5 mM glucose +5 mM (or as stated in the tables) of a neutral amino acid. The test incubations were all 10 mM lysine +5 mM glucose and of a duration of 0.4–0.6 min.

The data of Table 1 show that, with the exception of isoleucine, all amino acids tested were effective as trans-accelerators of the influx of lysine. It is noted that alanine is the most potent accelerator. The data of Tables 1 and 2 demonstrate that relatively the trans-effects of leucine and methionine are the same and independent of the concentration at which pre-incubation took place.

Sodium-Dependence of Trans-Effects of Alanine and Leucine

To test for sodium dependence of the trans-effects of alanine and leucine on J_{mc}^{lys} pre- and test incubations were performed in sodium-free media. To facilitate the reduction of tissue sodium the incubation medium was renewed several times during the 30 min of pre-incubation. Alternate mucosal areas were pre-incubated at 5 mM glucose and 5 mM glucose +40 mM alanine or 40 mM leucine. The test incubations were

Table 1. Trans-effects of neutral amino acids on J_{mc}^{lys} (µmol/cm^2 · hr ± SE (n=8)

	−	+/−	+
N-leu	1.23±0.11	1.62±0.13	1.92±0.12
N-val	1.18±0.09	1.63±0.18	1.82±0.11
Isoleu	1.04±0.12	0.91±0.07	0.95±0.03
Met	1.22±0.05	1.55±0.10	1.86±0.10
Leu	0.82±0.12	1.63±0.21	1.21±0.12
Val	1.22±0.12	1.16±0.07	1.38±0.11
ABA	1.73±0.09	1.44±0.12	2.43±0.13
Ala	1.06±0.10	1.80±0.15	1.89±0.22
Φ-ala	0.88±0.11	1.50±0.15	1.21±0.15
Ser	1.19±0.10	1.23±0.09	1.42±0.09
Homoser	–	–	–
Threo	1.20±0.07	1.40±0.16	1.51±0.15
Gly	1.45±0.08	1.19±0.12	1.70±0.15
AIB	–	–	–

Influx of lysine across the brush border membrane measured after pre-incubation at 5 mM glucose (−) or 5 mM glucose +5 mM of the appropriate neutral amino acid (+). The ratio (+/−) for the pairs is also stated. Each value is the mean of 8 observations. The neutral amino acids are listed from above in the order of decreasing affinity for the carrier of neutral amino acids as indicated by their efficiencies as inhibitors of J_{mc}^{ala}.

Table 2. Trans-effects of neutral amino acids on J_{mc}^{lys} (µmol/cm²· hr ± SE (n))

Preincubation		Test incubation		J_{mc}^{lys} (µmol/cm²· hr ± SE)	
Experimental	Control	Experimental	Control	Experimental	Control
5 mM Ala	KP	10 mM Lys	10 mM Lys	1.89±0.22 (8)	1.06±0.10 (8)
10 mM Lys+5 mM Ala	10 mM Lys	10 mM Lys+5 mM Ala	10 mM Lys	1.43±0.06 (4)	1.44±0.10 (4)
2 mM Leu	KP	10 mM Lys	10 mM Lys	1.95±0.21 (8)	1.31±0.14 (8)
10 mM Lys+2 mM Leu	10 mM Lys	10 mM Lys+2 mM Leu	10 mM Lys	1.37±0.12 (8)	1.36±0.08 (8)
10 mM Leu	KP	10 mM Lys	10 mM Lys	1.50±0.16 (4)	1.00±0.07 (4)
10 mM Lys+10 mM Leu	10 mM Lys	10 mM Lys+10 mM Leu	10 mM Lys	0.67±0.09 (4)	1.39±0.10 (4)
KP	KP	10 mM Lys+10 mM Leu	10 mM Lys	0.66±0.08 (7)	1.01±0.04 (7)
2 mM Met	KP	10 mM Lys	10 mM Lys	3.05±0.10 (8)	2.11±0.13 (8)

The conditions of pre- and test incubations were as stated in the table

Table 3. Effect of neutral amino acids on J_{ms}^{lys} (µmol/cm²·hr ± SE (5)

		−	+	+/−
N-leu		0.44±0.02 (4)	0.54±0.03 (4)	1.21±0.04 (4)
N-val		0.42±0.02 (4)	0.59±0.04 (4)	1.40±0.08 (4)
Isoleu		0.45±0.05 (3)	0.63±0.04 (3)	1.41±0.08 (3)
Met		0.47±0.04 (3)	0.78±0.05 (3)	1.69±0.10 (3)
	2 mM	0.35±0.04 (8)	0.84±0.06 (7)	2.4
Leu	5 mM	0.40±0.05 (7)	0.85±0.11 (7)	2.16±0.15 (7)
	10 mM	0.32±0.04 (8)	0.51±0.04 (8)	1.6
Val		0.44±0.07 (3)	0.46±0.07 (3)	1.04±0.01 (3)
ABA		0.39±0.03 (4)	0.40±0.02 (4)	1.05±0.05 (4)
Ala		0.49±0.02 (6)	0.55±0.02 (6)	1.12±0.05 (6)
Φ-ala		0.51±0.02 (4)	0.47±0.03 (4)	0.92±0.03 (4)
	5 mM	0.50±0.05 (3)	0.60±0.04 (3)	1.22±0.06 (3)
Homoser	10 mM	0.35±0.03 (4)	0.54±0.05 (4)	1.50±0.06 (4)
Threo		0.31±0.05 (4)	0.36±0.06 (4)	1.14±0.04 (4)
Gly		0.37±0.002 (2)	0.39±0.004 (2)	1.06±0.01 (2)

Unidirectional mucosa to serosa flux of lysine measured at 5 mM glucose +10 mM lysine (−), and after the addition of 5 mM (or as stated) of the appropriate neutral amino acid to the bathing solutions (+). The ratio between the fluxes after and before adding the neutral amino acid is indicated by (+/−). 2 and 10 mM leucine were used in paired experiments where leucine was absent throughout the flux measurements or present from the beginning. In these experiments J_{sm}^{lys} was also measured and found to be (µmol/cm²· hr ± SE (n)) 0.31±0.03 (8) and 0.23±0.03 (8) without and with 2 mM leucine, respectively, and 0.23±0.03 (8) and 0.25±0.02 (8) without and with 10 mM leucine. The order of the neutral amino acids is as described in Table 1.

at 10 mM lysine +5 mM glucose and of 0.4–0.6 min duration. It was found that in the alanine experiments J_{mc}^{lys} was 1.081±0.091 µmol/cm²· hr ($n=29$) in the control preparations and 1.140±0.088 µmol/cm²· hr ($n=31$) in the alanine-loaded preparations. In the leucine experiments J_{mc}^{lys} was 1.164±0.064 µmol/cm²· hr ($n=32$) in the control experiments against 1.426±0.067 µmol/cm²· hr ($n=31$) in the leucine-loaded preparations. Therefore the trans-effect of alanine is clearly sodium-dependent, but that of leucine only partially dependent on sodium.

Trans-effect of Alanine on J_{mc}^{gal}

The sodium-dependence of the trans-effect of alanine on J_{mc}^{lys} suggested that the mechanism of this trans-effect could be a hyperpolarization of the brush border membrane by alanine-coupled efflux of sodium. To test this possibility, the trans-effect of alanine on J_{mc}^{gal} was examined. Alternate mucosal areas were preincubated with glucose-free buffer with or without 5 mM alanine for 30 min. The 0.4–0.6 min test incubations were at 1 mM galactose without glucose or

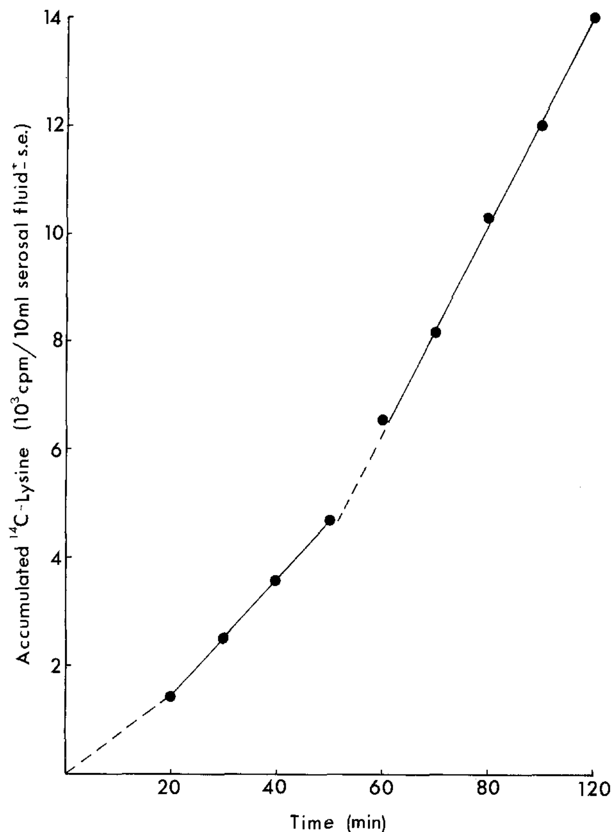

Fig. 1. Effect of 5 mM methionine on the unidirectional mucosa-to-serosa flux of lysine across the short circuited rat small intestine measured at 10 mM lysine +5 mM glucose. In the 50th min of incubation, 5 mM methionine was added to the bathing solutions. The dashed line between the 50- and 90-min observations represents a linear extrapolation of the right-hand limb of the curve. The slopes of the lines indicate fluxes of 0.52 and 0.89 μmol/cm^2·hr

amino acids. In the control preparations J_{mc}^{gal} was 0.303 ± 0.017 μmol/cm^2· hr ($n=12$), and in the alanine-loaded preparations J_{mc}^{gal} was significantly increased to 0.388 ± 0.017 μmol/cm^2· hr ($n=12$).

Simulation of the Conditions of J_{mc}^{lys} Prevailing under the Steady State of J_{ms}–J_{sm} Measurements

The magnitude of J_{ms}^{lys} is determined by its paracellular contribution and the fluxes J_{mc}, J_{cm}, and J_{sc} across the membranes of the epithelial cells [12]. Therefore, in order to locate the effects which lead to the leucine stimulation of J_{ms}^{lys}, it is a requirement that the steady-state values for J_{mc}^{lys} are measured with and without leucine present. This information is gained by measuring J_{mc}^{lys} after 30 min pre-incubation at the amino acid concentrations at which J_{ms}^{lys} and now J_{mc}^{lys} were measured. J_{mc}^{lys} was measured in paired experiments at 10 mM lysine+5 mM glucose and 10 mM lysine

Table 4. Steady-state tissue/medium distribution ratio of lysine

	−	+
10 mM Lys± 5 mM Met	2.83±0.12 (14)	2.10±0.10 (14)
10 mM Lys± 5 mM ABA	2.83±0.12 (14)	3.41±0.19 (14)
10 mM Lys± 5 mM Ala	1.75±0.08 (23)	2.41±0.12 (23)
10 mM Lys± 2 mM Leu[a]	3.30±0.24 (28)	2.11±0.20 (23)
10 mM Lys± 2 mM Leu[a] (Rings)	2.28±0.09 (19)	2.57±0.10 (24)
10 mM Lys±10 mM Leu[b]	2.14±0.10 (10)	1.75±0.08 (12)
1 mM Lys± 1 mM Leu[b]	9.73±0.83 (17)	7.18±0.64 (15)
1 mM Lys± mM Leu[b] (Rings)	11.62±0.72 (14)	14.55±0.72 (14)

Isolated mucosal tissues or rings of everted intestine were incubated for 40 to 80 min in paired experiments at 10 or 1 mM lysine with (+) or without (−) the neutral amino acid as stated in the Table.
[a] and [b] Vertical as well as horizontal pairing, otherwise the experimental series are not comparable.

+5 mM glucose +2 or 10 mM leucine, or 5 mM alanine after 30 min of pre-incubation with solutions of the same composition. It was found (Table 2) that the presence of 2 mM leucine or 5 mM alanine prevented the trans-effects on J_{mc}^{lys}, and that the presence of 10 mM leucine, which stimulates J_{ms} by 60%, even reduced J_{mc}^{lys} by 50%.

Effect of Leucine and Alanine on J_{ms}^{lys} and J_{sm}^{lys}

The effects of 2 and 10 mM leucine on J_{ms}^{lys} and J_{sm}^{lys} were measured in paired experiments where both unidirectional fluxes were measured at 10 mM lysine +5 mM glucose and 10 mM lysine +5 mM glucose +2 or 10 mM leucine.

The effect of 5 mM leucine or 5 mM alanine on J_{ms}^{lys} was examined by adding the appropriate amino acid to the incubation fluid immediately before taking the 70-min sample and continue the sampling for 50 min.

It was found (Table 3) that both 2 and 5 mM leucine doubled J_{ms}^{lys}, that this flux was increased by 60% by 10 mM leucine, but only by 13% by 5 mM alanine.

Effect of -Amino-Monocarboxylic Acids on J_{ms}^{lys}

The effects of several neutral amino acids on J_{ms}^{lys} (Table 3) were examined using the procedure described above for 5 mM leucine and alanine, except that the neutral amino acids were added after 50 min of incubation. Except for homoserine, which was used at 5 and 10 mM, the neutral amino acids were used at 5 mM. In all experiments, lysine and glucose were

present initially at 10 and 5 mM, respectively. It was found that all amino acids except α-amino-*n*-butyric acid (ABA), valine, and phenylalanine significantly increased J_{ms}^{lys}. Especially for leucine and methionine, the effect on J_{ms}^{lys} had a very rapid onset. This is illustrated by the experiment of Fig. 1.

Effect of α-Amino-Monocarboxylic Acids on [Lys]$_c$

In different series of paired experiments the steady-state mucosal uptake of lysine was measured at 10 mM lysine + 5 mM glucose + 2 mM leucine, 10 mM leucine or 5 mM alanine. It was found (Table 4) that both at 2 and at 10 mM leucine significantly reduced the lysine uptake, whereas alanine significantly enhanced it by 38%. These results suggested that the neutral amino acids, with respect to their effects on lysine transport, fall into two groups. One includes amino acids which stimulate J_{ms}^{lys} but reduce [Lys]$_c$ and one includes amino acids which increase [Lys]$_c$, but stimulates J_{ms}^{lys} less than corresponding to the effects on [Lys]$_c$. To test this possibility, the effects of 5 mM α-amino-*n*-butyric acid (ABA) and 5 mM methionine were examined, because this pair of amino acids had the desired effects on J_{ms}^{lys} and had very similar K_i values as cis-inhibitors of both lysine and alanine. In triplet experiments [Lys]$_c$ was measured at 10 mM lysine + 5 mM glucose and at 10 mM lysine + 5 mM glucose + 5 mM ABA or 5 mM methionine. In support of the grouping described above, it was found that in the control tissues, the ABA-, and the methionine-exposed tissues [Lys]$_c$ were 28.3 ± 1.2 (n = 14), and 34.1 ± 1.9 mM (n = 14), and 21.0 ± 1.0 mM (n = 14), respectively.

With the purpose of seeking an explanation for the differences between leucine stimulation of lysine uptake in rings of everted intestine [28] and leucine inhibition of this uptake by isolated mucosa [16], both types of preparation were prepared from the same rats and incubated in paired experiments at 1 mM lysine with or without 1 mM leucine and at 10 mM lysine with or without 2 mM leucine. In these experiments ^{3}H-methoxy-inulin was used as marker of the extracellular space. The results (Table 4) show, first, that leucine at both concentrations of lysine increased the steady-state uptake by the rings and decreased the uptake by the isolated mucosa, and, secondly, that at 1 mM lysine in the control-preparations the uptake is higher by the rings than by the isolated mucosa, whereas the opposite obtained at 10 mM lysine.

α-Amino-Monocarboxylic Acids as cis-inhibitors of J_{mc}^{lys} and J_{mc}^{ala}

In paired experiments J_{mc}^{lys} was measured at 1 mM lysine + 5 mM glucose + 0, 10 or 40 mM of the neutral amino acids listed in Table 5. From this table it is noted, first, that the order of affinity for the lysine transport mechanism is almost the same as often described for the neutral amino acids for their own transport system [21], and, secondly, it is seen that the estimates of K_i are the same whether these estimates are based on the inhibitory effects at 10 or at 40 mM. This agreement indicates fully competitive inhibition of lysine transport. However, it is seen that for alanine the K_i values are much higher than for the inhibitory effect of this amino acid against J_{mc}^{ala} (Table 6); in addition, methionine is a weaker inhibitor of J_{mc}^{lys} than leucine, while the opposite is true against J_{mc}^{ala}.

Previous experiments had shown that the affinity of alanine for the lysine carrier was very low (Table 5) and as the J_{max}^{lys} [14] is less than 10% of J_{max}^{ala} [13] the contribution by the lysine carrier to J_{mc}^{ala} at 1 mM alanine would be negligible. In previous experiments it had been impossible to demonstrate transport of alanine by the imino acid carrier [13]. On this background alanine was chosen to represent the neutral amino acids, and J_{mc}^{ala} was measured at 1 mM alanine + 5 mM glucose + 0, 10 or 40 mM of the competing amino acid, except in the case of α-amino-isobutyric acid (AIB) where 40 and 80 mM were used. The results of these experiments are shown in Table 6. It appeared that for all the more effective inhibitors of J_{mc}^{ala} the estimated K_i values were significantly higher at 40 than at 10 mM. There are at least three possible explanations for this phenomenon of apparently partially competitive inhibition: (i) A significant contribution to J_{mc}^{ala} by a process of diffusion for which the high MW PEG-4000 did not correct; (ii) a high degree of inhibition by depolarization of the electrical potential difference across the brush border membrane; (iii) in spite of previous results [13] alanine might to a significant degree move on the carrier of imino acids. The first possibility was tested in a series of experiments where ^{3}H-mannitol was used to correct for extracellular contamination, and J_{mc}^{ala} was measured at 1 mM alanine + 0, 10 or 40 mM leucine. In these experiments the fluxes were 0.92 ± 0.05 (n = 7), 0.27 ± 0.03 (n = 7), 0.16 ± 0.01 μmol/cm^2· hr (n = 7), respectively, corresponding to K_i values of 3.8 mM at 10 mM leucine and 7.7 mM at 40 mM leucine; neither fluxes nor K_i estimates to differ markedly from the results obtained using ^{3}H-PEG-4000 as tracer of extracellular contamination (Table 6). The second possibility was examined by assuming that in the latter series of experiments both 10 and 40 mM leucine, by depolarizing the luminal membrane, caused an additional 20% inhibition of J_{mc}^{ala}. This correction gives fluxes of 0.92, 0.32, and 0.19 μmol/cm^2· hr which correspond to the K_i values, 5.0 and

Table 5. Cis-inhibition of J_{mc}^{lys} by neutral amino acids

	0	K_i^{10}	10	K_i^{40}	40
N-leu	0.50±0.04 (6)	6.3	0.32±0.03 (5)	5.0	0.07±0.01 (5)
N-val	0.60±0.07 (6)	19.8	0.44±0.08 (5)	12.0	0.17±0.02 (5)
Isoleu	0.43±0.04 (6)	12.0	0.26±0.03 (5)	15.2	0.14±0.01 (5)
Met (0–10–20)	0.50±0.01 (4)	12.2	0.31±0.01 (4)	11.2	0.18±0.10 (4)
Leu (0–10–20)	0.62±0.07 (8)	4.7	0.24±0.02 (7)	4.4	0.14±0.01 (7)
Val	0.40±0.10 (5)	52.4	0.35±0.08 (5)	32.8	0.21±0.06 (5)
ABA	0.42±0.08 (5)	15	0.26±0.05 (5)	12.5	0.12±0.02 (5)
Ala (0–15–30)	0.63±0.05 (8)	168.5	0.59±0.08 (4)	120.2	0.53±0.04 (4)
Φ-ala	0.46±0.12 (4)	7.4	0.23±0.08 (4)	8.6	0.10±0.02 (4)
Ser	0.43±0.05 (5)	25.1	0.33±0.04 (5)	36.7	0.24±0.02 (5)
Homoser	0.55±0.10 (5)	31.0	0.45±0.12 (5)	34.9	0.30±0.10 (5)
Threo	0.43±0.05 (3)	44.0	0.30±0.06 (4)	48.0	0.21±0.01 (4)
Gly	0.34±0.03 (5)	99.6	0.32±0.02 (5)	134	0.28±0.03 (5)
AIB	0.47±0.03 (4)	92.4	0.44±0.05 (5)	35.3	0.26±0.06 (5)

J_{mc}^{lys} was measured in paired experiments at 1 mM lysine +0 (0), 10 (10), or 40 (40) mM of the appropriate neutral amino acid except for methionine, leucine, and alanine where the concentrations used are stated in parentheses. The K_i values are calculated from mean values for J_{mc}^{lys} using the equation $J^o/J^i=(K_t+[S])/(K_t+[S]+K_t\cdot[I]/K_i)$, assuming a K_t for lysine of 3 mM [14]. The order of the neutral amino acids is as described in Table 1.

Table 6. Cis-inhibition of J_{mc}^{ala} by neutral amino acids

	0	K_i^{10}	K_i^{40}	40	
N-leu	1.04±0.14 (6)	2.8±0.4(6)/1.9	0.22±0.02 (6)	7.4± 1.4(6)/3.0	0.15±0.02 (6)
N-val	0.98±0.14 (5)	4.0±0.4(4)/3.2	0.28±0.01 (5)	7.2± 0.8(4)/3.1	0.15±0.02 (5)
Isoleu	1.09±0.14 (5)	5.6±1.4(4)/4.8	0.37±0.08 (5)	15.1± 4.9(4)/6.6	0.22±0.03 (5)
Met	0.72±0.07 (4)	6.1±0.7(3)/4.9	0.27±0.04 (4)	11.9± 1.4(3)/8.5	0.19±0.04 (4)
Leu	1.00±0.08 (6)	5.5±1.2(4)/5.8	0.37±0.04 (5)	8.6± 2.0(4)/6.0	0.20±0.03 (5)
Val	0.74±0.13 (6)	7.7±0.6(4)/5.2	0.29±0.08 (5)	15.9± 7.0(4)/4.9	0.16±0.03 (4)
ABA	1.11±0.07 (6)	6.5±0.3(4)/7.0	0.45±0.02 (5)	9.5± 0.4(4)/6.7	0.22±0.02 (5)
Ala (0–9–39)	1.56±0.14 (7)	17.0	1.04±0.12 (7)	17.4	0.50±0.03 (8)
Φ-ala	1.10±0.08 (6)	16.0±1.8(4)/18.1	0.64±0.03 (5)	18.9± 1.9(4)/16.6	0.35±0.02 (5)
Ser	1.20±0.10 (6)	12.7±1.7(4)/20.8	0.73±0.06 (5)	30.1± 5.3(4)/34.6	0.54±0.02 (5)
Homoser	–	–	–	–	–
Threo	1-09±0.16 (5)	30.0±8.5(4)/45.4	0.78±0.00 (5)	38 ± 7(4)/38	0.50±0.04 (5)
Gly (0–80)	1.43±0.17 (8)	–	–	127 ±17(4)	0.84±0.06 (8)
AIB (0–40–80)	1.00±0.06 (5)	186.7±73.5(3)	0.81±0.07 (5)	154 ±38 (3)	0.63±0.05 (5)

J_{mc}^{ala} was measured at 1 mM alanine +0 (0), 10 (10), or 40 (40) mM (or as stated in parentheses) of the appropriate neutral amino acid. The "0", "10", and "40" measurements were for each inhibitor concentration distributed in 3 or 4 pairs within which the k_i values were calculated as described in Table 5, using a k_t for alanine of 20 mM [13]. Except for glycine, alanine and AIB which have k_i values against imino acids of, respectively, 37±7, 23±4, and 78±23 (B.G. Munck & S.N. Rasmussen, *unpublished results*) the k_i values were recalculated as described in the text and stated as the right-hand member of the pairs of K_i values. The order of the neutral amino acids is as described in Table 1.

9.1 mM. The third possibility was tested by measuring J_{mc}^{ala} at 1 mM alanine +5 mM glucose +40 mM leucine with or without 40 mM β-alanine. In these experiments β-alanine reduced J_{mc}^{ala} from 0.16±0.02 ($n=12$) to 0.08±0.010 ($n=12$) μmol/cm$^2\cdot$ hr, indicating that the reason for the apparently, partially competitive inhibition of J_{mc}^{ala} by the neutral amino acids must be that alanine is also transported by the imino acid carrier. Previous results [13] have shown that 20 mM is a good estimate of K_t for β-alanine for the imino acid carrier, indicating that in the present experiments this carrier contributed 0.1 μmol/cm$^2\cdot$ hr to J_{mc}^{ala}. Corrected for this contribution and then for the electrongenic effect of leucine the fluxes measured with ^{3}H-mannitol as tracer become: 0.82, 0.21, and 0.07 μmol/cm$^2\cdot$ hr which correspond to K_i values of 3.1 and 3.3 mM at 10 and 40 mM leucine, respectively. These results indicate that the relative affinities of

these amino acids for the carrier of neutral amino acids are best judged on the basis of the K_i values calculated from the flux data corrected as described.

Discussion

Confirming and supplementing previously reported observations [16, 22, 26, 28] it is here demonstrated that the stimulating effects of neutral amino acids on the epithelial transport of basic amino acids belong in two categories: one characterized by markedly increased transepithelial net transport and reduced steady-state uptake of the transported basic amino acid, and one characterized by a significantly increased steady-state uptake and none, or only a small increment, of the transepithelial net transport.

In the steady state of transepithelial transport of lysine, all the neutral amino acids tested inhibit J_{cm}^{lys}. Otherwise an increased net flux could not be maintained under the conditions of either unchanged or reduced J_{mc}^{lys}, nor could an increased steady-state epithelial uptake be maintained under the conditions of unchanged or reduced J_{mc}^{lys} and unchanged or slightly increased J_{ms}^{lys}. In the cases of alanine and ABA the steady-state uptake of lysine was increased relatively more than J_{ms}^{lys}, also when this flux is corrected for a paracellular contribution [15]. These observations indicate a possibly competitive inhibition by neutral amino acids of lysine efflux across the basolateral membrane. Leucine and methionine may have a similar effect which then is overshadowed by a parallel, enhancing effect on this step of lysine transport. Three compartment analyses of the data of leucine-lysine interactions in rabbit ileum [16] and rat jejunum [13] suggested an increase in the mobility of a carrier and its lysine-complex or a changed intracellular compartmentation of lysine as possible mechanisms of the increased J_{ms}^{lys}. A changed compartmentation could be effected by competitive exclusion of lysine from the intracellular organelles. However, one would expect ABA and leucine to be equally effective in this respect; the data therefore favor the first interpretation.

In agreement with previous observations on rabbit ileum [16] and rat jejunum [14], the neutral amino acids all proved to be regular competitive inhibitors of J_{mc}^{lys}. Cis-stimulation was never observed. As outlined below these observations suggest that the results on rings of everted intestine which have been interpreted in terms of cis-stimulation of J_{mc}^{lys} [27] can rightly be assumed to represent a subepithelial conservation of the leucine- or methionine-induced increments of J_{ms}^{lys}.

The data of Table 2 and a comparison of data of Tables 1 and 3 demonstrate that the trans-stimulation of J_{mc}^{lys} is unrelated to the stimulation of J_{ms}^{lys}. This phenomenon was first observed with leucine [16] which is a high affinity cis-inhibitor of J_{mc}^{lys}. Assuming symmetric carrier systems, it could therefore be explained as an example of accelerative exchange-diffusion, and consistent with this interpretation it was found to persist when preloading and influx measurement were both performed in sodium-free media. However, as confirmed by the present study (Table 1) alanine was found to be the most potent trans-stimulant of J_{mc}^{lys} [22], although it is a very poor cis-inhibitor of this flux (Table 5). This discrepancy indicated that other mechanisms were involved in the trans-effects on J_{mc}^{lys}. The present results which show that all the neutral amino acids tested, except isoleucine, were effective trans-stimulators strengthened this view. The observations of electrogenic, sodium-coupled influx of sugars and amino acids across the brush border membrane [30, 33], the stimulation of sodium-coupled uptake of sugars by brush border microvesicles preloaded with sugars and sodium [17], and the observation of electrical hyperpolarization of the Ehrlich ascites tumor cell under the net efflux of amino acids [4] as well as the apparently proton coupled trans-stimulation by galactoside of proline uptake by *Escherichia coli* [2] point to an electrogenic mechanism as essential for the trans-effect on J_{mc}^{lys}. The present observation of almost complete sodium-dependence of the trans-effect of alanine and that of a trans-effect of this amino acid on J_{mc}^{gal} provides strong support for the view that at least some of the effective amino acids primarily accelerate J_{mc}^{lys} through an electrical hyperpolarization of the brush border membrane induced by their sodium-coupled efflux across this membrane. Leucine appears to have a partly sodium-independent trans-effect. However, the cytoplasm is unlikely to be sodium-free, and leucine has a high affinity for its own carrier; it is therefore conceivable that also the remaining trans-effect of leucine is sodium-dependent and electrogenic. Finally an electrogenic mechanism would also tie together the observations that alanine, although it has the highest K_i against J_{mc}^{lys}, is the most effective trans-stimulant (Table 1) and the amino acid with the highest J_{max} across the brush border membrane observed so far [13].

The present results on the sodium-dependence of transeffects on J_{mc}^{lys} contradict earlier reports [22, 24, 25]; but this difference is explained by the fact that in the latter studies only the test-incubations were sodium-free media, while the preloadings were at normal sodium concentrations.

At 1 mM lysine the transepithelial net flux of lysine is higher than at 10 mM [15]. Therefore the subepithelial space-incubation fluid concentration difference is highest at 1 mM lysine. In the everted ring

which forms a ring-shaped everted sac [34], this difference must be enhanced, especially when the net transport of lysine is stimulated by leucine. The data of Table 4 confirm the conflict between data obtained for isolated mucosa and rings of everted intestine [16, 28]. The different relative uptakes at 1 and at 10 mM lysine are consistent with the above evaluation of the ring-preparation. Therefore these observations strongly suggest that the different relative tissue uptakes observed with the two techniques will be accounted for by the necessary accumulation in the subepithelial space and the serosal space of the everted rings. The time course of the effect of methionine (Fig. 1) or leucine [6*a*.] demonstrate that this artefact suffices to explain why methionine or leucine appears to stimulate the initial rate of epithelial uptake of lysine, when the parameter is judged by 2 min uptake in rings of everted intestine [27]. The discrepancy between the results obtained with the presently used techniques and those with rings of everted intestine can, as outlined above, be explained without being too speculative. The situation is somewhat different with respect to the difference from results obtained with isolated enterocytes from rat small intestine. With this technique [22] several neutral amino acids, leucine included, increased the 2 to 15 min uptake of lysine. In interpreting these results it is important to bear in mind that they originate from some of the first successful applications of this technique. The reports clearly showed: first, that the isolated enterocyte had a very short period of reasonably well sustained function [21] (already after 10–15 min of incubation the cellular accumulation was declining); secondly, even at their maximum the degrees of accumulation were much lower than the stable values reached by the isolated mucosa [15]. Thus the isolated enterocyte was a very leaky cell in which the presence of leucine or methionine possibly would not significantly further increase the efflux of lysine. In this case the inhibitory effect of intracellular neutral amino acids on J_{cm} and/or J_{cs} would become manifest. The present data (Table 5) supplement previous data on the inhibitory effect of neutral amino acids on J_{mc}^{lys} [14]. The order of affinity is in its details different from that indicated by ability of the same amino acid to inhibit J_{mc}^{ala}. This difference confirms the previous conclusion that lysine is not transported by the principal carrier of neutral amino acids [14]. The similarity of the K_i estimates at 10 and at 40 mM of the inhibitor indicates a simple competitive inhibition. This interpretation is consistent with the proposal that neutral amino acids may be transported by the carrier of basic amino acids, for which direct evidence has been reported for leucine [10, 16], alanine [23], and valine [20].

The duality in leucine transport by rabbit ileum [16] has recently been confirmed and extended to several neutral amino acids [32]. The previous study [16, 14] and the present results indicate that the second carrier of neutral amino acids may be that of the basic amino acids.

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