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LYSINE TRANSPORT IN THE GUINEA-PIG SMALL INTESTINE

BJARNE G. MUNCK

Institute of Medical Physiology, Dept. A, University of Copenhagen, Panum Institute, Blegdamsvej 3C, DK-2200 Copenhagen N (Denmark)

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Interactions between cationic and neutral amino acids in transport across the brush-border membrane, J_{mc}, of the small intestine have been examined using preparations from the distal rabbit ileum and the rat and guinea-pig mid-small intestine. (1) In the guinea pig, the dependence of J_{mc}^{Lys} on the concentration of lysine is best described in terms of two saturable transport mechanisms in addition to free diffusion. (2) It is shown that the discrepancy between cis-effects of low concentrations of neutral amino acids on the J_{mc} of cationic amino acids, cis-stimulation in the guinea pig contra cis-inhibition in the rabbit and rat, represents species differences. In the guinea pig, imposing sodium-free conditions turns cis-stimulation into cis-inhibition. (3) It is demonstrated that in rat and guinea pig, leucine is transported both by the transport system(s) for cationic amino acids and by transport system(s) which cannot be inhibited by cationic amino acids.

Introduction

In rabbit and rat small intestine α-amino-monocarboxylic acids, neutral amino acids, inhibit competitively the influx of cationic amino acids across the brush-border membrane [1,2]: cis-inhibition. In these two species neutral amino acids can also act as trans-stimulators of influx of cationic amino acids, and certain neutral amino acids stimulate the unidirectional mucosal to serosal flux of the cationic amino acids under conditions, which strongly indicate that the stimulation results from a cis-stimulation of efflux across the basolateral cell membrane [2,3]. In the guinea-pig small intestine it was found that the uptake of cationic amino acids by rings of the intestine could be increased by low concentrations of neutral amino acids, although at higher concentrations the same amino acids would inhibit the transport [4]. Disregarding possible species differences, methodological shortcomings were proposed to account for the conflict between the inhibition of influx of cationic amino acids seen in rat and rabbit small intestine and the stimulation observed for the guinea pig [2,4].

As the postulates of methodological weaknesses appeared unsatisfying, the present study was undertaken in order to study the guinea-pig small intestine by the same methods as had been used on the rat and rabbit small intestine. The results indicated that species differences were involved, and this indication was confirmed by supplementing rat and rabbit experiments.

Materials and Methods

Female albino guinea pigs (body weight 300–400 g), male albino rats (body weight 175 g), and female, albino rabbits (body weigh 2500–3000 g) were used. Prior to the experiments the animals were maintained with free access to food and water. The animals were anaesthetized with intraperitoneally or intravenously administered pentobarbital. Hereafter, the abdomen was opened,

the entire small intestine was removed, and the animal was killed by transsection of the heart. From the guinea pigs and rats the mid 30 cm of the total small intestine, and from the rabbits the most distal 30 cm of the ileum were used for the experiments. These were performed at 37°C in a phosphate buffer with 140 mM Na^+, 8 mM K^+, 2.6 mM Ca^{2+}, 1 mM Mg^{2+}, 140 mM Cl^-, 1 mM SO_4^{2+}, and 8 mM $H_2PO_4^- + HPO_4^{2-}$, Sodium-free solutions were prepared by substituting choline chloride for sodium chloride. Unless otherwise stated, the solutions contained 5 mM D-glucose. The incubation fluids were aerated and stirred by 100% O_2. the chemicals were of analytical grade; sugars and amino acids were of the highest, commercially available purity. ^{14}C-labelled lysine, leucine, D-galactose, mannitol and tetraethylammonium and ^3H-labelled poly(ethyleneglycol) (M_n 4000) were obtained from New England Nuclear Co.

Unidirectional influx across the brush-border membrane, J_{mc}, was measured as previously described, except that [^{3}H]poly(ethyleneglycol) 4000 was used as extracellular marker instead of [^{3}H]methoxyinulin [5]. In all experiments but those made to compare the influx of mannitol (1 mM mannitol) with that of galactose (1 mM galactose + 200 mM glucose), mannitol was used to compensate for osmotic differences whenever sugars or amino acids were used at concentrations at or in excess of 40 mM.

The fluxes of sugars and amino acids were calculated on the basis of measured fluxes of radioactive tracers. The radioactivity of appropriate samples was analysed in a TriCarb fluid scintillation spectrometer, using Lumagel as scintillation fluid.

The results are stated as mean values ± S.E., with the number of observations in parentheses. P values less than 0.05 according to Student's t-test are taken as indication of statistical significance. The estimates of the kinetics of influx of lysine across the brush-border membrane were made by a nonlinear, least-squares analysis of the fit of the data to a model of one or two Michaelis-Menten terms plus a passive contribution. The errors of these estimates are ± S.D.

Experiments and Results

Diffusional contributions to J_{mc}

The magnitude of a passive diffusional contribution to the measured rates of influx across the brush-border membrane was examined by three means. (1) The influx of tetraethylammonium, J_{mc}^{TEA}, was measured at 1 mM tetraethylammonium; (2) Influx of mannitol, J_{mc}^{Man}, was measured at 1 mM mannitol; (3) Influx of D-galactose, J_{mc}^{Gal}, was measured at 1 mM D-galactose in the presence of 200 mM D-glucose. The results of these experiments were: $J_{mc}^{TEA} = 0.026 \pm 0.003$ ($n = 15$) μmol/cm^2 per h; $J_{mc}^{Man} = 0.061 \pm 0.005$ ($n = 16$) μmol/cm^2 per h; $J_{mc}^{Gal} = 0.028 \pm 0.006$ ($n = 16$) μmol/cm^2 per h. In the rat agreement has been found between J_{mc}^{TEA} and the passive permeability of lysine [6,7]. Therefore, J_{mc}^{TEA} is taken as a measure of the passive permeability of lysine also in the guinea pig small intestine, J_{mc}^{Man} and J_{mc}^{Gal} were measured in paired experiments. The data therefore demonstrate that J_{mc}^{Man} overestimates the passive permeability of sugars and, possibly, that of amino acids.

A possible role for depolarization of the electrical potential difference across the brush-border membrane in the mutual inhibition between cationic and neutral amino acids

The degree to which lysine inhibition of J_{mc}^{Leu} might be caused by a depolarization of the brush-border membrane potential was examined by measurements of J_{mc}^{Gal} at 1 mM D-galactose plus 10 mM leucine with or without 200 mM lysine. It was found that lysine caused a statistically insignificant, 13% reduction of J_{mc}^{Gal} from 0.197 ± 0.014 ($n = 8$) to 0.172 ± 0.013 ($n = 8$) μmol/cm^2 per h. In identical experiments in the rat, lysine reduces J_{mc}^{Gal} statistically insignificantly by 15% from 0.14 ± 0.03 ($n = 8$) to 0.12 ± 0.02 ($n = 8$) μmol/cm^2 per h. It must be pointed out that, unlike the effect of lysine on J_{mc}^{Leu}, which is measured in the presence of 5 mM D-glucose, the effect on J_{mc}^{Gal} must necessarily be measured in the absence of glucose

Influx of lysine across the brush-border membrane

J_{mc}^{Lys} was measured in paired experiments at concentrations between 0.5 and 100 mM lysine. The observations, shown in Fig. 1, cannot be

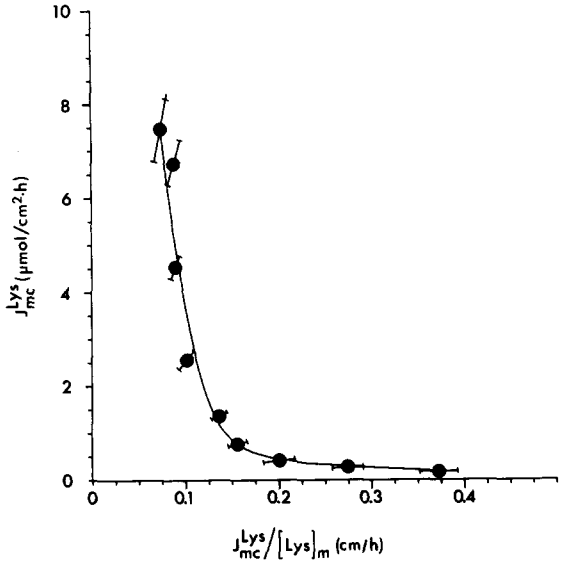

Fig. 1. Influx of lysine across the brush-border membrane of the guinea-pig mid-small intestine. Each point represent the mean of eight observations. The curve is described by Eqn. 1.

accounted for in terms of one saturable transport process plus free diffusion. The non-linear, least-squares analysis was applied to the data on J_{mc}^{Lys} assuming that the diffusional contribution was described by the permeability to tetraethylammonium as measured by J_{mc}^{TEA}, and without any assumption regarding the magnitude of this fraction of J_{mc}^{Lys}. The results are described respectively by Eqns. 1 and 2:

$$J_{mc}^{Lys} = \frac{(0.20 \pm 0.04)\,[Lys]_m}{(0.41 \pm 0.16) + [Lys]_m} + \frac{(8.9 \pm 1.39)[Lys]_m}{(93 \pm 21) + [Lys]_m} + (0.026 \pm 0.026)[Lys]_m \ \mu mol/cm^2 \cdot h \quad (1)$$

$$J_{mc}^{Lys} = \frac{(0.15 \pm 0.05)[Lys]_m}{(0.14 \pm 0.17) + [Lys]_m} + \frac{(1.80 \pm 1.08)[Lys]_m}{(21.5 \pm 15.5) + [Lys]_m} + (0.06 \pm 0.01)[Lys]_m \ \mu mol/cm^2 \cdot h \quad (2)$$

Clearly, particularly the estimates of the kinetic constants for the low-affinity transport are influenced by the magnitude of the passive permeability of lysine. But by the chi-square test, the fit between observations and the equations is for both characterized by a P value of 0.8.

Effects of leucine on J_{mc}^{Lys}

Guinea pig. Leucine was tested as inhibitor of J_{mc}^{Lys} at 1 mM lysine, where, according to Eqn. 1, 60% of J_{mc}^{Lys} should be by the high-affinity carrier, and at 20 mM lysine, where 90% should be by the low-affinity carrier. At 1 mM lysine, J_{mc}^{Lys} was measured at 0, 2, 10 or 20 mM leucine, and at 20 mM lysine with 0, 40, 80 or 160 mM leucine. The results are shown in Fig. 2. This figure demonstrates that at both concentrations of lysine, leucine is only a partial inhibitor of J_{mc}^{Lys}. In addition Fig. 2A shows that 2 mM leucine was not enough to cause any inhibition of J_{mc}^{Lys} as measured at 1 mM lysine. This lack of inhibition by 2 mM leucine indicated that, in agreement with the observed stimulation [4] of lysine uptake by rings of the guinea-pig small intestine, at lower concentrations leucine might indeed stimulate J_{mc}^{Lys}. Therefore,

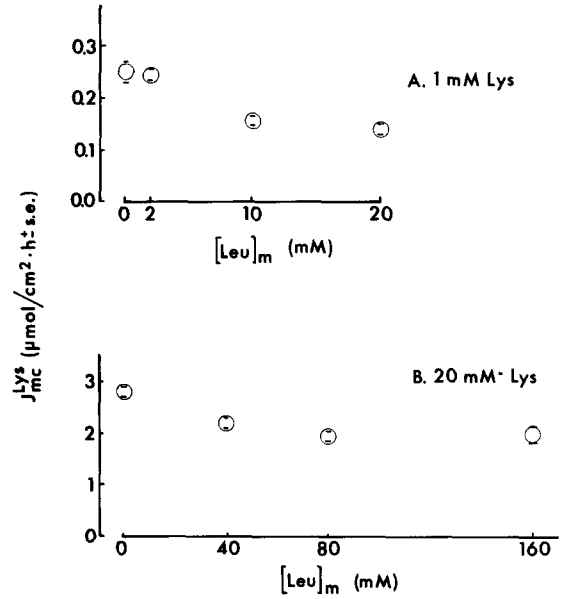

Fig. 2. Effects of leucine on J_{mc}^{Lys}. The data for 1 mM lysine represent means of eight measurements; those for 20 mM lysine are means of eight observations.

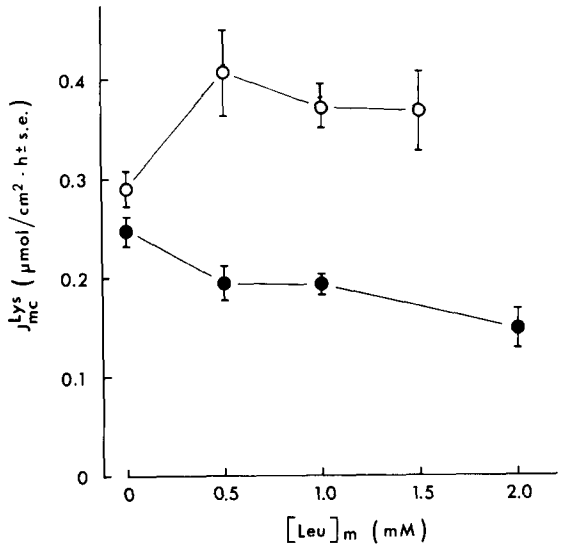

Fig. 3.Effects of low leucine concentrations on J_{mc}^{Lys}. At 140 mM Na^+, O; at zero Na^+, ●. All points represent means of eight measurements.

J_{mc}^{Lys} was measured at 1 mM lysine in the presence of 0, 0.5, 1.0 and 1.5 mM leucine. These measurements were performed using exposure times of 0.5 min, these experiments were repeated under sodium-free conditions.

The results are shown in Fig. 3. Here it is seen that at 140 mM sodium, using exposure times of 0.5 min leucine statistically significantly stimulates J_{mc}^{Lys} when present at 0.5 and 1.0 mM. Essentially the same effect was demonstrated using exposure times of 0.25 min (Fig. 4). Finally the data of Fig. 3 show that in the absence of sodium 0.5, 1.0 and 1.5 mM leucine all inhibit J_{mc}^{Lys}. Thus with the present technique, it is confirmed that a neutral amino acid can act as a cis-stimulator of the influx of lysine across the guinea-pig brush-border membrane. This effect, however, clearly is sodium dependent.

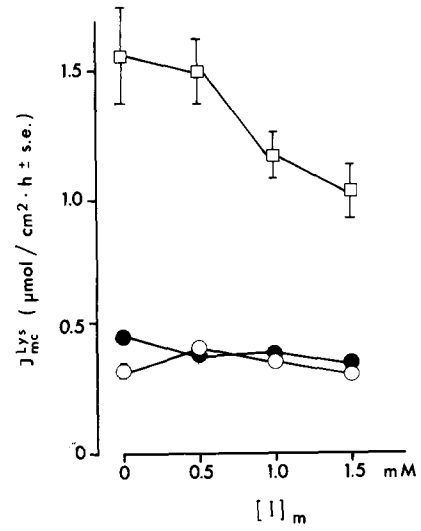

Fig. 4. Effects of low concentrations of methionine on J_{mc}^{Lys} (1 mM lysine) in the rabbit ileum (□), and of leucine in guinea-pig (O) or rat (●) mid-small intestine. All points represent means of eight observations.

Rabbit and rat. In previous studies on lysine transport by rat jejunum and rabbit ileum, neutral amino acids had not been used as inhibitors at concentrations below 1 mM. Therefore, the above results raised the possibility that, also in rat and rabbit, small intestine neutral amino acids might act as cis-stimulators of J_{mc}^{Lys}. This possibility was examined by measuring J_{mc}^{Lys} at 1 mM lysine in rat mid small intestine and distal rabbit ileum. In the rat, leucine was added at concentrations of 0.5, 1.0 and 1.5 mM; in the rabbit, methionine was used at the same concentrations. The results are shown in Fig. 4, where, for comparison, the 0.25 min results from the guinea pig are included. Clearly, in both rat and rabbit small intestine neutral amino acids act exclusively as cis-inhibitors. Fig. 4, then, demonstrates a major species difference with respect to the interaction between neutral and cationic amino acids in transport across the brush-border membrane.

Effects of lysine on J_{mc}^{Leu}

Guinea pig and rat. To examine the possibility that neutral amino acids might inhibit the transport of lysine without being transported by the transport system used by cationic amino acids, lysine was studied as inhibitor of the transport of leucine. J_{mc}^{Leu} was measured at 10 mM leucine in the presence of 0, 25, 50, 100 or 200 mM lysine. For the rabbit ileum lysine has previously been demonstrated to be an inhibitor of the transport of neutral amino acids (Ref. 1 and Munck, B.G., unpublished data). But previously described inhibitory effects of cationic amino acids on the transport of neutral amino acids by the rat small intestine [5,9] cannot unambiguously be interpreted as evidence of inhibition of influx across the

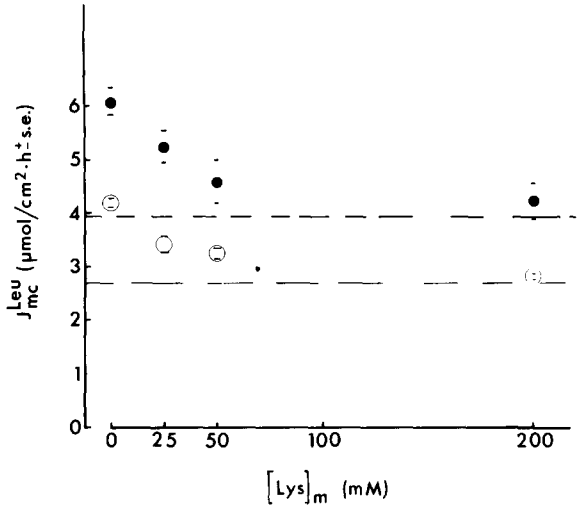

Fig. 5. Effects of lysine on J_{mc}^{Leu} measured at 10 mM leucine + 5 mM D-glucose in rat (●) and guinea pig (○) mid-small intestine. All points represent means of four observations. The dashed lines represent the lysine-resistant contributions to J_{mc}^{Leu}.

brush-border membrane. Therefore, lysine was examined as inhibitor of J_{mc}^{Leu} both in rat and in guinea-pig mid-small intestine. The results are shown in Fig. 5. This figure shows that in both species lysine is a partial inhibitor of J_{mc}^{Leu}. In both species, the maximum inhibition greatly exceeds the estimates of lysine's ability to inhibit influx across the brush-border membrane through a reduction of the electrical potential difference across this membrane. The results, then, provide conclusive evidence that in both rat and guinea pig small intestine cationic and neutral amino acids share one or more transport mechanisms.

Discussion

The present study was undertaken with the purpose of examining whether the discrepancy between the cis-stimulation of J_{mc}^{Lys} by neutral amino acids observed in the guinea-pig small intestine [4] and the cis-inhibition observed in rat jejunum [2] and rabbit ileum [1] had its origin in methodological differences or in genuine species differences. The studies of lysine transport by the guinea pig intestine had been performed using small rings of the intestinal tissues comprising all layers of the intestinal wall and exposing connective tissues, muscle tissues as well as the basolateral membrane of the epithelium directly to the incubation solutions. In contrast, in the studies on rabbit and rat small intestine, only the luminal surface of the brush-border membrane had been exposed. This evidently made it possible to disregard species differences as the reason for the discrepancy and to focus on the methodological aspect. However, the data of Fig. 3 confirm with the present technique the previous observations [4] on the guinea-pig small intestine. In addition, the observation of equal degrees of *cis*-stimulation in 0.25 and 0.5 min incubations virtually eliminates the possibility that the apparent cis-stimulation might have been caused by an effect at the basolateral membrane of leucine which on the carrier of neutral amino acids had reached the cytoplasmic side of the brush-border membrane. The data of Fig. 5 just as clearly demonstrate that in rabbit and rat small intestine, the cis effects of neutral amino acids on J_{mc}^{Lys} are exclusively those of cis-inhibition. It is therefore possible to conclude that with respect to the cis effect of neutral amino acids on J_{mc}^{Lys}, a major difference exists between guinea-pig small intestine on the one hand and rabbit and rat small intestine on the other.

In contrast to the previous observation in the guinea pig, where cis-stimulation, in 10 min incubations of rings of intestinal tissues, was found to be independent of sodium [4], the present study (Fig. 3) has demonstrated that in the absence of sodium, leucine becomes a cis-inhibitor of J_{mc}^{Lys} also in the guinea-pig small intestine.

The transport of cationic amino acids by the guinea pig had not previously been characterized, neither had it been determined whether neutral amino acids could be transported by the mechanism of transport of cationic amino acids. An elucidation of these two questions would be substantially eased by estimates of the passive permeability of lysine and of the extent to which lysine might interfere with other sodium-coupled transport through reductions of the electrical potential difference across the brush-border membrane. The question of passive permeability was not unequivocally resolved. In both rat and rabbit small intestine, close agreement has been found between estimates of the passive permeability of lysine and J_{mc}^{TEA} [6–8]. In the guinea pig (Fig. 2),

the leucine-resistant lysine transport corresponds to a permeability of 0.1 μmol/cm^2 per h per mM, 4-times that of tetraethylammonium and twice the permeability of mannitol. The difference between J_{mc}^{Man} and J_{mc}^{Gal} measured at 1 mM D-galactose in the presence of 200 mM D-glucose clearly shows that J_{mc}^{Man} overestimates the passive permeability of hexoses. It cannot, however, be determined whether J_{mc}^{TEA} provides a better estimate of the passive permeability of lysine than that of the non-linear regression analysis of the data on lysine transport, which is almost identical to the estimate of the permeability of mannitol and to estimates of the passive permeabilities of β-alanine and *N*-methylaminoisobutyric acid [10].

The question of the possible role of changing electrical potential difference across the brush-border membrane in the mutual inhibition between leucine and lysine was more decisively answered. The effect of lysine on J_{mc}^{Leu} was measured in the presence of 5 mM D-glucose. Compared with 1 mM D-galactose 5 mM D-glucose will give rise to a greater influx of sodium across the brush-border membrane, and thus to a greater increase in its electrical conductance [12]. Relatively to the situation with 1 mM D-galactose 5 mM D-glucose will therefore attenuate the potential difference effect of lysine, and the lysine inhibition of J_{mc}^{Gal} as measured in the presence of 10 mM leucine will overestimate the potential difference-related fraction of the lysine inhibition of J_{mc}^{Leu}.

Both in rat and in guinea-pig lysine reduced J_{mc}^{Leu} by more than 33%. When this is compared with the 13 and 15% reductions of J_{mc}^{Gal}, it becomes clear that both in guinea pig and rat, as previously shown in the rabbit [1], cationic and neutral amino acids share at least one transport mechanism, and that in addition both species possess at least one transport system for neutral amino acids which is inaccessible to cationic amino acids.

In both rat and rabbit, J_{mc}^{Lys} can be completely inhibited by neutral amino acids [8], and it is evident that all lysine carriers are shared by neutral amino acids [8]. In the guinea pig the obviously only partial inhibition of lysine transport by leucine, together with leucine's ability to act as a cis-stimulant of J_{mc}^{Lys} make it impossible to ascribe the effects of leucine with certainty to the individual carrier of lysine.

Acknowledgement

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