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INTERACTIONS BETWEEN LEUCINE AND LYSINE TRANSPORT IN RABBIT ILEUM

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SUMMARY

1. The presence of 2 mM leucine in the mucosal solution markedly stimulates the rate of net lysine transport across short-circuited segments of rabbit ileum. This stimulatory effect is due entirely to an increase in the unidirectional flux of lysine from mucosa to serosa; the flux from serosa to mucosa is unaffected by leucine.

2. High concentrations of lysine inhibit leucine transport across the brush border, and high concentrations of leucine similarly inhibit lysine transport. These observations suggest that the processes mediating the transport of these two amino acids across the brush border have overlapping specificities.

3. The stimulatory effect of leucine on lysine transport is due, primarily, to an increase in the unidirectional flux of lysine out of the cell across the serosal and/or lateral membranes. This phenomenon cannot be readily explained in terms of known carrier models and suggests a direct noncompetitive interaction between leucine and a carrier mechanism responsible for lysine movement across the serosal boundary of the epithelium.

4. Preloading the intestinal tissue with leucine alone markedly enhances the unidirectional influx of lysine from the mucosal solution across the brush border. Since preloading with lysine has no effect on lysine influx and preloading with leucine has no effect on leucine influx, this phenomenon appears to be another indication of an effect of leucine on the lysine transport mechanism.

INTRODUCTION

In recent years it has become apparent that the earlier concept that two distinct transport systems are responsible for neutral and basic amino acid transport by animal cells is no longer entirely valid. Evidence that the transport of neutral and basic amino acids are mediated by transport mechanisms with overlapping specificities for these two groups of amino acids has been presented for kidney[1], small intestine[2–4] and Ehrlich ascites tumor cells[5]. In most instances, the evidence for interactions between basic and neutral amino acids consists of the demonstration that the transport of an amino acid from one group is inhibited by an amino acid from the other group and *vice versa*. Recently, however, ROBINSON AND FELBER[2] and MUNCK[4] have reported an interaction between basic and neutral amino acids in small intestine that is characterized by the ability of the neutral amino acid to stimulate the

transport of the basic amino acid. Thus, MUNCK[4] has shown that the rate of lysine transport across everted sacs of rat ileum is markedly enhanced by the presence of a low concentration of leucine in the mucosal medium. Although these studies provide conclusive evidence for some interaction between leucine and the transmural transport of lysine, it is clear that studies of transmural transport alone cannot provide detailed insight into the mechanisms of this interaction. The rate of net transmural transport is a complex function of unidirectional flows across both the mucosal and serosal membranes of the intestinal cell, therefore it is necessary to localize the primary effects of leucine on lysine transport in order to further clarify their interaction.

The purpose of the present investigation was to examine the interaction between leucine and lysine transport across isolated rabbit ileum using techniques that permit an analysis of the events occurring at the mucosal and serosal boundaries of the tissue.

METHODS

Male and female New Zealand white rabbits, that had been maintained on normal food intake, were sacrificed by intravenous injection of pentobarbital. A section of distal ileum was removed, opened along the mesenteric border and rinsed free of intestinal contents using normal buffer.

The methods and apparatus employed for the determination of (a) transmural fluxes of leucine and lysine across short-circuited segments of the tissue[6,7]; (b) unidirectional influxes of leucine and lysine from the mucosal solution across the brush border and into the epithelium[8]; and, (c) intracellular accumulation of leucine and lysine by mucosal strips* of rabbit ileum[9], have been described in detail previously.

Unless otherwise indicated, the composition of the buffer used for washing, perfusion and incubation in each of the above procedures was: NaCl, 140 mM; $KHCO_3$, 10 mM; K_2HPO_4, 1.2 mM; KH_2PO_4, 0.2 mM; $CaCl_2$, 1.2 mM and $MgCl_2$, 1.2 mM. The gas mixture employed was O_2–CO_2 (95:5, v/v) and the pH of the buffer at 37° was between 7.0 and 7.2. All amino acids were in the L form and were obtained from Sigma Chemical (grade A). L-[^{14}C]Lysine, L-[^{14}C]leucine and [^{3}H]inulin were obtained from New England Nuclear (Boston, Mass.).

RESULTS

Transmural fluxes of leucine and lysine

The unidirectional transmural fluxes from mucosa-to-serosa (J_{ms}) and serosa-to-mucosa (J_{sm}), and the net fluxes (J_{net}) of lysine and leucine across short-circuited segments of rabbit ileum are given in Table I. In the experiments in which 10 mM lysine or 2 mM leucine were present alone, the concentrations of these amino acids in the mucosal and serosal bathing solutions were identical. As is shown in Table I, when both surfaces of the tissue are perfused with normal buffer containing 10 mM lysine, J_{ms} slightly exceeds J_{sm} and there is a small but significant net flux from mucosa to serosa in the absence of an electrochemical potential difference for lysine. When both bathing solutions contain 2 mM leucine, J_{ms} is much greater than J_{sm} and there is a highly significant net flux from mucosa to serosa. When the mucosal

* The mucosal strip consists of the epithelial cell layer, the lamina propria and a portion of the muscularis mucosa. The submucosa and muscle layers are removed as described previously[9].

TABLE I

TRANSMURAL FLUXES OF LYSINE AND LEUCINE ACROSS SHORT-CIRCUITED RABBIT ILEUM

All fluxes are in units of μmoles/h·cm^2 ± S.E. Each experiment involved 3–5 sampling periods.

	Number of experiments	J_{ms}	*Number of experiments*	J_{sm}	J_{net}
Lysine fluxes					
10 mM lysine	7	0.30 ± 0.05	5	0.20 ± 0.03	0.10 ± 0.06
10 mM lysine + 2 mM leucine	7	0.60 ± 0.11	4	0.20 ± 0.03	0.40 ± 0.12
Leucine fluxes					
2 mM leucine	3	0.53 ± 0.05	5	0.03 ± 0.00	0.50 ± 0.05
2 mM leucine + 10 mM lysine	3	0.15 ± 0.05	5	0.05 ± 0.01	0.10 ± 0.05

and serosal solutions contain 10 mM lysine *plus* 2 mM leucine, the J_{ms} of lysine is doubled whereas the J_{sm} is unaffected. This increase in J_{ms} results in a 4-fold increase in the rate of net lysine transport from mucosa to serosa, an effect which is in excellent agreement with the 3–4-fold increase in the rate of lysine transport across everted sacs of rat ileum reported by MUNCK[4]. Further, leucine need only be added to the mucosal solution in order to elicit this stimulation of lysine transport; the presence of leucine in the serosal solution alone is ineffective. The effect of 2 mM leucine on the J_{ms} and J_{sm} of lysine after steady-state unidirectional fluxes of lysine had been achieved is shown in Fig. 1. As can be seen, the addition of leucine to the mucosal solution (arrow) brings about a 2-fold increase in J_{ms} after a lag period of approximately 10 min; J_{sm} is unaffected.

As is also shown in Table I, 10 mM lysine significantly inhibits the net transmural flux of leucine. This effect is the result of a marked reduction in the J_{ms} of leucine as well as a small increase in the J_{sm} of leucine. This inhibitory effect of lysine on transmural leucine transport was not observed in the case of rat ileum[4], but has been reported for uptake of leucine by everted rings of rat small intestine[10].

Unidirectional influxes of lysine and leucine

The unidirectional influxes of leucine from the mucosal solution into the epithelium (J_{mc}^{Leu}) are given as a function of the leucine concentration in the mucosal medium in Table II and are plotted in Fig. 2. These data cannot be described by a single saturable process that conforms to Michaelis–Menten kinetics, however, they can be adequately described by two saturable processes that operate in parallel. The following equation, which is represented by the curve in Fig. 2, appears to adequately describe the concentration dependence of J_{mc}^{Leu}

$$J_{mc}^{Leu} = [Leu]_m \left[\frac{3.0}{0.3 + [Leu]_m} + \frac{4.0}{6.0 + [Leu]_m} \right] \quad (1)$$

where $[Leu]_m$ is the leucine concentration in the mucosal medium.* It should be

* Eqn. 1 does not provide a unique fit to the experimental data, and other parameters can be chosen that provide an equally adequate description of the influx process. Thus, the maximal velocities and half-saturation concentrations given in Eqn. 1 are subject to some uncertainty.

noted that is previously reported studies, leucine influx across the brush border of rabbit ileum appeared to describe a single saturable process with a maximum influx of 6.3 ± 1.2 (S.E.)μmoles/h·cm^2 and a K_t (the leucine concentration at which influx

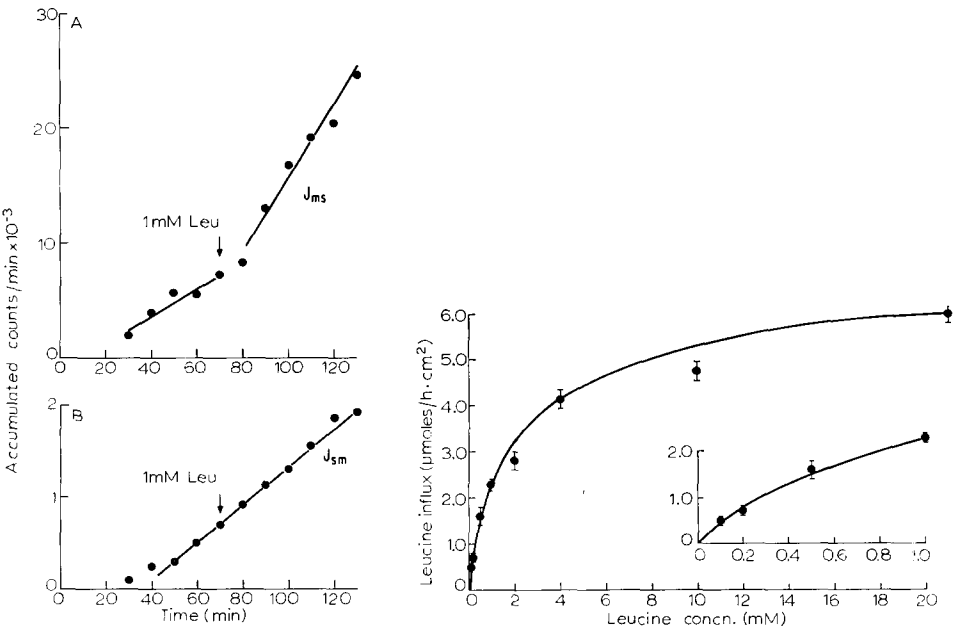

Fig. 1. Effect of 1 mM leucine in the mucosal solution on the J_{ms} (A) and J_{sm} (B) of lysine. Leucine was added to the mucosal solution (arrows) after steady-state transmural lysine fluxes were achieved; steady-state fluxes are achieved between 30 and 40 min after the addition of [^{14}C]lysine to the mucosal or serosal solutions (zero time).

Fig. 2. Leucine influx (J_{mc}^{Leu}) as a function of the leucine concentration in the mucosal medium. The inset simply expands the concentration range of 0–1.0 mM. The curve is calculated from Eqn. 1. The parameters given in Eqn. 1 adequately describe leucine influx over a wide concentration range. All errors are S.E.

TABLE II

UNIDIRECTIONAL LEUCINE INFLUX

The mucosal solution for both preincubation and influx determination consisted of either normal buffer containing 140 mM Na or a Na-free buffer in which NaCl was replaced by choline chloride.

Leucine concn. (mM)	*Number of influx determinations*	J_{mc}^{Leu} (μmoles/h·cm^2) *Na$^+$ (140 mM)*	*Na$^+$ (0)*
0.1	4	0.50 ± 0.06	0.10 ± 0.02
0.2	4	0.72 ± 0.04	0.18 ± 0.02
0.5	4	1.59 ± 0.19	0.52 ± 0.07
1.0	4	2.30 ± 0.08	0.89 ± 0.19
2.0	8	2.79 ± 0.18	—
4.0	8	4.00 ± 0.20	
10.0	3	4.74 ± 0.18	—
20.0	3	5.96 ± 0.16	—

is half-maximal) of 4.2 mM (ref. 11). These data were obtained over a leucine concentration range of 3.3–20 mM. It is clear from Table II and Fig. 2, that examination of leucine influx at lower concentrations discloses a second saturable process. This does not significantly affect the previously reported maximum influx, however, the previously reported K_t is intermediate between the K_t's of the two saturable processes described by Eqn. 1. This points out the importance of examining influx kinetics over a wide concentration range and reenforces the urgings of CHRISTENSEN[12] with respect to the analysis and interpretation of kinetic data.

The kinetics of lysine influx (J_{mc}^{Lys}) as a function of lysine concentration in the mucosal medium ($[Lys]_m$) have been described previously[7]. Suffice it to say that lysine influx can also be described by means of 2 saturable processes operating in parallel and is given by the following equation

$$J_{mc}^{Lys} = [Lys]_m \left[\frac{0.4}{0.3 + [Lys]_m} + \frac{4.6}{10.0 + [Lys]_m}\right] \quad (2)$$

Studies of lysine influx from a medium rendered Na^+-free by replacement with choline indicated that the first of these saturable influx processes (maximum influx = 0.4 μmoles/h·cm^2) is unaffected by Na^+ and that only the influx process characterized by the maximal influx of 4.6 μmoles/h·cm^2 is Na^+-dependent[7]. We have previously demonstrated that leucine influx from a medium containing 3.3–20 mM leucine is markedly inhibited in the absence of Na^+ (ref. 11). In view of the present results, disclosing a second saturable influx process that predominates at low leucine concentrations, it seemed important to determine whether this influx process is influenced by Na or whether, as for the case of lysine, it is unaffected by Na^+. As shown in Table II, leucine influx at low leucine concentrations is markedly inhibited by removal of Na^+ from the mucosal medium. From these and previous[11] results, we conclude that both leucine influx processes are dependent upon Na^+.

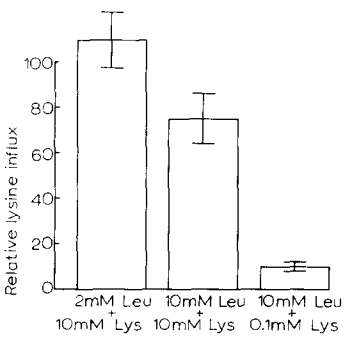

Fig. 3. The effect of leucine on lysine influx at various leucine and lysine concentrations expressed as percent of control (*i.e.* lysine influx in the absence of leucine is assigned a value of 100). Errors are S.E.

The effect of leucine on lysine influx is shown in Fig. 3. These data were obtained using paired tissues from the same animal. In one of the paired tissues lysine influx was determined in the absence of leucine (control) and in the other tissue lysine influx was determined with either 2 mM or 10 mM leucine in the mucosal solution. It is seen that 2 mM leucine does not affect J_{mc}^{Lys} from a solution containing 10 mM lysine. On

the other hand, 10 mM leucine significantly inhibits J_{mc}^{Lys} from a solution containing 10 mM lysine, and lysine influx from a solution containing 0.1 mM lysine and 10 mM leucine is only 10% of that observed from a solution containing 0.1 mM lysine alone. Thus, lysine influx is inhibited by relatively high concentrations of leucine but is not affected detectably when the mucosal solution contains 10 mM lysine and only 2 mM leucine.

The effects of various preincubation conditions on lysine influx are given in Table III. In these experiments, the paired control tissues were preincubated for 30 min in either the normal buffer (Na^+–Ringer) or a buffer rendered Na^+-free by replacement of NaCl with choline–chloride (choline–Ringer). The paired test tissue contained, in addition to the indicated buffer, either lysine, leucine or a combination of these amino acids at the concentrations shown. It is seen that lysine influx is not affected by preincubation of the tissue in the presence of 4 mM lysine, or 10 mM

TABLE III

EFFECT OF PREINCUBATION CONDITIONS ON UNIDIRECTIONAL LYSINE INFLUX

Numbers in parentheses indicate number of influx determinations. All errors are S.E. The details are given in the text.

Preincubation medium		*Influx medium*		J_{mc}^{Lys} ($\mu moles/h \cdot cm^2$)	
Control	*Test*	*Control*	*Test*	*Control*	*Test*
Na^+–Ringer	Na^+–Ringer + 4 mM Lys	Na^+–Ringer + 10 mM Lys	Na^+–Ringer + 10 mM Lys	2.7 ± 0.3 (4)	2.7 ± 0.5 (4)
Na^+–Ringer	Na^+–Ringer + 10 mM Lys + 2 mM Leu	Na^+–Ringer + 10 mM Lys	Na^+–Ringer + 10 mM Lys + 2 mM Leu	1.9 ± 0.1 (14)	2.0 ± 0.1 (14)
Na^+–Ringer	Na^+–Ringer + 2 mM Leu	Na^+–Ringer + 10 mM Lys	Na^+–Ringer + 10 mM Lys	2.9 ± 0.3 (15)	3.8 ± 0.2 (16)
Choline–Ringer	Choline–Ringer + 30 mM Leu	Choline–Ringer + 10 mM Lys	Choline–Ringer + 10 mM Lys	1.5 ± 0.1 (4)	2.8 ± 0.1 (4)

TABLE IV

EFFECT OF PREINCUBATION CONDITIONS ON UNIDIRECTIONAL LEUCINE INFLUX

Numbers in parentheses indicate number of influx determinations. All errors are S.E. Experimental details are given in the text.

Preincubation medium		*Influx medium*		J_{mc}^{Leu} ($\mu moles/h \cdot cm^2$)	
Control	*Test*	*Control*	*Test*	*Control*	*Test*
Na^+–Ringer	Na^+–Ringer	Na^+–Ringer + 2 mM Leu	Na^+–Ringer + 2 mM Leu + 10 mM Lys	2.8 ± 0.2 (8)	2.1 ± 0.1 (8)
Na^+–Ringer	Na^+–Ringer + 10 mM Lys	Na^+–Ringer + 2 mM Leu	Na^+–Ringer + 2 mM Leu	2.6 ± 0.2 (4)	2.3 ± 0.1 (4)
Na^+–Ringer	Na^+–Ringer + 2 mM Leu	Na^+–Ringer + 4 mM Leu	Na^+–Ringer + 4 mM Leu	4.2 ± 0.2 (4)	3.9 ± 0.2 (4)

lysine *plus* 2 mM leucine. These data support the previously reported observation[7] that lysine influx is not subject to exchange diffusion or a transconcentration effect at the concentrations studied. However, preincubation of the tissue with 2 mM leucine alone results in a highly significant stimulation of lysine influx (row 3). Further, this effect is not dependent upon the presence of Na^+ in the mucosal medium. As shown in row 4 of Table III, preincubation of the tissue with choline–Ringer containing 30 mM leucine markedly enhances lysine influx from a Na^+-free mucosal medium*.

The effects of lysine and of various preincubation conditions on leucine influx are given in Table IV. The presence of 10 mM lysine in the mucosal solution results in a 25% inhibition of J_{mc}^{Leu} from a medium containing 2 mM leucine. On the other hand, preincubation of the tissue for 30 min with either 10 mM lysine or 2 mM leucine does not affect leucine influx.

The data shown in Fig. 3 and Tables III and IV can be summarized as follows: (a) relatively high concentrations of either leucine or lysine inhibit the influx of the other, however, lysine influx from a solution containing 10 mM lysine is not affected by the presence of 2 mM leucine; (b) preloading the tissue with lysine does not affect lysine influx nor does preloading the tissue with either leucine or lysine affect leucine influx; (c) preloading the tissue with leucine alone enhances lysine influx, however, preloading with leucine *plus* lysine has no effect upon lysine influx.

TABLE V

ACCUMULATION OF LYSINE AND LEUCINE BY MUCOSAL STRIPS OF RABBIT ILEUM

The extracellular amino acid concentrations are the final steady-state values; the initial concentrations of lysine and leucine were 10 mM and 2 mM, respectively. Numbers in parentheses indicate number of mucosal strips and the errors are S.E.

Extracellular amino acid concn. (mM)	*Steady-state intracellular concentration*	
	Lysine (mM)	*Leucine (mM)*
9 mM lysine	57 ± 3 (16)	—
1.5 mM leucine	—	23 ± 2 (8)
9 mM lysine + 1.8 mM leucine	50 ± 3 (16)	9.7 ± 0.3 (8)

Intracellular accumulation of lysine and leucine by mucosal strips

The intracellular concentrations of lysine and/or leucine following incubation of mucosal strips for 60 min in normal buffer initially containing either 10 mM lysine, 2 mM leucine or a combination of these two amino acids are given in Table V. Since the tissue to medium ratio was not infinite, the amino acid concentrations declined slightly during the incubation; the final concentrations, given in Table V, were determined by assaying an aliquot of the medium for ^{14}C at the conclusion of the incubation. Intracellular concentrations were calculated using values of 0.30 cm^3/g wet wt. for the extracellular space and 0.20 for the dry-to-wet wt. ratio as described

* 30 mM leucine was included in the preincubation medium because an Na^+-free, choline–Ringer was used. Previous studies suggest that after 30 min the intracellular concentration of leucine under these preincubation conditions would be comparable with that achieved using 2 mM leucine in Na^+–Ringer[9].

previously[7]. To facilitate comparisons, the effect of leucine on intracellular accumulation of lysine and the effect of lysine on the accumulation of leucine were evaluated simultaneously on paired tissues from the same animal. In rows 1 and 3 of Table V it is seen that the presence of 2.0–1.8 mM leucine in the incubation medium results in a small but significant decline in the steady-state intracellular lysine concentration. Further, as can be seen by comparing rows 2 and 3 of Table V, the presence of 9–10 mM lysine in the incubation medium results in a 50% decrease in the steady-state intracellular leucine concentration.

DISCUSSION

The present results support two major conclusions. The first is that the mechanisms that are responsible for the transport of lysine and leucine across the intestinal brush border into the epithelium against electrochemical potential differences are not functionally distinct but have overlapping specificities. This conclusion is based on the findings that high concentrations of either leucine or lysine inhibit the unidirectional influx of the other. Further, 10 mM lysine inhibits the transmural transport and intracellular accumulation of leucine when the latter amino acid is present at the relatively low concentration of 2 mM. Although, strictly speaking, these observations do not permit the conclusion that the observed inhibitions are truly "competitive" in the classic sense, this is still the most probable explanation for these phenomena. Since neither leucine influx nor lysine influx conform to simple Michaelis–Menten kinetics, an unequivocal demonstration of classic competitive inhibition would be extremely difficult, particularly if both terms in the influx expressions for leucine (Eqn. 1) and lysine (Eqn. 2) were influenced by the presence of the other amino acid.

The second conclusion confirms the observation of MUNCK[4] that the presence of a low concentration of leucine in the mucosal solution markedly stimulates the rate of net transmural transport of lysine from mucosa to serosa. In addition, these results indicate that the enhanced rate of net transmural lysine transport is solely the result of an increase in the mucosa-to-serosa unidirectional flux; the unidirectional flux of lysine from serosa-to-mucosa is unaffected by leucine. Further, leucine must exert this stimulatory action through its presence in the mucosal medium and/or its presence in the cell. The addition of leucine to the serosal medium has no effect on lysine transport, and, as shown in Fig. 1, the effect of leucine on the J_{ms} of lysine is observed within a few minutes after the addition of leucine to the mucosal medium. It is unlikely that a significant concentration of leucine could collect in the serosal tissues during this brief lag period. The question is: How does the presence of leucine in the mucosal solution and/or in the cell bring about an increase in the J_{ms} for lysine?

A possible answer to this question emerges from an analysis of the steady-state bidirectional fluxes of leucine and lysine across the mucosal and serosal borders of the epithelium. These fluxes are illustrated in Fig. 4A and are related by the following equations[7,8].

$$J_{ms} = J_{mc}J_{cs}/(J_{cm} + J_{cs}) \tag{3a}$$

$$J_{sm} = J_{sc}J_{cm}/(J_{cm} + J_{cs}) \tag{3b}$$

$$J_{net} = J_{ms} - J_{sm} = J_{mc} - J_{cm} = J_{cs} - J_{sc} \tag{3c}$$

We may first examine the consequences of Eqn. 3c which simply states that in a composite membrane system consisting of a series array of membranes, the steady-state net flux across the entire system must be equal to the net flux across each of the component membranes. Thus, under steady-state conditions the increase in net

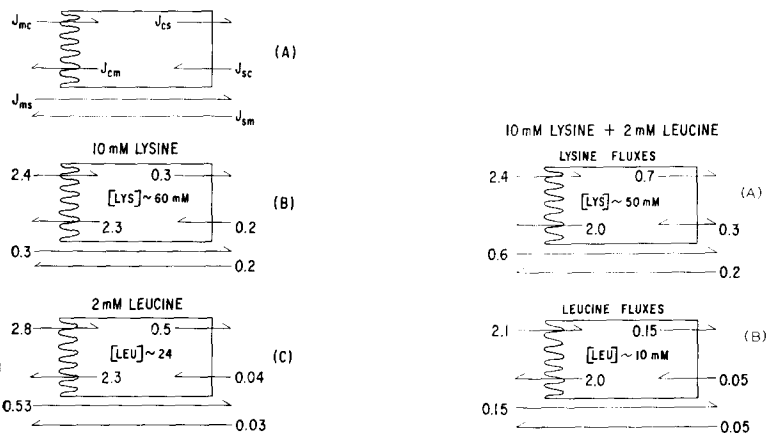

Fig. 4. (A) The unidirectional fluxes across intestinal epithelium as related by Eqns. 3; (B) The unidirectional fluxes of lysine in the presence of 10 mM lysine; (C) The unidirectional fluxes of leucine in the presence of 2 mM leucine. All fluxes are given in μmoles/h·cm^2 and were calculated from J_{ms}, J_{sm} and J_{mc} using Eqns. 3.

Fig. 5. The unidirectional fluxes of lysine (A) and leucine (B) in the presence of 10 mM lysine and 2 mM leucine.

transmural lysine transport must be associated with an equivalent increase in the net fluxes across the mucosal and serosal membranes. Now, an increase in the net flux across the mucosal membrane can result from either a relative increase in J_{mc}, a relative decrease in J_{cm} or a combination of these changes. However, as is shown in Table III (row 2) J_{mc}^{Lys} is not increased by the presence of 2 mM leucine when the tissue has been preincubated with leucine and lysine for 30 min (these influx conditions most closely stimulate the conditions under which steady-state transmural fluxes are determined). Thus, the increased net flux across the mucosal membrane must be attributed solely to a decrease in J_{cm}^{Lys}. This decrease in J_{cm}^{Lys} was suggested by MUNCK in his original studies of this phenomenon[4], and he proposed that the stimulation of lysine flux by leucine could be due to competitive inhibition of lysine efflux out of the cell across the brush border resulting from a high intracellular level of leucine. This in turn, would lead to an increased intracellular lysine concentration and an increase in the rate of lysine efflux out of the cell across the serosal border. It is clear from the present data that this explanation is insufficient. Thus we find that in the presence of 10 mM lysine and 2 mM leucine, high intracellular levels of leucine are not achieved and that, if anything, there is a drecrease in the steady-state intracellular lysine concentration.

The unidirectional fluxes of lysine in the absence of leucine and of leucine in the absence of lysine, calculated using Eqns. 3, are shown in Figs. 4B and 4C. The unidirectional fluxes of lysine and of leucine in the presence of 10 mM lysine *plus* 2 mM leucine are shown in Fig. 5. The unidirectional fluxes of leucine in the presence

of 10 mM lysine are qualitatively consistant with primary inhibition of leucine influx across the brush border by lysine. Thus, J_{mc}^{Leu}, the intracellular leucine concentration and both J_{cm}^{Leu} and J_{cs}^{Leu} are decreased in the presence of 10 mM lysine. At present we cannot comment any further on the effect of lysine on leucine fluxes because we have no information on the kinetic characteristics of J_{cm}^{Leu} and J_{cs}^{Leu} as functions of intracellular leucine concentrations.

The most striking feature in the comparison of unidirectional lysine fluxes in the presence and absence of 2 mM leucine (Figs. 4B and 5) is that J_{cs}^{Lys} is markedly increased in the presence of leucine in spite of the fact that the intracellular lysine concentration is decreased. Further, we have previously shown that in the absence of leucine J_{cs}^{Lys} appears to be a saturable function of the intracellular lysine concentration with a maximum value of 0.3–0.4 μmole/h·cm^2. Yet, in the presence of leucine the calculated value of J_{cs}^{Lys} is almost twice this previously estimated maximum value[7].

Several explanations for this increase in J_{cs}^{Lys} can be suggested. First, it is possible that intracellular lysine is compartmentalized so that the overall levels measured do not reflect the local lysine concentration that determines J_{cs} (*i.e.* the so-called "transport pool" of lysine). Were this the case, our previous analysis of the kinetics of lysine efflux across the serosal border of the cell could be invalid, and the decrease in the intracellular lysine concentration in the presence of leucine observed in this investigation could actually be associated with an increase in the lysine transport pool which in turn results in an increased J_{sc}^{Lys}. This possibility cannot be ruled out, but it is not subject to experimental evaluation at this time. An alternative explanation is that intracellular leucine in some way affects the process or processes responsible for lysine efflux across the serosal membrane. At least two observations suggest this possibility. First, as was seen in Table III (rows 3 and 4) preloading of the tissue with leucine alone markedly stimulates lysine influx into the tissue. This cannot be attributed to a classical hetero-exchange phenomenon since preloading with lysine does not stimulate lysine influx, and preloading with either leucine or lysine does not stimulate leucine influx. We are at present unable to account for this phenomenon in terms of any of the classical carrier models, and are unware of any previous demonstration of hetero-exchange in the absence of homo(or auto)-exchange. Second, the stimulatory effect of leucine on lysine transport by small intestine appears to be highly specific. Thus, B. G. MUNCK (unpublished observations) has observed that only leucine and, to a lesser extent, methionine significantly stimulate lysine transport by everted sacs of rat jejunum; no stimulation was observed with isoleucine, valine, serine, aspartic acid or sarcosine. Since isoleucine, valine, serine and leucine have very similar transport characteristics[13], the specificity of leucine with respect to the stimulation of lysine transport suggests that this is a property of leucine (and perhaps methionine) that is not directly related to its transport. Thus, intracellular leucine may interact non-competitively with the lysine transport mechanism(s) and result in configurational changes that increase the rate at which the lysine–carrier complex translocates across the membrane.

Recently, SCRIVER AND MOHYUDDIN[14] have described an interaction between proline and α-aminoisobutyric acid uptake by rat kidney cortex slices that resembles, in some respects, the leucine–lysine interaction in small intestine. These authors have shown that proline and hydroxyproline enhance α-aminoisobutyric acid uptake

by the kidney slices when α-aminoisobutyric acid is present in low concentrations. In addition, preloading the tissue with proline stimulates α-aminoisobutyric acid uptake but preloading with α-aminoisobutyric acid has no effect on α-aminoisobutyric acid uptake. Finally, the effect of proline appears to be highly specific and unrelated to its transport inasmuch as glycine, which is believed to share the imino acid transport mechanism in kidney, does not affect α-aminoisobutyric acid uptake. These authors have suggested that proline binds to the α-aminoisobutyric acid–carrier complex and influences influx in a manner similar to that proposed for the interaction between Na^+ and α-aminoisobutyric acid transport; the interaction between proline and α-aminoisobutyric acid transport is believed to take place at a site that is not identical with the reactive site for proline transport. These observations together with the present results suggest that an effect of one amino acid on the transport of another need not represent an interaction at the sites that are involved in the transport of both of these amino acids but could be due to a noncompetitive interaction between one amino acid and the transport agency that serves another. As is seen in Figs. 4B and 5, the increase in J_{cs}^{Lys} due to the presence of leucine (0.3–0.4 μmole/h·cm^2) is greater than J_{cs}^{Leu} (0.15 μmole/h·cm^2). Thus, the mechanism by which leucine enhances lysine efflux out of the cell does not appear to result in a simultaneous increase in leucine efflux out of the cell; that is, the agency with which leucine interacts to enhance lysine efflux out of the cell does not appear to be involved in leucine transport.

We have previously demonstrated that J_{cs} is the rate-limiting step for transmural lysine transport and that J_{ms}^{Lys} approximates J_{cs}^{Lys}. The present results are consistent with this analysis and suggest that the effect of leucine on J_{ms}^{Lys} (and J_{net}^{Lys}) is due primarily to a stimulation of J_{cs}^{Lys} by intracellular leucine. Further speculation on the mechanism by which leucine stimulates lysine transport seems, at present, unproductive. It is quite clear from the present investigation that the usual transport mechanisms do not adequately explain this highly specific interaction. Further investigation of the mechanism by which intracellular leucine alone stimulates lysine influx across the brush border and of the effects of other amino acids and leucine analogs on lysine transport could provide additional insight into this phenomenon.

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