BBA 75430

INTERACTIONS BETWEEN LYSINE, Na^+ AND Cl^- TRANSPORT IN RAT JEJUNUM

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(Received November 24th, 1969)

SUMMARY

The effect of L-lysine (10 mM) on transmural electrical potential difference and short-circuit currents was studied in jejunal and ileal preparations from rat small intestine. It was seen that the jejunal response was much less than the ileal one but rose to the ileal level when Cl^- was substituted for by SO_4^{2-}, whereas the ileal response was independent of the type of anion.

In the short-circuited jejunum the lysine (10 mM) transport was found to be active and Na^+ dependent. It was further associated with an increase of the net flux of Na^+ from the mucosal to the serosal side, both in Cl^- and in SO_4^{2-} media. With the short-circuited jejunal preparation it was also demonstrated that lysine (10 mM) increased the net flux of Cl^- from the mucosal to the serosal side. With the short-circuited ileal preparation lysine did not influence the Cl^- net flux.

In the absence of lysine the short-circuited jejunum exhibited significant Cl^- secretion.

It is concluded that rat jejunal transport of lysine is active and Na^+ dependent and that the failure to observe lysine-induced increments in jejunal short-circuiting current in excess of what can be accounted for by the net flux of lysine results from a lysine-induced increase in net Cl^- flux from the mucosal to the serosal side of the gut wall, that is by an inhibition of Cl^- secretion.

Based on current evidence it is concluded that measurements of changes in electrical transmural potential difference and short-circuiting current are at best poor and often plainly misleading substitutes for actual flux measurements.

INTRODUCTION

Transport of lysine by rabbit small intestine is well established as being active and in part Na^+ dependent[1,2]. For rat small intestine there is considerable evidence that basic amino acids at concentrations below about 3 mM can be transported against an electrochemical potential difference (PD)[3–5], and there is some evidence that this process is Na^+ dependent[4]. Neither in rabbit nor in rat small intestine has the effect of basic amino acids on Na^+ transport been studied.

With the everted-sac preparation of rat jejunum it was found[6] that unilateral addition of 15 mM lysine·HCl to the medium bathing the mucosal surface did not

Abbreviations: PD, potential difference; I_{sc}, short-circuiting current.

significantly affect the transmural electric PD. These observations were interpreted as evidence that rat jejunal lysine transport is Na^+ independent and passive.

The obvious conflict between this conclusion and that one would make from several studies[3-5,7-10] on transfer of basic amino acids into and across rat small intestinal epithelium was intriguing. It seemed possible that the transmural PD response to lysine·HCl became negligible because of a change in net Cl^- transport. But it was clear that further discussion of the conclusion[6] drawn from PD measurements should be based on measurements of unidirectional transmural fluxes of lysine, Cl^-, and Na^+. This report is based on such measurements which showed that lysine in rat jejunum is actively transported by a Na^+-dependent mechanism, that the net Na^+ transport is increased during lysine transport but that also transmural net flux of Cl^- by lysine is increased enough to explain the very low lysine-induced change in transmural short-circuiting current (I_{sc}).

MATERIALS AND METHODS

L-Lysine·HCl and L-lysine as free base were obtained from Sigma Chemical Co. L-Lysine·H_2SO_4 was obtained in solution, by titrating a solution of the free base to pH 7.4 with concentrated H_2SO_4. L-[^{14}C]lysine and [*methoxy*-^{3}H]inulin were obtained from New England Nuclear Corporation Boston, Mass., $^{22}Na^+$ as a NaCl solution from The Radiochemical Centre, Amersham, Great Britain. $^{36}Cl^-$ as a NaCl solution from the research establishment of The Danish Atomic Energy Commission at Risø, Denmark.

Male albino rats with a body weight of 400–500 g were used. The rats had free access to food and water until anesthetized by intraperitoneal injection of sodium pentothal. The entire small intestine was torn from the mesentery. From around the midpoint a 10–15-cm length of intestine was taken to represent the jejunum. From this piece which was opened along the mesenteric border two preparations were made according to the modification of SCHULTZ and co-workers[11,12] of the apparatus of USSING AND ZERAHN[13] for unidirectional flux measurements on short-circuited membranes. The exposed area of each preparation was 0.62 cm^2. The preparation was performed so that *in situ* in the rat the two exposed areas would never have been more than 2 cm apart. Accordingly, they are for each rat assumed to constitute a pair of identical membranes.

Ileal preparations were made in the same way from the most distal 10–15 cm of the small intestine. All experiments were done with equal volumes of identical solutions bathing the two sides of the tissues. When substances were added during an experiment, the same amounts were added on both sides. During flux measurements the tissues were kept short-circuited. This was not the case in the experiments in which changes in I_{sc} were studied. I_{sc} was calculated from the medium resistance R_1 between the agar bridges used for PD measurements and the medium *plus* tissue resistance R_2 between these bridges, and the current I needed to reduce the PD between the bridges to zero: $I_{sc} = I \times R_2/(R_2-R_1)$.

The unidirectional fluxes of a substance S from the mucosal to the serosal side, J_{ms}, and from the serosal to the mucosal side, J_{sm}, were measured for L-lysine, Na^+ and Cl^- using the appropriate radioactive isotopes. These were added 5–10 min after the tissues had been mounted. This event represents the zero time of the flux

experiments. Sampling of the initially unlabeled solution was begun immediately after the addition of isotope to the opposite bathing solution. Samples of 1 ml were taken at 5- or 10-min intervals during experimental periods of 65–90 min. The samples were dissolved in the solution described by BRAY[14] and counted in a Packard liquid-scintillation spectrometer.

Net fluxes, J_{net}, were calculated from unidirectional fluxes measured on tissues from the same animal. A net flux to the serosal side is regarded as positive, and changes in net fluxes are described accordingly as increases or decreases.

Mucosal tissue isolated as described for rabbit small intestine[15] was used for measurements of lysine uptake. 100–200 mg wet wt. mucosal tissue were incubated for 40–70 min in a volume of 5 ml with [^{14}C]lysine and [*methoxy*-^{3}H]inulin. After the incubation the tissue was divided into three approximately equal pieces. Two of these were weighed and extracted overnight in 0.1 M HNO_3. The extract and samples of the initial and final incubation medium were assayed for ^{14}C and ^{3}H simultaneously. The third piece of tissue was weighed and dried at 105° to constant weight. From these data the tissue concentration was calculated assuming (1) that the ratios between total tissue water and tissue wet weight were the same for all three parts of each incubated tissue, (2) that inulin is an ideal marker of extracellular volume, which (3) equilibrates with the extracellular space of the tissues within the period of incubation. The first and third assumption were warranted by the experimental data. It is further assumed that lysine equilibrates between the incubation medium and the extracellular space of the incubated tissue. The buffer used for preparation of the tissues and for the experimental incubations was a modified Krebs phosphate buffer (pH 7.4), 8 mM phosphate, 140 mM Cl^-, 1.2 mM SO_4^{2-}, 140 mM Na^+, 8 mM K^+, 2.6 mM Ca^{2+}, and 1.2 mM Mg^{2+}. The incubations took place at 37°. The incubation solutions were oxygenated and circulated by an O_2–air lift. Na^+-free experiments were done with media in which NaCl had been replaced by choline chloride. Cl^--free media were made by replacing NaCl with Na_2SO_4 *plus* mannitol, and $CaCl_2$ with $Ca(NO_3)_2$, and by using lysine·H_2SO_4 instead of lysine·HCl.

EXPERIMENTS AND RESULTS

Lysine effects on jejunal I_{sc}

In one series of experiments (Table I) one of each pair of jejunal tissues was mounted in Cl^- medium, the other in SO_4^{2-} medium. The PD and I_{sc} were read every minute; when the tissues were electrically stable, lysine·HCl and lysine·H_2SO_4, respectively, were added to both sides of the tissues to a concentration of 10 mM. When a state of steady or steadily declining I_{sc} was reached, either alanine or glucose was added to reach the same concentration as that of lysine, later again, respectively, glucose or alanine was added. Alanine and glucose were used to check the functional integrity of the preparations and the possibility that SO_4^{2-} substitution might have a generalized effect on the electrical response to sugar and amino acid transport. It appeared that the effects on I_{sc} of alanine and glucose were the same in Cl^- as in SO_4^{2-} media, whereas the effects of lysine differed markedly. In Cl^- media lysine induced a rather small but significant ΔI_{sc} of 4 μA. In SO_4^{2-} media lysine induced a ΔI_{sc} of 12 μA, which as will be seen contrary to that induced in Cl^- media is well above the current equivalent of the transmural net flux of lysine as measured in

TABLE I

AMINO ACID AND GLUCOSE-INDUCED CHANGES IN I_{sc} AND ELECTRICAL PD ACROSS JEJUNAL AND ILEAL PREPARATIONS

The numbers represent means ± S.D. with number of experiments in parentheses. The order of the abbreviations in the first column indicates the order in which the corresponding substances were added to the bathing solutions.

	Medium	*Lysine*		*Glucose*		*Alanine*	
		ΔPD (*mV*)	ΔI_{sc} (μA)	ΔPD (*mV*)	ΔI_{sc} (μA)	ΔPD (*mV*)	ΔI_{sc} (μA)
Jejunum	Cl^-	0.3 ± 0.2 (10)	4 ± 3 (10)	1.7 ± 0.9 (8)	22 ± 15 (8)		
(Lys-Glc)	SO_4^{2-}	1.0 ± 0.3 (12)	12 ± 5 (12)	1.8 ± 0.7 (10)	18 ± 11 (10)		
Jejunum	Cl^-	0.6 ± 0.2 (6)	11 ± 6 (6)	3.0 ± 0.9 (6)	33 ± 10 (6)	1.1 ± 0.7 (4)	20 ± 11 (4)
(Glc-Lys-Ala)	SO_4^{2-}	0.5 ± 0.2 (6)	9 ± 5 (6)	3.6 ± 1.0 (6)	41 ± 13 (6)	0.8 ± 0.2 (4)	13 ± 6 (4)
Ileum	Cl^-	0.9 ± 0.6 (5)	11 ± 5 (5)	2.2 ± 1.4 (5)	22 ± 13 (5)	2.9 ± 1.6 (5)	33 ± 20 (5)
(Lys-Ala-Glc)	SO_4^{2-}	1.1 ± 0.5 (5)	11 ± 3 (5)	2.0 ± 0.7 (5)	21 ± 6 (5)	2.7 ± 0.8 (5)	31 ± 7 (5)

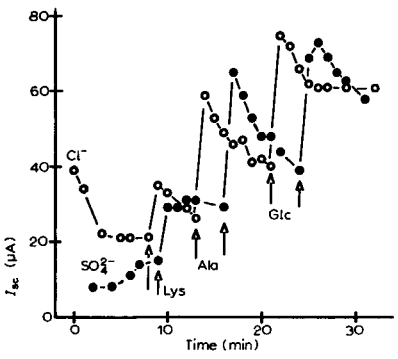

Fig. 1. Ileal short-circuit response in SO_4^{2-} and Cl^- media to successive addition of L-lysine (Lys), L-alanine (Ala), and D-glucose (Glc), each added to a concentration of 10 mM on both sides of the preparation.

SO_4^{2-} media. In another series of experiments the media were made 10 mM with respect to glucose before lysine was added; otherwise this series was identical to the first. It was found (Table I) that the ΔI_{sc} induced by lysine was the same in Cl^- as in SO_4^{2-} media and identical with that induced in the SO_4^{2-} media of the first series.

Lysine effects on ileal I_{sc}

In view of the observation[16] that for both rabbit and rat there is a marked difference between jejunal and ileal ionic flux responses to galactose transport, it seemed of interest to examine whether there would be any difference between the lysine effects on ileal I_{sc} in Cl^- and SO_4^{2-} media. This was investigated in experiments with pairs of ileal tissues, which were conducted as described for the first series of jejunal experiments. The I_{sc} responses to lysine, alanine, and glucose were the same in the two types of media (Table I), and they were identical to the responses found for jejunal tissues in SO_4^{2-} media. One ileal experiment is illustrated by Fig. 1, which also illustrates the procedure of these experiments.

Transmural fluxes of lysine

With rat small intestine lysine transport against a concentration difference has not been demonstrated at concentrations above 2–3 mM (ref. 5). For this reason it was found necessary in the present context to study the unidirectional transmural fluxes of lysine at the 10 mM concentration used throughout this study. J_{ms} and J_{sm} were measured in paired experiments on tissues from the same rat by means of [^{14}C]lysine. Samples from the initially unlabeled solution were taken every 10 min through 70–90 min. The fluxes of radioactive isotope reached a steady state after about 30 min of incubation. Each steady-state unidirectional flux is therefore estimated on the basis of 4–6 measurements. The unidirectional fluxes of lysine were measured in Cl^- media, SO_4^{2-} media, and in Na^+-free media, in which sodium was replaced by choline. Only jejunal tissues were used for these experiments.

The results of these experiments (Fig. 2) show that in the rat jejunum at a normal Na^+ concentration and at a rather high lysine concentration of 10 mM there is a significant lysine net flux of $0.22 \pm$ S.D. $= 0.13$ μmole$\cdot$h$^{-1}\cdot$cm^{-2} ($n = 5$) $P < 0.05$ in Cl^- medium and $0.16 \pm$ S.D. $= 0.08$ μmole$\cdot$h$^{-1}\cdot$cm^{-2} ($n = 6$) $P < 0.01$ in SO_4^{2-} media, although the experimental design was such as to eliminate an electrochemical PD for lysine. Substituting choline for Na^+ did not influence J_{sm}^{Lys} but reduced the net flux to zero by reducing J_{sm}^{Lys} to equality, with this flux resulting in a lysine net flux of $-0.01 \pm$ S.D. $= 0.02$ μmole$\cdot$h$^{-1}\cdot$cm^{-2} ($n = 6$). These results show that the rat jejunum possesses a Na^+-dependent mechanism for active transport of lysine.

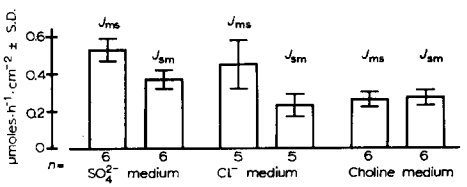

Fig. 2. Unidirectional fluxes of lysine (10 mM) across the short-circuited jejunum at 140 mM Na^+ in SO_4^{2-} and in Cl^- media and at 0 mM Na^+ in choline Cl^- media. n is the number of experiments.

TABLE II

TRANSMURAL FLUXES OF Cl^- AND Na^+

J^0 and J^+ indicate transmural fluxes of Na^+ and Cl^- in absence and presence of lysine, respectively. ΔJ_{net} indicates the effect of lysine on J_{net}, *i.e.* $\Delta J_{net} = J^+_{net} - J^0_{net}$. P is estimated according to Student's t-tests and indicates the significance of the deviation from 0 in the case of J_{net} and ΔJ_{net}, from 1.00 in the case of J^+/J^0 for Na^+, and from 1.07 in the case of J^+/J^0 for Cl^-. (Cl^-) and (SO_4^{2-}) indicate that the Na^+ fluxes were measured in Cl^- and SO_4^{2-} media, respectively. The values are means given with their standard deviation. The number of observations are given in parentheses. Fluxes are given as μequiv·h^{-1}·cm^{-2}.

		J_{ms}	J^+_{ms}/J^0_{ms}	J_{sm}	J^+_{sm}/J^0_{sm}	J_{net}	ΔJ_{net}
Jejunum	Cl^- (J^0)	6.5 ± 0.9 (7)		9.6 ± 1.7 (7)		− 3.1 ± 1.3 (7) $P < 0.001$	
			1.37 ± 0.15 (7)		1.11 ± 0.08 (7)		1.4 ± 1.0 (7)
	Cl^- + lysine (J^+)	8.9 ± 1.8 (7)	$P < 0.01$	10.6 ± 1.0 (7)	$P > 0.20$	− 1.7 ± 1.7 (7) $P < 0.05$	$P < 0.02$
Ileum	Cl^- (J^0)	8.8 ± 1.1 (4)		11.0 ± 2.4 (4)		− 2.2 ± 1.8 (4)	
			1.20 ± 0.10 (4)		1.15 ± 0.06 (4)		0.1 ± 0.8 (4)
	Cl^- + lysine (J^+)	10.6 ± 1.3 (4)	$P > 0.05$	12.7 ± 2.6 (4)	$P > 0.05$	− 2.1 ± 1.9 (4)	
Jejunum	Na^+ (Cl^-) (J^0)	8.5 ± 3.3 (9)		8.7 ± 2.6 (9)		− 0.2 ± 1.7 (9)	
			1.22 ± 0.13 (9)		1.09 ± 0.10 (9)		1.0 ± 1.1 (9)
	Na^+ + lysine (Cl^-) (J^+)	10.2 ± 3.3 (9)	$P < 0.02$	9.4 ± 2.7 (9)	$P < 0.05$	0.8 ± 1.0 (9) $P < 0.05$	$P < 0.05$
Jejunum	Na^+ (SO_4^{2-}) (J^0)	10.5 ± 1.5 (5)		8.9 ± 0.4 (5)		1.6 ± 1.5 (5) $P < 0.05$	
			1.11 ± 0.03 (5)		1.04 ± 0.08 (5)		1.1 ± 0.3 (5)
	Na^+ + lysine (SO_4^{2-}) (J^+)	11.8 ± 1.6 (5)	$P < 0.01$	9.1 ± 0.5 (5)	$P > 0.3$	2.7 ± 1.4 (5) $P < 0.02$	$P < 0.001$

Intracellular accumulation of lysine

Mucosal tissues were prepared from the middle third of the intestine, incubated at 10 mM lysine, at 37°, in an oscillating water bath during continuous O_2 bubbling. It was found that the degree of accumulation did not change between the 40th and 70th min of incubation, neither did the ratio of total tissue water/tissue wet weight or the ratio of extracellular water/tissue wet weight. The tissue uptake is therefore given as the mean value for tissues incubated for 40–70 min. The distribution ratio was 3.3 ± 0.9 ($n = 8$) corresponding to an intracellular concentration of 30 mM lysine. This could be regarded as further evidence of an active jejunal lysine transport. But one must be careful of this conclusion as the lysine distribution could be determined exclusively by the electrical PD between the incubation medium and the intraepithelial space. For the Greek tortoise[17] and for the hamster jejunum[18] this PD has been measured to be 10–30 mV. The value for the rat jejunum is unknown. It would have to be −28 mV to explain the observed distribution.

The present results on lysine uptake by rat small intestinal epithelium quantitatively agree quite well with those reported for rabbit ileum[2]. They differ, however, rather markedly from data reported for lysine uptake by intestinal rings[4], which in the course of a 60-min incubation seemed not even to reach equilibrium with respect to the lysine concentration of the incubation medium.

Transmural unidirectional Na^+ fluxes

The effect of a 10-mM concentration of lysine on the jejunal unidirectional fluxes of Na^+ was studied in order to see whether Na^+ might be cotransported with this amino acid. Both unidirectional fluxes were measured in paired experiments on tissues from the same rat. It was found that these fluxes in 5–15 min reached a steady state which would be maintained for more than 90 min. Accordingly, the effect of lysine on these fluxes was studied in experiments where lysine was added to the bathing media during the 35th min of Na^+ flux measuring, whereupon the Na^+ flux was measured for 30 min more. The effect of lysine is expressed by the ratio (J^+/J^0) between the steady-state Na^+ flux (J^+) after adding lysine and the steady-state Na^+ flux (J^0) of the same preparation before lysine was added. Each preparation thus provided one J^+/J^0 value. These experiments were performed with Cl^- and with SO_4^{2-} media. It appeared (Table II) that in the SO_4^{2-} media lysine increased the ratio for J_{ms} significantly above 1, whereas J_{sm} was not changed. Also the net flux of Na^+ was increased significantly. In Cl^- media (Table II), the J^+/J^0 ratios for J_{ms} and J_{sm} were both significantly increased, that of J_{sm} significantly more than that of J_{ms}, and the Na^+ net flux was increased significantly.

Transmural unidirectional Cl^- fluxes

The experiments discussed so far have shown that lysine transport is active, Na^+ dependent, and associated with an additional net flux of Na^+. In SO_4^{2-} media the current equivalent of the combined lysine net flux and lysine-induced $\Delta J_{net}^{Na^+}$ is 16 μA which compares rather well with the mean value of 12 μA for the lysine-induced ΔI_{sc}. However, in Cl^- media the corresponding values are 14 and 3 μA. This rather marked discrepancy suggested that lysine might induce an increase in Cl^- net flux and thus counteract the I_{sc} effect by the simultaneously induced increase in net cationic flux. The validity of this suggestion was examined by an experimental

approach identical with that used for measuring the effect of lysine on Na^+ fluxes in Cl^- media, except that $^{36}Cl^-$ was used instead of $^{22}Na^+$. By the addition of lysine·HCl during the experiments, the Cl^- concentrations were raised from 140 to 150 mM. The ratio between Cl^- fluxes after and before lysine addition must therefore be compared to 1.07 instead of 1.00. In these experiments it was found (Table II) that lysine significantly increased $J_{ms}^{Cl^-}$, whereas $J_{sm}^{Cl^-}$ was increased only insignificantly above 1.07. Lysine thus induced a significant increase in $J_{net}^{Cl^-}$ which, as required by the explanation suggested for the very low lysine-induced I_{sc}, is similar to the $\Delta J_{net}^{Na^+}$. The ileal net flux of Cl^- is unchanged by lysine. This is in accordance with the observation (Table I) that lysine induces the same ΔI_{sc} in Cl^- as in SO_4^{2-} media.

Relations between transmural ion fluxes and I_{sc}

The variability between individual rats is so pronounced that the number of experiments generally is too small to allow a detailed analysis of the relation between I_{sc} and J^{Cl^-} and J^{Na^+}. An exception, however, is provided by 45 experiments in which either Cl^- or Na^+ fluxes were measured for 35 min before lysine was added and in which also the I_{sc} was measured. In these experiments Cl^- or Na^+ isotopes were added 5–10 min after the tissues were mounted. The mean I_{sc} was then 44 ± (S.D. = 10) μA, with a flux equivalent of 2.6 ± 0.6 μequiv·h^{-1}·cm^{-2}; 35 min later the flux equivalent of the I_{sc} was 1.5 ± 0.6 μequiv·h^{-1}·cm^{-2}. In these experiments the net flux of Na^+ did not differ significantly from zero, whereas the Cl^- net flux (3.1 ± 1.3 μequiv·h^{-1}·cm^{-2}) is very similar to the initial I_{sc}.

DISCUSSION

It has now been shown that lysine in rat jejunum is actively transported by an at least partly Na^+-dependent mechanism and that its transport leads to an increased transmural net flux of Na^+. Further, explaining why in Cl^- media the lysine-induced ΔI_{sc} is too low to account for both the net flux of lysine and the increased transmural Na^+ net flux, it was observed that lysine induced an increase of the transmural Cl^- net flux, that is, a decrease of Cl^- secretion. Because of this effect on Cl^- transport, the lysine-dependent ΔI_{sc} is in fact so much reduced that making the Cl^- concentration 15 mM higher on the mucosal than on the serosal side by adding lysine·HCl to a concentration of 15 mM (ref. 6) might suffice to increase the transmural Cl^- net flux enough to reduce the lysine-dependent ΔI_{sc} to an insignificant level. It thus seems possible to reconcile the data and conclusion reported here with the observations of KOHN *et al.*[6], though not with their conclusion, regarding the rat jejunal transport of basic amino acids.

With the aim of making the results comparable to those obtained for rabbit small intestine, lysine[2] was used at a concentration of 10 mM. It appeared that the unidirectional transmural fluxes of lysine and their dependence on Na^+ as well as the epithelial accumulation of lysine are very much the same in rat as in rabbit small intestine[2]. Further unpublished results have shown that leucine in the isolated rat small intestinal epithelium reduces accumulation of lysine to the same degree as was found for isolated rabbit small intestinal epithelium[19]. For lysine transport in the small intestine it thus seems that methodological differences are more important than species differences.

The demonstration that lysine increases the transmural Cl^- net flux supplements the observation[16] that galactose decreases this flux and thereby disturbs the usual relation between sugar-induced changes in I_{sc} and transmural Na^+ net fluxes. These observations emphasize that the model described for coupling between Na^+ transport and transport of, *e.g.* sugars and amino acids[20,21] does not imply that transport of these substances necessarily generates changes in I_{sc} or transmural PD equivalent to changes in transmural Na^+ net flux. Simultaneously induced changes in other ion fluxes or a net charge of the transported substance[22] itself can disguise an effect on the Na^+ flux and/or on PD and I_{sc}.

For rat ileum our data on unidirectional transmural Cl^- fluxes are in accordance with those of CLARKSON AND TOOLE[23] and of SCHULTZ *et al.*[24] as far as J_{ms} and J_{sm} are not significantly different. In the case of rat jejunum our observations agree with results obtained for the small intestine of the Greek tortoise[25] in demonstrating an active secretion of Cl^-. This observation is not in accordance with the conclusion of TAYLOR *et al.*[16] but agrees well with their data (ref. 16; Table I). They as we observed with sugar- and amino acid-free experiments a significant I_{sc} in spite of an insignificant difference between the unidirectional transmural Na^+ fluxes. The data reported do not reveal the mechanisms by which lysine affects Cl^- transport. However, the short-circuiting is transmural. This means that parts of the electrical PD's between the cell interior and the fluids bathing the mucosal and serosal surfaces remain or are at least not controlled. At the same time lysine ions are accumulated to a rather high concentration intraepithelially. These factors could result in an increased intraepithelial Cl^- concentration provided primarily from the mucosal side, which could give a higher efflux to the serosal side, that is an increased J_{ms} as demonstrated. This could happen if lysine increased mainly the brush border membrane's passive permeability to Cl^-.

In summary the data reported here suffice to make lysine fit into the group of substances which in the intestinal epithelium are actively transported by Na^+-dependent systems. They further demonstrate some jejuno-ileal differences regarding Cl^- transport. Perhaps more important, the observations show how easy methodological differences can create a basis for assuming species differences, just as they confirm the notion[16] that a discussion of the models described to explain the role of Na^+ and Na^+ transport in intestinal absorption of different substances must be based on detailed flux measurements in addition to measurements of PD and I_{sc} changes.

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