BBA 75922

AMINO ACID TRANSPORT BY RAT SMALL INTESTINE

GALACTOSE INHIBITION OF TRANSEPITHELIAL NET TRANSPORT AS A RESULT OF STIMULATION OF BIDIRECTIONAL EFFLUX FROM THE EPITHELIUM

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(Received November 11th, 1971)

SUMMARY

1. The effects of galactose on jejunal and ileal transport of amino acids have been reexamined. Influx across the brush border membrane (J_{mc}) steady-state epithelial uptake $[A]_c$ and steady-state unidirectional transmural fluxes (J_{ms}, J_{sm}) have been measured. Both leucine and proline have been used.

2. J_{mc} and J_{ms} were found to be unaffected but $[A]_c$ was markedly reduced and J_{sm} just as markedly increased.

3. It is concluded that the apparently inhibitory effects of galactose on intestinal amino acid transport result from a galactose-induced stimulation of amino acid efflux across the brush border membrane and across the serosal face of the epithelial cell membrane.

4. Attention is called to the possibility that these effects are specific for the *in vitro* situation. Only if this is not the case should the possibility of cooperative interactions between sugars, amino acids and their carriers be seriously reconsidered.

5. It is shown that everted rat jejunum sacs as far as amino acid transport is concerned, maintain their functional integrity for more than 180 min.

INTRODUCTION

The possible nature of the well-documented[1-3] inhibitory action of actively transported non-metabolizable sugars on intestinal transport of amino acids has recently been reexamined and discussed by Alvarado and co-workers[4-6]. They conclude[5,6] that the best explanation is that both groups of substances use the same carrier for passage through the small intestinal epithelium luminal membrane in the hamster mouse, guinea pig, rabbit and rat. This carrier, accordingly, is described as multifunctional.

The opposite viewpoint was described by Newey and Smyth and their co-workers[1,7] who suggested that the mutual inhibition between these two groups could be explained as the epithelium being unable to provide enough energy to simultaneously maintain control values for the transport of an amino acid and a sugar.

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This view was in principle supported by Chez *et al.*[2], Munck[8,9] and Read[10] who for rabbit, rat, and fish, respectively, found no evidence that sugars inhibit amino acid transport by decreasing influx across the brush border. Neither was it consistent with the multifunctional carrier hypothesis that galactose inhibition of proline transfer from the mucosal to the serosal fluid of an everted rat jejunum sac was not apparent until after 30 min of incubation, when the serosal fluid concentration had surpassed that of the mucosal fluid[8]. These observations are all consistent with the view[2,11] that sugars act by increasing efflux across the brush border membrane. The possible mechanisms of this sugar–amino acid interaction have been discussed very recently[11].

The evidence[9] of the inability of sugars to induce a significant change in amino acid net flux, when added to the mucosal fluids after a steady-state distribution of amino acid was established between tissue and bathing fluids, has recently[5] been seriously questioned on the basis of the assumption that everted rat jejunum sacs were not functionally viable for the time used for the experiments (100 min).

Irrespective of the validity of this assumption and the criticism based upon it, obviously the information gained from these experiments is of limited value for a discussion of influx processes. This, however, is the case for all information concerning the interaction between sugars and amino acid transport in the small intestine except for the data on influx across the brush border of the rabbit ileum[2,12] and the low temperature data of Read[10] for fish intestine. It was therefore decided to study in detail the effect of galactose on amino acid transport by rat small intestine in order to obtain unequivocal information on influx across the brush border membrane and to obtain data comparable to those for rabbit intestine[2,12].

The study comprises measurements of influx across the brush border membrane, steady-state unidirectional transmural fluxes, and steady-state intracellular accumulation by the isolated mucosa of both ileum and jejunum from the rat.

The results show that galactose does not inhibit influx across the brush border membrane but inhibits transport by increasing efflux across this membrane. Thus it is demonstrated that in the rat there is no evidence that sugars and amino acids are transported by a common multifunctional carrier.

The question of the functional viability of the everted sac preparation is of general interest alone because of the vast number of studies based on this method. It was therefore examined whether prolonged incubation had a significant deleterious effect on the function of everted rat jejunum. This was found not to be the case for periods of incubation of 150–190 min.

MATERIALS AND METHODS

All experiments reported here have been made at 37 °C, during aeration with pure oxygen using Krebs phosphate media with 8 mM phosphate at pH 7.4. Inorganic chemicals were of analytical grade. Amino acids and sugars were of the highest purity available. ^{14}C-labelled amino acids were obtained from New England Nuclear Co. It has previously been shown[8,9,13] that rat small intestinal tissues do not significantly metabolize the amino acids leucine, proline and lysine used in the present study. ^{3}H-labelled methoxyinulin has been used as an extracellular space marker.

All experiments have been made using tissues from male albino rats of 125–150 g

body weight. The rats used for the sac experiments were fasted for 18–24 h prior to use. Otherwise the rats which were kept in the laboratory for at least 18 h had free access to food and water. All preparations were made from pentobarbital anaesthetized animals. Sacs of everted rat jejunum were prepared and used with the test tube technique as previously[8,9] described.

Steady-state epithelial uptake $[A]_c$ and steady-state transmural unidirectional fluxes (J_{ms}, J_{sm}) were measured on jejunal and ileal tissues using the preparation of isolated mucosa and the Ussing–Zerahn technique, respectively, as previously described[13].

Influx across the brush border membrane (J_{mc}) was measured using the technique described by Schultz *et al.*[14] with the only modification that the area of exposed gut was reduced from 1.13 to 0.62 cm^2 serosal surface.

By ileal tissues are meant the distal 10 cm of the small intestine. By jejunal tissues are meant the mid 10–20 cm of the total small intestine.

The statistical evaluation of the results is based on the students t-test[15]. All data are given as means ± S.E. with number of experiments in parentheses.

EXPERIMENTS AND RESULTS

Time dependence of the functional integrity of everted sacs

The time course of lysine distribution between the serosal and mucosal fluids of everted rat jejunum sacs was studied to test the functional viability of this preparation with respect to amino acid transport. The purpose was to examine whether a previous study[9] was a reliable basis for dissent with the conclusion made recently by Robinson and Alvarado[6]. From each rat two 7–8 cm long sacs were prepared. Both sacs were filled with 1 ml medium without sugars or amino acids. One sac was incubated for 150 min in the same medium. It was then transferred to 10 ml 1 mM lysine medium with ^{14}C-labelled lysine, and the transport of lysine was followed for

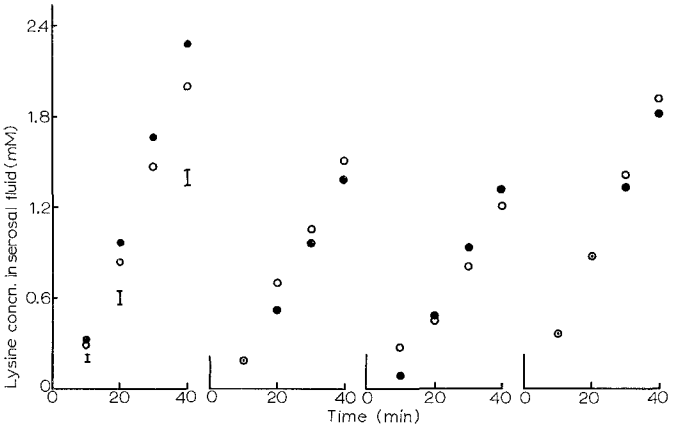

Fig. 1. Lysine accumulation in serosal fluids (initially 1 ml, free from lysine) of 7–8-cm long sacs of everted jejunum incubated in 10 ml at an initial lysine concentration of 1 mM, without preincubation (●), or after 150 min of preincubation in sugar and amino acid free media (○). The vertical bars in the left part of the figure show the mean ± S.E. ($n = 8$) for lysine transfer to the serosal fluid during incubation at 1 mM lysine *plus* 17 mM glucose in the mucosal fluid and 17 mM glucose in the serosal fluid as initial conditions.

40–60 min by sampling every 10th min from the serosal and the mucosal fluids. The other sac was incubated for 180–200 min in 10 ml 1 mM lysine medium with ^{14}C-labelled lysine. Here transport was studied by sampling every 10th–20th min. The results of these experiments are given in Fig. 1, which shows the increase in lysine concentration in the serosal fluid during the period from the 150th to the 190th min of experimental sac incubation and during the first 40 min of control sac incubation. For comparison the figure includes the results of a separate series of experiments in which sacs similarly prepared were incubated at 1 mM lysine *plus* 17 mM glucose. It is seen that incubation for 150 min in glucose-free media does not reduce the ability of sacs of everted rat jejunum to transport amino acids. The rate of lysine disappearance from the mucosal fluids is also the same whether measured during the first 40 min of incubation or after a preincubation period of 150 min. The distribution ratios for lysine between the serosal and mucosal fluids were in these experiments either continually increasing from, or maintained at, the high value reached during the 2nd h of incubation.

Influx of proline and leucine across the brush border membrane

The relation of influx to incubation time was examined and found to be linear for the 1st min of incubation. All data reported here were obtained using incubation periods of 0.50–0.70 min. The procedure was tested by looking for saturability of proline influx. The level at which saturation is complete or nearly complete was not established. But approach to saturation was clearly demonstrated by experiments in which tissues from each of three rats were exposed to proline concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 mM (Fig. 2). It was further checked whether transconcentration effects occur in transport of neutral amino acids. For this purpose alternate areas were preincubated either with amino acid-free medium or with 1 mM leucine for 30 min whereupon influx at 1 mM leucine was measured. There was no evidence of transconcentration effects. It is therefore considered a valuable test of the procedure and of the reliability of the lack of transconcentration effects on leucine influx that leucine was found to have a transconcentration effect on lysine influx

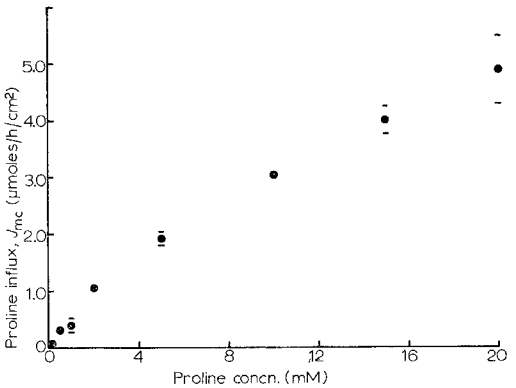

Fig. 2. Rat jejunal J_{mc} of proline *vs* proline concentration in test solution. Each point represents the mean of three observations. On each of the three rats used J_{mc} was measured at all eight different concentrations. ± S.E. is indicated by horizontal bars except when 2 S.E. is less than the diameter of the circle used to indicate the magnitude of the flux.

TABLE I

EFFECTS OF 28 mM GALACTOSE ON JEJUNAL AND ILEAL TRANSPORT CHARACTERISTICS FOR LEUCINE AND PROLINE

Fluxes are in μmoles/h per cm^2. Tissue accumulation in mM. All errors are given as the S.E. Numbers of observations are in parentheses. J_{cs} is calculated using the equations $J_{net} = J_{ms} - J_{sm} = J_{mc} - J_{cm}$; $J_{ms} = (J_{mc} \cdot J_{cs})/(J_{cm} + J_{cs})$. The data for 20 mM proline are included to assist in interpretation and discussion of the other data.

		J_{mc}	J_{ms}	J_{sm}	J_{cs}	$[A]_c$
1mM leucine (ileum)	Mannose	1.60 ± 0.26 (8)	0.32 ± 0.08 (5)	0.015 ± 0.002 (5)	0.33	24 ± 2 (20)
	Galactose	1.44 ± 0.18 (8)	0.46 ± 0.07 (5)	0.021 ± 0.002 (5)	0.47	14 ± 1 (22)
1 mM leucine (jejunum)	Mannose	1.12 ± 0.06 (16)	0.31 ± 0.04 (9)	0.017 ± 0.004 (10)	0.32	15 ± 1 (23)
	Galactose	1.06 ± 0.04 (16)	0.33 ± 0.02 (10)	0.029 ± 0.005 (10)	0.34	9 ± 0.5 (22)
1 mM proline (jejunum)	Mannose	0.70 ± 0.08 (8)	0.14 ± 0.03 (5)	0.015 ± 0.002 (6)	0.14	13 ± 1 (24)
	Galactose	0.66 ± 0.06 (8)	0.17 ± 0.02 (5)	0.030 ± 0.002 (6)	0.18	5 ± 0.3 (22)
10 mM proline (jejunum)	Mannose	3.44 ± 0.40 (6)	1.19 ± 0.32 (5)	0.18 ± 0.03 (6)	1.28	40 ± 2 (24)
	Galactose	3.32 ± 0.30 (6)	1.11 ± 0.21 (6)	0.34 ± 0.03 (6)	1.28	20 ± 1 (24)
20 mM proline		6.20 ± 0.72 (11)	1.92 ± 0.16 (11)	0.49 ± 0.04 (12)	2.14	63 ± 3 (24)

TABLE II

EFFECTS OF DIFFERENT EPITHELIAL PRELOADINGS ON AMINO ACID INFLUX ACROSS THE JEJUNAL BRUSH BORDER

All preincubations were 30 min in duration. Errors are given as the S.E. Number of observations are in parentheses.

Measured flux	*Preincubation medium*	*Test incubation medium*	J_{mc} (μmoles/h per cm^2 ± S.E.)
J_{mc}^{Lys}	Krebs–phosphate	Krebs–phosphate + 10 mM lysine	1.37 ± 0.14 (8)
	Krebs–phosphate + 2 mM leucine	Krebs–phosphate + 10 mM lysine	1.95 ± 0.21 (8)
J_{mc}^{Leu}	Krebs–phosphate	Krebs–phosphate + 1 mM leucine	1.77 ± 0.17 (8)
	Krebs–phosphate + 1 mM leucine	Krebs–phosphate + 1 mM leucine	1.84 ± 0.06 (8)
J_{mc}^{Leu}	Krebs–phosphate	Krebs–phosphate + 28 mM mannose + 1 mM leucine	1.68 ± 0.19 (4)
	Krebs–phosphate + 28 mM galactose	Krebs–phosphate + 28 mM galactose + 1 mM leucine	1.48 ± 0.13 (4)

comparable to that previously found for rabbit ileum[16]. For this test alternate areas were preincubated for 30 min either with amino acid-free medium or at 2 mM leucine. For all areas influx of lysine was measured at 10 mM lysine and no leucine. The influx values are shown in Table II. The ratio between the test and control was 1.54 ± 0.12 ($n = 8$).

The effect of 28 mM galactose was examined for J_{mc} of proline at 1 and 10 mM using jejunal preparations and of leucine at 1 mM using jejunal and ileal preparations. The effect of galactose preloading on jejunal J_{mc} of leucine at 1 mM was also examined. For the former experiments all areas were preincubated without sugars or amino acids and alternate areas were incubated with the appropriate amino acid with or without galactose (28 mM). For the latter experiments alternate areas were preincubated for 30 min in Krebs phosphate medium or at 28 mM galactose. Influx of leucine was then measured at 1 mM leucine and 1 mM leucine *plus* 28 mM galactose, respectively. The results of these experiments are shown in Tables I and II. It is seen that in all cases influx of amino acid across the brush border membrane is unaffected by galactose.

Steady-state epithelial accumulation of proline and leucine

Proline uptake by isolated jejunal mucosa was measured in paired experiments at 1 mM proline and 1 mM proline *plus* 28 mM galactose and at 10 mM proline and 10 mM proline *plus* 28 mM galactose. Further, in paired experiments leucine uptake by jejunal and ileal isolated mucosa was measured at 1 mM leucine and 1 mM leucine *plus* 28 mM galactose. The results stated in Table I demonstrate that in all cases amino acid uptake was markedly reduced in the presence of galactose.

Steady-state unidirectional fluxes of leucine and proline across the short circuited jejunum or ileum

Using four preparations from each rat, J_{ms} and J_{sm} of each amino acid are simultaneously measured in the absence and presence of galactose (28 mM). Jejunal fluxes of proline were measured at 1 and 10 mM. Jejunal and ileal fluxes of leucine were measured at 1 mM. After the 30th min of incubation ionic and amino acid fluxes are all at steady-state. Therefore from the values of the short circuiting current and the potential differences read between the 30th and 40th min of incubation, the steady-state electrical resistance has been calculated for the jejunal and ileal preparations incubated at 1 mM leucine and 1 mM leucine *plus* 28 mM galactose. The results of the flux measurements are shown in Table I. They demonstrate that in all cases J_{sm} was increased. In the jejunal preparations the electrical resistance was significantly reduced by galactose from 80 ± 5 $\Omega \cdot cm^2$ to 40 ± 3 $\Omega \cdot cm^2$ (both from 14 observations). In the ileal preparations the values were 56 ± 3 and 50 ± 2 $\Omega \cdot cm^2$ (from 8 observations). The difference between these two values is not statistically significant.

DISCUSSION

The data of Table I show that neither in the ileum nor in the jejunum of the rat does galactose inhibit proline or leucine influx, and that in the jejunum leucine influx is also insensitive to the presence of glucose. As for rabbit ileum[2,12] there is thus

no evidence that sugars and amino acids interact by partial or complete competitive inhibition during passage of the brush border membrane of rat small intestine.

It is also seen (Table I) that the steady-state epithelial uptake and transmural net fluxes of leucine and proline are reduced by galactose. These observations confirm and extend studies in which other amino acids and other species[1-3,18,12] were used.

It has been proposed[17] that the galactose inhibition of steady-state epithelial uptake of amino acid resulted from a transinhibition of J_{mc} caused by the galactose-induced increase in epithelial sodium concentration. However, this possibility seems ruled out by the lack of effect of galactose preincubation on the subsequent influx of leucine. Nor does the fact that J_{ms} is unaffected by galactose whereas J_{sm} is increased support the hypothesis of transinhibition. The latter results show that the sugar-induced decrease[1,7] in amino acid transfer to the serosal fluid of sacs incubated with initially identical solutions on both sides of the gut wall results only from an increased J_{sm}.

The effects of galactose (28 mM) on proline (10 mM) transport by sacs of everted rat jejunum have previously been studied[8]. In these studies the serosal fluids were initially free from sugars and amino acids. It was surprisingly found that an inhibition of proline accumulation in the serosal fluid was first evident some time after the serosal fluid concentration of proline had reached a level comparable to that of the mucosal fluid. These results are consistent with the present observation that J_{sm} but not J_{ms} is affected by galactose.

A transinhibition effect being ruled out, the reduced steady-state epithelial accumulation with unchanged J_{mc} and J_{ms}, and increased J_{sm} show that the effect of galactose must be to stimulate efflux of amino acids across the brush border membrane and across the serosa facing part of the epithelial cell membrane as well. In accordance with the sodium coupling hypothesis[11] the effect on J_{cm} can be explained[2,12,17] as a result of the galactose-induced increase in epithelial sodium concentration and decrease in potassium concentration[18]. Existing evidence seems to rule out a sodium or sodium-potassium dependence of J_{cs}[11]. There is thus no explanation available for the galactose-induced increase* in J_{cs}.

The interpretation[16] of the data on $J_{mc'}$, $J_{ms'}$, J_{sm} and steady-state $[Pro]_c$, which leads to the conclusion that J_{cs} is stimulated in the presence of galactose, rests at least partly on the assumption that the nonepithelial part of the gut wall and the serosal side medium constitute one well-stirred compartment and neglects the possibility of substantial fluxes through the tight junctions of the epithelium. The assumption of a well-stirred subepithelial space is unrealistic and the data for J_{ms} and J_{sm} differ qualitatively from those reported[2,12] for rabbit ileum where galactose inhibits J_{ms} of alanine and phenylalanine but leaves J_{sm} unaffected. It is therefore of interest to consider alternatives to a stimulation of J_{cs} as explanations of coexistence of reduced $[A]_c$ and unchanged J_{ms}.

It has been found[20] that proline (20 mM), glucose (28 mM) and galactose (28 mM) decrease the electrical resistance of rat jejunum prepared according to the Ussing–Zerahn technique[21], and that 20 mM proline stimulated J_{ms} and J_{sm} of

* In this discussion J_{cs} of proline and leucine are considered stimulated by the presence of galactose because they are maintained at control values in spite of the very marked galactose induced reduction of $[A]_c$.

thiourea by 70 and 30%, respectively. The increments in electrical conductivity and the increase of J_{sm} of thiourea indicate an increase in passive permeability. That J_{ms} of thiourea is increased much more than J_{sm} is very suggestive of a solvent drag effect and indicates that proline induces a net flow of water across the Ussing rat jejunum preparation. This increase in water flow will be accompanied by an expansion of the intercellular spaces and can thus at least partially explain the increased passive permeability. If the fluxes of proline and leucine through intercellular shunts between the mucosal and serosal side solutions were substantial compared to the expected galactose induced J_{cs} reduction, these increments in passive permeability might be responsible for maintenance of control J_{ms} in spite of reduced J_{cs}. Such a mechanism might also explain the data in Table I which demonstrate that J_{sm} of proline increases more than proportionally with the concentration. It is difficult to evaluate whether a shunt effect of the tight junction is realistic or quantitatively important in amino acid transport. However, some evidence can be mustered against its having a decisive effect. There was no increase in net water transport by the sacs which showed the delayed effect of galactose on proline transfer to the serosal fluid. But more weight in this regard should be given to the observations on galactose effects on rat ileum leucine transport (Table I). The effects are the same as with the jejunal preparations, but the electrical resistance was not significantly reduced by galactose. That is, although there is no indication of increased permeability of an intercellular shunt the amino acid flux data indicate a galactose stimulation of J_{cm} and J_{cs}.

Furthermore, the data of Table I indicate that J_{cs} at 10 mM proline *plus* 28 mM galactose would have been reduced by 0.64 μmole/cm^2 per h or more if it had not in some way been stimulated by the presence of galactose. This expected reduction exceeds by far the total J_{sm}. Its absence can therefore not be explained by an increased flux through an intercellular shunt pathway, although such a contribution to J_{ms} would increase the present estimates of J_{cs}. The total evidence then favours the conclusion that in fact stimulation of J_{cm} and J_{cs} are the effects of galactose on rat small intestinal amino acid transport.

Observations[22], confirmed by Robinson and Alvarado[6], indicate that glucose, although it stimulates[1] rat jejunal net transport of amino acids, inhibits amino acids uptake by the rat jejunum tissues. These partly contradictory sets of observations became almost completely inconsistent when it was found[8] that glucose did not reduce the amino acid concentration reached in the serosal fluids of everted rat jejunum sacs. The data were, however, reconciled by the demonstration[23] that glucose inhibits rat jejunal tissue amino acid uptake by inhibiting the subepithelial accumulation without affecting the epithelial accumulation. The suggestion[23] that this effect of glucose is secondary to its stimulation of transepithelial water transport has been further supported by the demonstration (B. G. Munck, unpublished observations) that glucose does not inhibit leucine uptake by rings cut from rat ileum, which, when prepared as everted sacs, does not show glucose stimulation of water transport[24].

For their recent study Robinson and Alvarado[6] used the same technique as I used some time ago. After letting an everted intestine sac accumulate a sugar or an amino acid, another sugar or amino acid is added to the mucosal side. By using this technique and adding the second substance during the 30th min of incubation Robinson and Alvarado were able to induce net efflux to the mucosal fluid from the

sac within 5–10 min. Both sugars and amino acids were effective against each other. By adding the second substance during the 90th min of incubation I found an efflux from sac to mucosal fluid within the first 10 min after the addition only by adding amino acids to amino acids and sugars to sugars. Robinson *et al.*[5] have interpreted this difference between our results and theirs to signify that everted rat jejunum sacs in the absence of exogenous glucose do not maintain functional integrity with respect to amino acid or sugar transport for the 100–120 min of my experiments. The data of Fig. 1 show that this interpretation is not correct.

As has been shown for rabbit ileum[2,12] and fish small intestine[10], the data presented here show that galactose does not reduce amino acid transport by rat small intestine by inhibiting influx across the brush border membrane. Contrarily, the effects of galactose are to stimulate efflux of amino acids across both the brush border and the serosal face of the epithelial cell membrane.

This conclusion is essentially the same as that reached by Reiser and Christiansen[25] on somewhat less direct evidence. The data for rabbit ileum and those reported here indicate very strongly that the techniques used by Robinson, Alvarado and co-workers have not allowed them to measure J_{mc}. It is therefore not unreasonable to conclude that so far it has not been shown for any species that any sugar inhibits the J_{mc} of any amino acid.

All published data, including the *in vivo* results of Cividanes *et al.*[26], are consistent with the interpretation presented here. Nevertheless, the difference between the data of Cividanes *et al.*[26] and those of Bingham *et al.*[7], who found that *in vivo* the transport of amino acids was uninhibited by galactose, is of interest. It emphasizes the important question whether the effects of galactose on J_{cm} and J_{cs} are peculiar for the *in vitro* situation or whether they should be expected to also occur in the intact rat small intestine. The basis for this question is that the *in vitro* situation differs from that *in vivo* by an inadequate clearance[23] of the subepithelial space for metabolites and transported substances of all kinds, and, perhaps, by a less adequate supply of oxygen[20]. Only if the sugar effects on J_{cs} and J_{cm} are independent of metabolic disturbances is it reasonable to consider further the possibility that these effects are expressions of cooperative interactions[27] between sugars, amino acids and the mediators of epithelial transport.

In conclusion it can be stated that the central question regarding sugar–amino acid interactions during intestinal transport is how the active sugars stimulate membrane transport of amino acids.

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