Biochimica et Biophysica Acta 896 (1987) 295–304
Elsevier

BBA 73189

Electrophysiology of L-lysine entry across the brush-border membrane of *Necturus* intestine

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(Received 11 August 1986)

Key words: Amino acid transport; Apical membrane; Membrane potential; Lysine; Sodium ion effect; (*Necturus* intestine)

Microelectrode measurements of apical membrane potentials (V_a) in absorptive cells of isolated *Necturus* intestine showed that, in the presence or absence of external Na^+, 10 mM lysine added to the mucosal medium caused rapid depolarization followed by slower repolarization of V_a. In Na^+-free media the effects of 10 mM lysine on V_a were abolished by 10 mM leucine which alone had no effect on V_a under these conditions. This indicates that uncoupled electrodiffusion of lysine plays little or no role in lysine entry across the brush-border membrane. When external Na^+ was > 10 mM the maximum depolarization of V_a ($\Delta V_a'$) induced by [Lys] ranging from 5 to 30 mM was a simple saturable function of [Lys]. In Na^+-free media, the relationship between $\Delta V_a'$ and [Lys] was biphasic. At first, $\Delta V_a'$ increased with increasing [Lys] reaching a maximum at 10 mM lysine. When [Lys] was further increased, $\Delta V_a'$ declined progressively to reach zero or near zero values. A single transport pathway model is proposed to account for rheogenic lysine entry across the brush-border membrane in the presence and absence of Na^+. This postulates an amino acid transporter in the membrane with two binding sites. One is an amino acid site specific for the α-amino-α-carboxyl group. The other is a Na^+ site. Neutral amino acids (e.g. leucine) compete with lysine for the amino acid site. The Na^+ site has some affinity for the ε-amino group of lysine. When external Na^+ is high the Na^+ site is essentially 'saturated' with Na^+ and formation of a mobile complex between an amino acid and the transporter depends in a saturable fashion on amino acid concentration. In Na^+-free media or in media containing low [Na^+]; (i) at low external [Lys] the ε-amino group of a lysine molecule (simultaneously attached to the amino acid site) interacts with the Na^+ site to form a mobile complex, (ii) as external [Lys] is increased, attachment of different lysine molecules to each site of an increasing number of transporters to form nontransported or poorly transported complexes results in substrate inhibition of the rheogenic lysine transport process.

Introduction

There is abundant evidence that Na^+-coupled transport processes in the brush-border membrane mediate accumulative entry of neutral amino acids from the luminal medium into the absorptive cells of the small intestine and the proximal renal tubule [1]. Moreover, electrophysiologic studies [2] strongly indicate that these processes are rheogenic and are characterized by a rapid depolarization of the apical cell membrane *.

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* This appears to be the primary electrical event associated with Na^+-coupled amino acid entry [2]. However, because the paracellular shunt pathway in these tissues is highly conducting, a concomitant depolarization of the basolateral cell membrane is also observed.

With neutral amino acids, electrophysiologic analysis of Na^+-coupled entry is simplified by the fact that amino acid transport across the cell membrane, unless it is obligatorily linked to the concomitant movement of Na^+ or other ions, should be electroneutral. Experiments in Na^+-free media have confirmed this prediction [2]. With cationic amino acids, the electrophysiology of the entry process is subject to additional complexities. Radioisotope flux measurements have disclosed the presence, in both the kidney and the intestine, of Na^+-dependent and Na^+-independent luminal entry pathways for basic amino acids [3–11]. In principle, both types of entry process could be rheogenic, i.e. involve net charge transfer from the luminal medium to the cell interior. Alternatively, Na^+-independent amino acid entry might involve 1/1 exchange (countertransport) of a univalent cation (e.g. K^+, H^+) with the amino acid that enters the cell and thus be electrically silent.

In vivo experiments have provided evidence that is, apparently, consistent with either rheogenic or electroneutral Na^+-independent luminal entry of basic amino acids in the proximal renal tubule. In newt (*Triturus pyrrhogaster*) kidney, Hoshi et al. [12] observed that, in the presence of luminal Na^+, introduction of L-alanine (alanine) or L-lysine (lysine) into the lumen of the proximal tubule evoked a rapid and sustained depolarization of the peritubular membrane potential. In the absence of luminal Na^+ the depolarizing effect of alanine was abolished but that of lysine persisted. This suggests the presence, in the brush-border cell membrane, of a rheogenic Na^+-independent entry pathway for lysine. In contrast to these results, Samaržija and Frömter [13] found that, in the proximal tubule of rat kidney, the depolarization of the peritubular membrane induced by the addition of the basic amino acids lysine, arginine, and ornithine to the luminal fluid was abolished when the luminal perfusate was Na^+-free. This was interpreted as indicating that, in this tissue, conductive pathways for the uncoupled flow of basic amino acids across the brush-border membrane of the epithelial cells are either absent or quantitatively negligible.

In rabbit intestine, tracer flux studies suggest the existence of a complex array of transport pathways for the entry of amino acids across the brush-border membrane of the absorptive cells [6]. One of these is a high-affinity, low-capacity system for which neutral and cationic amino acids compete in the presence or absence of Na^+. Another is a low-affinity, high-capacity carrier for which lysine and neutral amino acids compete at physiological Na^+ concentrations but which appears to be inaccessible to neutral amino acids when Na^+ is absent from the mucosal bathing solution. A second high-affinity transport system for which neutral and cationic amino acids can compete has also been identified.

To date, the electrophysiologic correlates of cationic amino acid transport across the brush-border membrane of intestinal epithelial cells do not appear to have been systematically investigated. This report describes experiments in which the effects of lysine, added to the mucosal medium, on the apical membrane potential (V_a) in absorptive cells of *Necturus* intestine were studied. Experiments were performed in the presence and in the absence of external Na^+. Under both sets of conditions a lysine-induced depolarization of V_a was observed, indicating that rheogenic lysine entry across the brush-border memrbane occurs both in the presence and in the absence of Na^+. In both these circumstances lysine entry appeared to be carrier-mediated, i.e. no evidence was found for significant uncoupled electrodiffusive lysine movement into the cells. In the presence and absence, respectively, of external Na^+ the relationship between $\Delta V_a'$, the maximal depolarization induced by lysine, and luminal lysine concentration ([Lys]) was strikingly different. At physiological concentrations of external Na^+, $\Delta V_a'$ appeared to be a saturable function of [Lys]. In Na^+-free solutions, or when the external Na^+ concentration was low (5 mM), $\Delta V_a'$ at first increased to a maximum value with increasing [Lys] and then declined as [Lys] was farther increased. To account for these results, a single-cararier model which can, under appropriate conditions, effect the cotransport of one Na^+ ion together with one lysine molecule or the transport of one lysine molecule alone across the cell membrane is proposed. A preliminary account of some of these results was given elsewhere [6].

Materials and Methods

Necturus maculosis were obtained from Graska Biological Supplies (Oshkosh, WI) and kept in a large aquarium at 4°C. Animals were killed by a blow on the head followed by double transection of the spinal cord. A median abdominal incision was performed and a segment of intestine, approx. 10 cm long and immediately distal to the common bile duct, was excised. This was opened along its mesenteric border and rinsed of its luminal contents with oxygenated Ringer's solution. A section of this segment, stripped of its external muscle layers by blunt dissection, was mounted, mucosal surface upward, in a divided chamber of the type described by Garcia-Diaz et al. [15]. During experiments, the mucosal and serosal surfaces of the tissue were continuously superfused by gravity at room temperature (23 ± 1°C) from separate fluid reservoirs. The area of tissue exposed to the superfusates was 0.38 cm^2. A mechanical stopcock near the inlet to the upper half of the divided chamber permitted the mucosal bathing solution to be changed rapidly during impalement of an absorptive cell with a microelectrode.

In experiments where [Lys] was ≤ 10 mM, the control Ringer's solution contained (in mM), NaCl 100, KH_2PO_4 0.3, K_2HPO_4 2.3, calcium gluconate 1.8, mannitol 21. In low-Na^+ or Na^+-free media, Tris chloride replaced NaCl. Lysine was added as its chloride and, when this was done, NaCl or Tris chloride was omitted in an amount sufficient to keep the chloride content of the medium constant. When Tris chloride was omitted in this way, sufficient mannitol was added to maintain constant osmolality. When NaCl was removed it was replaced by an equivalent amount of sodium gluconate.

In experiments with 20 or 30 mM lysine the NaCl content of the control Ringer's solution was reduced to 80 mM and its mannitol content was increased to 61 mM. This was done so that these higher concentrations of lysine chloride could be incorporated in the mucosal medium without increasing its total osmolality. Under these conditions, other adjustments in the composition of the bathing media were made as already described. As shown in the Results (see Fig. 2A), it seems highly unlikely that a reduction of the external Na^+ concentration from 100 to 80 mM would significantly affect the response of V_a to mucosal lysine.

In some experiments 10 mM L-alanine (alanine) or 10 mM L-leucine (leucine) was added to the mucosal bathing medium. When this was done, the mannitol content of this medium was adjusted to maintain constant osmolality. The pH of all the bathing solutions was adjusted to 7.3 with Tris and all solution reservoirs were bubbled with 100% O_2 throughout each experiment.

Open-tip microelectrodes were used to measure V_a in absorptive cells. A Brown-Flaming microelectrode puller (Sutter Instrument Co., San Francisco, CA) was used to pull these microelectrodes from borosilicate glass tubing (Kwik-Fil 1B120F glass capillaries, WPI, Inc., New Haven, CT). Before pulling, capillaries were cleaned by overnight immersion in 1 M HNO_3. Following this, they were thoroughly rinsed with distilled water and air dried in an oven. Microelectrodes were back filled with 3 M KCl. When immersed in the control Ringer's solution used in this study they had tip resistances that ranged approximately from 100 to 160 mΩ. As reported earlier [15], with microelectrodes having tip resistances in this range, no apparent effects on cell potentials due to KCl leakage [16] were found in the present study.

To impale cells, a microelectrode was attached to the slave cylinder of a Narishige MO-10 hydraulic micromanipulator. This cylinder was, in turn, attached to a Narishige MM-33 manual micromanipulator. The MM-33 was used to bring the microelectrode close to a villus at a point near the villus tip. This was done under microscopic observation. Final advancement of the microelectrode and cell impalement though the apical membrane were effected with the MO-10 micromanipulator. The criteria used to determine the acceptability of impalements were those described by Garcia-Diaz et al. [15].

Electrical measurements were made as described by Garcia-Diaz et al. [15,17]. Briefly, the transepithelial potential (V_T) was monitored by two calomel half cells conected through 3 M KCl agar bridges to the mucosal and the serosal bathing solution, respectively. The tissue was maintained in the open-circuit mode except when transepithelial current (I_T) pulses were applied via two silver wire rings built into the chamber. The

microelectrode was connected through an Ag/AgCl wire and a guarded coaxial cable to a high impedance ($> 10^{15}$ Ω) FET electrometer (Analog Devices 515L) with capacitance neutralization. An electronic current-clamp device [17] was used to control the I_T pulses and to calculate the transepithelial resistance, R_T, and the fractional apical voltage ratio, F_{Va}. The latter quantity is the ratio ($\Delta V_a/\Delta V_T$) of the changes in apical membrane potential and transepithelial potential produced by an I_T pulse. I_T pulses had a duration of 1.1 s. The interval between successive pulses was 2.2 s. Pulse intensity was 15 μA (39.5 $\mu A/cm^2$) in the serosal to mucosal direction. During the intervals between I_T pulses, the current-clamp device delivered a second series of pulses (0.5 μA, 0.7 s) through the microelectrode. The deflections in V_a induced by these provided a continuous record of microelectrode resistance (R_{el}) during an experiment (see, for example, Fig. 1). Virtual constancy of F_{Va} during impalement and of R_{el} before, during, and after impalement are important factors in assessing the validity of intracellular potential recordings in leaky epithelia such as the small intestine and the gallbladder [15,17].

All electrical potentials were measured with respect to the grounded mucosal bathing solution. Numerical values of R_T, V_T, V_a, and F_{Va} were continuously displayed on digital panel meters. These parameters were also recorded simultaneously on a four-channel strip-chart recorder (Gould-Brush, Model 2400S). The current-clamp device provided automatic compensation of R_T and F_{Va} for external solution resistance.

Numerical results are presented as mean values $\pm$ S.E. These are followed in parentheses by the number of animals (tissues) involved and, where appropriate, the total number of microelectrode impalements. The Student's t-test was employed to analyze the significance or otherwise of observed differences between sets of data. The number of animals was used to make these comparisons. Where more than one successful impalement was made with a single intestinal preparation, an average value was included in the final calculations.

Results

Initial experiments were performed with 10 mM lysine and were designed primarily to study the effect of lysine, added to the mucosal medium, on V_a in *Necturus* intestine together with the Na^+ dependence of any effect observed. In these experiments the electrical responses of V_a to lysine

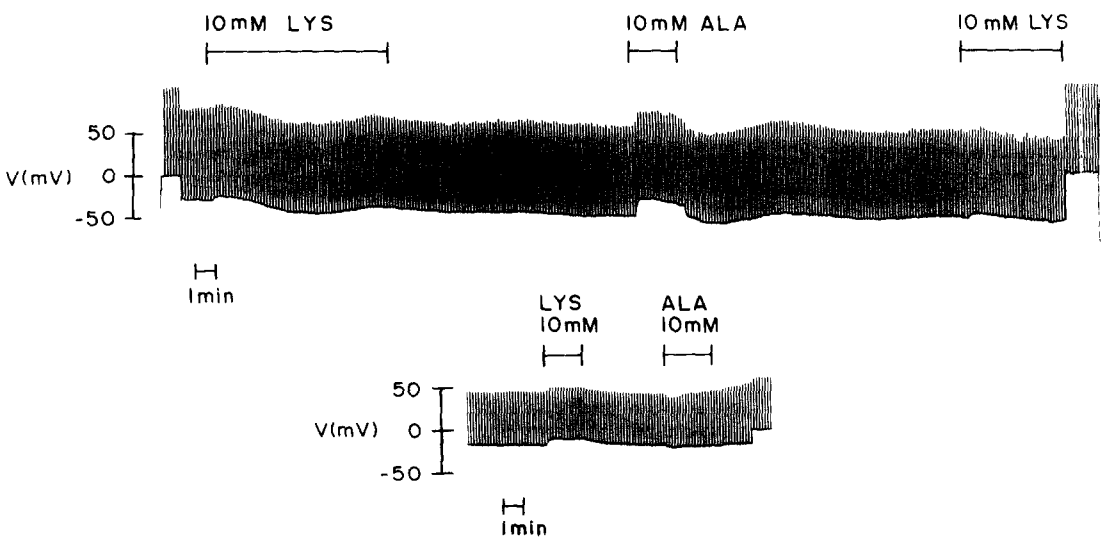

Fig. 1. Upper tracing: effects of 10 mM lysine and 10 mM alanine (added to the mucosal medium during the times shown by the horizontal bars above the record) on V_a in *Necturus* intestine in the presence of 100 mM external Na^+. Lower tracing: A similar experiment performed in the absence of external Na^+. The vertical bars on each tracing represent the response of V_a to periodic pulses of current through the microelectrode and are a measure of the constancy of the microelectrode resistance throughout the impalements (for details see text).

and to the neutral amino acid L-alanine (alanine) were compared under the same conditions. The electrophysiological effects of alanine on *Necturus* intestine have been well documented by Schultz and his collaborators [18,19].

The results of two of these experiments are shown in Fig. 1. In one experiment (upper tracing) the effects of lysine and alanine on V_a were compared during a single impalement in a tissue bathed by a medium containing 100 mM Na^+. During this impalement, a single exposure of the mucosal surface of the tissue to 10 mM alanine was bracketed by two similar exposures to 10 mM lysine. The results obtained during exposure of the tissue to alanine were essentially similar to those reported by Gunter-Smith et al. [18]. First there was a relatively rapid depolarization of V_a. This reached a maximum value ($\Delta V_a'$), following which V_a repolarized more slowly towards its initial value. The effect of 10 mM lysine on V_a was qualitatively similar to this, i.e. an initial depolarization followed by a slower return to the initial value. (The slow repolarization of V_a, following its initial depolarization by alanine has been extensively discussed by Schultz and his collaborators [18, 19] who have argued convincingly that it is due to changes in the electrophysiologic properties of the basolateral cell membrane. Since the present study was concerned primarily with lysine entry across the brush-border membrane of the absorptive cell, the analogous repolarization of V_a in the presence of lysine (Fig. 1) was not investigated in detail.) However, in the experiment shown in Fig. 1 (upper tracing), the $\Delta V_a'$ value induced by 10 mM lysine appeared to be considerably smaller than that elicited by the same concentration of alanine. (Following removal of alanine and after repolarization to the control level in the presence of lysine (Fig. 1: upper tracing) V_a hyperpolarized transiently. This was also noted in other experiments but was not studied in detail.) This was confirmed in further experiments of the same kind, i.e. where the tissue was exposed to alanine and to lysine during a single impalement. (In these experiments, the order in which the tissue was exposed to alanine and to lysine was randomized.) The results obtained were as follows: Following exposure of the mucosal surface of the tissue to 10 mM alanine, V_a depolarized from -39 ± 3 to -23 ± 3 mV ($\Delta V_a' = 16 \pm 4$ mV, $n = 6, 7$). In the presence of 10 mM lysine, a significantly smaller depolarization of V_a (-37 ± 4 to -31 ± 4 mV, $\Delta V_a' = 6 \pm 1$ mV, $n = 5, 5$) was observed.

The lower tracing in Fig. 1 shows an experiment similar to the one illustrated by the upper tracing of this figure, except that, in the experiment shown in the lower tracing NaCl, in the bathing media was completley replaced by Tris chloride. Inspection of this tracing clearly shows that the initial depolarization, followed by a slower repolarization, of V_a by 10 mM lysine persists in the absence of external Na^+. On the contrary, as one would expect [2], removal of external Na^+ completely abolished the effects of 10 mM alanine on V_a. The complete Na^+ dependence of the changes in V_a induced by alanine was confirmed in two further experiments with different tissue preparations. On the other hand, in seven impalements with three tissues, lysine induced a $\Delta V_a'$ of 6 ± 1 mV in the absence of external Na^+. This was identical to the corresponding value of $\Delta V_a'$ observed in solutions containing 100 mM Na^+. However, because of the small sample sizes involved, this agreement might be to some extent fortuitous.

These results indicate the presence, in the brush-border membrane of *Necturus* intestine absorptive cells, of one or more Na^+-independent rheogenic entry processes for lysine. However, they provide no information concerning the specific nature of these processes and are, in fact consistent with several different explanations. They could be interpreted as indicating that lysine entry into the cell, both in the presence and in the absence of Na^+, occurs via one or more simple diffusive pathways. Alternatively they could be construed as reflecting the presence, in *Necturus* intestine brush-border membranes, of Na^+-independent carrier-mediated systems for lysine transport analogous to those described for the small intestine of the rabbit [6]. Finally they could, despite the aparent similarity of the results initially obtained in the presence and in the absence of Na^+, be due to the operation of different lysine transport systems under these two sets of conditions.

Additional experiments were designed to distinguish between these possibilities. To determine if rheogenic lysine entry in the absence of Na^+ is

diffusive or carrier-mediated, the following approach was chosen. By analogy with the results reported for rabbit intestine [6] it was reasoned that if lysine entry into *Necturus* intestine under these conditions occurs, in whole or in part, via a carrier-mediated mechanism, neutral amino acids might compete with lysine for this entry pathway. Moreover, since the effects of neutral amino acids on the electrical parameters of the small intestine are completely Na^+ dependent [2], one would anticipate that, in the absence of external Na^+, competition by a neutral amino acid for the lysine transporter in the brush-border membrane would reduce or abolish lysine-induced depolarization of V_a. L-Leucine (leucine) which is known to inhibit mucosal lysine influx in rabbit intestine under Na^+-free conditions [6] was used to test this prediction. Since the effects of leucine alone, when added to the mucosal bathing medium, on V_a in *Necturus* intestine did not appear to have been reported, initial experiments were performed to show that this amino acid did in fact elicit the expected Na^+-dependent depolarization of V_a. In media containing 100 mM Na^+, addition of 10 mM leucine to the mucosal solution depolarized V_a from -35 ± 1 to -18 ± 3 mV ($n = 2$, 8). In the absence of external Na^+ the depolarizing effect of leucine was completely abolished. Under these coditions V_a remained unchanged at -30 ± 1 mV ($n = 2$, 10) when 10 mM leucine was added to the mucosal bathing medium. Of more immediate consequence to the pesent study was the finding that, in the absence of external Na^+, incorporation of 10 mM leucine into the mucosal bathing medium completely abolished the depolarizing effect of 10 mM lysine on V_a ($\Delta V_a' = 0.5 \pm 1$ mV: $n = 5$, 7). This strongly indicates that, in *Necturus* intestine, as in rat proximal tubule [13], rheogenic lysine entry occurs via a carrier-mediated mechanism rather than by simple conductive pathways.

To determine if a single Na^+-independent mediated transfer process is responsible for rheogenic lysine entry both in the presence and in the absence of Na^+, the response of V_a to mucosal lysine concentrations ranging from 5 mM to 30 mM was examined under three sets of conditions. These were, (i) a high external Na^+ concentration (100 or 80 mM), (ii) complete absence of external Na^+ and, (iii) a low external Na^+ concentration (5 mM). It was reasoned that if rheogenic lysine

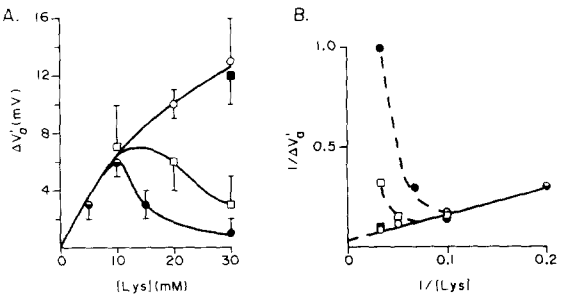

Fig. 2. (A) Effect of [Lys] on $\Delta V_a'$ (the maximum lysine-induced depolarization of V_a) at various external Na^+-concentrations. ○, 100 or 80 mM Na^+; ●, Na^+-free media; □, 5 mM Na^+; ■, 10 mM Na^+. Vertical bars correspond to ±S.E. (B) Double-reciprocal plot of the data shown in (A).

entry is mediated by a single saturable transport process under all three conditions then one would expect the relationship between $\Delta V_a'$ and mucosal lysine to be independent of external [Na^+]. The results of these experiments are shown in Fig. 2A. It is apparent from this figure that, in the presence of 80 mM or 100 mM Na^+, $\Delta V_a'$ increased continuously but non-linearly with increasing [Lys]. This is consistent with a single saturable process for lysine entry under these conditions and agrees with the results of similar electrophysiologic studies on the uptake, in the presence of high luminal Na^+ concentrations, of basic amino acids from the lumen of the proximal renal tubule in the newt [12] and the rat [13]. This conclusion is supported by the double-reciprocal plot, shown in Fig. 2B, of the data from the upper curve of Fig. 2A. This plot gives a $\Delta V_{max}'$ of 25.6 mV and a K_m of 31 mM for the rheogenic lysine entry process.

In Na^+-free media a more complex biphasic dependence of $\Delta V_a'$ on mucosal lysine concentration was found (bottom curve of Fig. 2A). Under these conditions $\Delta V_a'$ at first increased with increasing [Lys] until it reached a maximum at about 10 mM lysine. When [Lys] was further increased $\Delta V_a'$ declined again until, at 30 mM lysine, its average value (1 ± 0.5 mV, $n = 6$, 7) did not differ significantly from zero. At 5 mM external Na^+ (Fig. 1A, middle curve) the relationship between $\Delta V_a'$ and [Lys] was still biphasic but was intermediate between those found at high external Na^+ concentrations and in Na^+-free media, respectively. As indicated in Fig. 2A, when external Na^+ was increased to 10 mM, $\Delta V_a'$ in the presence of 30 mM lysine (12 ± 2 mV, $n = 4$, 4) did

not differ significantly from its value (13.3 ± 3 mV, $n = 5, 9$) at an external Na^+ concentration of 80 mM. Because of this, results obtained at external Na^+ concentrations of 80 and 100 mM, respectively, were considered to be equivalent (Figs. 2A and 2B).

Discussion

At the outset, it should be emphasized that the present study is concerned only with those mechanisms for lysine entry across the brush-border membranes of absorptive cells in *Necturus* intestine that are rheogenic, i.e. that involve net change transfer across the membrane and can therefore be considered as current generating events. Electroneutral modes of lysine entry across the brush-border membrane (e.g. coupled entry by 1/1 cotransport with an anion such as Cl^- or OH^- or by 1/1 exchange for a cation such as K^+ or H^+) can neither be detected nor excluded by studies such as those described herein. Within these limitations, the results presented in this paper clearly indicate that low concentrations of lysine (≤ 10 mM), when added to the mucosal bathing medium, depolarize V_a in absorptive cells of *Necturus* intestine and that the depolarizing effect of Lys does not depend on external Na^+. This finding can be interpreted in a straightforward way in terms of a rheogenic entry of lysine across the brush-border membrane of the intestinal absorptive cells. Moreover, the fact that, in the absence of external Na^+, the depolarizing effect of lysine on V_a can be completely inhibited by the neutral amino acid leucine strongly indicates that rheogenic lysine entry under these conditions occurs predominantly via a carrier-mediated mechanism (or mechanisms). Thus, as reported for the rat proximal tubule [13], simple diffusive entry appears to play little if any role in the uptake of lysine across the brush-border membrane in *Necturus* intestine.

In the presence of high external Na^+ concentrations, the relationship between $\Delta V_a'$ and mucosal [Lys] shown in Fig. 2A indicates that rheogenic lysine entry across the brush-border membrane of *Necturus* intestine is also carrier mediated. Fig. 2B further indicates that, under these conditions, lysine entry is due to a single saturable process that obeys simple Michaelis-Menten kinetics, i.e. a process where the final response observed ($\Delta V_a'$) is mediated by the degree to which a single population of receptor sites in the brush-border membrane is occupied by lysine molecules. A similar model has been proposed for basic amino acid uptake, in the presence of high luminal Na^+ concentrations, from the lumen of the proximal renal tubule [12,13]. In the absence of external Na^+ and at mucosal [Lys] values ≤ 10 mM, the relationship between $\Delta V_a'$ and [Lys] was essentially similar to that observed with high external Na^+ concentrations (Fig. 2A). However, under these conditions when [Lys] was increased progressively beyond 10 mM it appeared to become increasingly self-inhibitory towards the transport process responsible for its rheogenic entry across the brush-border membrane of the absorptive cells. A similar pattern was observed at an external Na^+ concentration of 5 mM except that self-inhibition by lysine occurred over a higher range of lysine concentrations (Fig. 2A). At an external Na^+ concentration of 10 mM the response of V_a to 30 mM lysine was identical to that observed at 80 mM Na^+.

Thus, overall, the data shown in Fig. 2A appear to be consistent with a single carrier-mediated transport process that is responsible for rheogenic lysine entry across the brush-border membrane in *Necturus* intestine both in the presence and in the absence of external Na^+. In the absence of external Na^+, high concentrations of lysine inhibit the entry process but this inhibitory effect can be overcome by the addition of Na^+ to the external medium. Although the range of observations shown in Fig. 2A is somewhat restricted for detailed graphical analysis * a double-reciprocal plot

* In particular, points corresponding to very low (< 5 mM) values of [Lys] are lacking in Fig. 2A. Extension of the present study into this range of [Lys] was not attempted for two reasons. First, one would expect that $\Delta V_a'$ values under these conditions should be very small (< 3 mV) and that the technical uncertainties in their measurement would be proportionately large. Second, because of the slow repolarization of V_a (following its initial depolarization) in the presence of mucosal lysine (Figs. 1A and 1B) $\Delta V_a'$ is, at best, an approximate index only of the rate of rheogenic lysine entry across the brush-border membrane. Under conditions where $\Delta V_a'$ is small and the rate of depolarization of V_a can be expected to be correspondingly slow the error introduced by this approximation could seriously compromise any attempt at detailed analysis of the data.

such as that shown in Fig. 2B suggests a reasonable model for rheogenic lysine entry in this tissue that can serve as a basic for further investigation. This plot (Fig. 2B) is strongly reminiscent of the kinetics of enzyme reactions that are subject to substrate inhibition of the kind described by Webb [20] as Type A. This kind of substrate inhibition typically arises where the formation of an active enzyme-substrate complex involves multipoint attachment of the substrate to the active center of the enzyme molecule and where, at high substrate concentrations, two or more substrate molecules may bind to single binding sites in the active center and form inactive complexes.

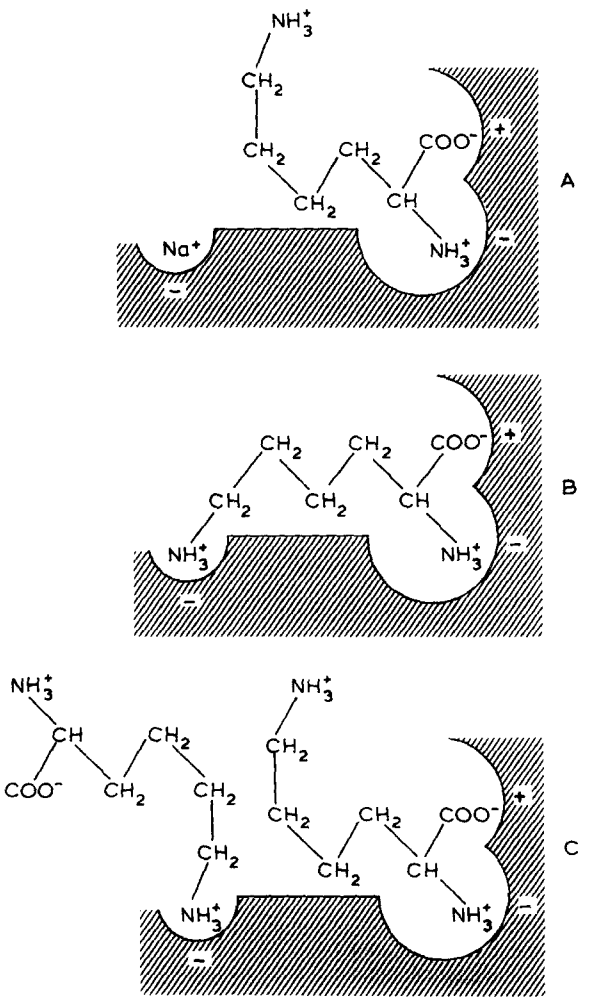

Fig. 3. Schematic model for rheogenic lysine entry across the brush-border membrane of *Necturus* intestine. For details see text.

Based on this idea and on a mechanism for Na^+-coupled amino acid entry into Ehrlich ascites cells proposed by Christensen [21,22], a model for rheogenic lysine entry across the brush-border membrane of *Necturus* intestine can be proposed by which all the data presented in the present paper can be explained, qualitatively at least, in terms of a single saturable entry process. This model is illustrated schematically in Fig. 3. Its essential features are as follows: An amino acid transporter in the brush-border membrane with two separate binding sites is proposed. One site is specific for the α-amino-α-carboxylic group of amino acids. Neutral (e.g. alanine, leucine) and basic amino acids can compete for this site. The other site has a high affinity for Na^+ but is also assumed to have some affinity for the ϵ-amino group of basic amino acids like lysine.

In terms of this model, the characteristics of rheogenic lysine entry across the brush-border membrane reported herein may be visualized as shown in Fig. 3. At normal physiological Na^+ concentrations (Fig. 3A), the Na^+ site is essentially saturated. Therefore, under these conditions, formation of a mobile complex between an amino acid and the transporter molecule (and its electrophysiologic consequences, e.g. $\Delta V_a'$) would be expected to depend on mucosal amino acid concentration in a simple saturable fashion (Figs. 2A and 2B). In the absence of external Na^+ the formation of a complex between a neutral amino acid (e.g. leucine) and the transporter would leave the Na^+ site unoccupied. (Since no direct measurements of alanine or leucine transport were made in this study, the mobility of such a complex in the membrane remains an open question.) On the other hand, under these conditions, lysine can form a complex in which a single lysine molecule is attached to both sites and that has a finite mobility in the membrane (Fig. 3B). Thus the depolarization of V_a by low (≤ 10 mM) concentrations of lysine in the absence of external Na^+ is readily explained. As the concentration of lysine in the mucosal medium is progressively increased under these conditions one can visualize a situation in which an increasing number of transporters have their amino acid and their Na^+ sites occupied by different lysine molecules (Fig. 3C). If complexes of this type, because of steric

factors or for other reasons, have zero or near zero mobility in the membrane the 'self-inhibitory' effect of increasing [Lys] in the absence of Na^+ (Figs. 2A, 2B) is readily understood. The model diagrammed in Fig. 3 can also explain the remaining two major observations reported in this study, the blockade by 10 mM leucine of the depolarizing effect of 10 mM lysine on V_a in the absence of Na^+ and the displacement by a low external Na^+ concentration towards higher [Lys] of the domain in which the self-inhibitory effects of this compound become apparent (Fig. 2A, middle curve). The latter observation can be interpreted in terms of competition between the ϵ-amino group of lysine and Na^+, at low concentrations, for the Na^+ site in the amino acid transporter.

Assuming that the model shown in Fig. 3 also applies to the rheogenic transport of basic amino acids in other leaky epithelia, a possible explanation for an apparent discrepancy observed in the electrophysiologic studies of Hoshi et al. [12] and of Suzuki and Fromter [13] emerges. To recapitulate, Hoshi et al. [12] found that in the proximal tubule of the newt, lysine when added to the luminal perfusate depolarized the peritubular cell membrane potential both in the presence and in the absence of luminal Na^+. In similar experiments Suzuki and Fromter [13] observed that the depolarizing effect of basic amino acids (added to the luminal perfusate) on the peritubular cell membrane potential in rat proximal tubule was absolutely dependent on luminal Na^+. However, in both these studies single concentration only of basic amino acids were tested in the absence of luminal Na^+. If one assumes that, in these systems, the relationship between ΔV and the concentration of the amino acid in the absence of Na^+ is qualitatively similar to that shown in Fig. 2A but that this relationship can vary quantitatively for different tissues and different amino acids, these apparently contradictory results are readily reconciled in terms of the model shown in Fig. 3.

Clearly, much additional work will be required to establish the quantitative role played by the mechanism proposed in Fig. 3 in the overall uptake of lysine by absorptive cells of *Necturus* intestine, particularly in view of the complex array of transport pathways that appear to be involved in cationic amino acid transport across the brush-border membrane of mammalian intestine [6]. The model outlined in Fig. 3 does, however, pose a number of questions that are directly accessible to experimental study. Among these are the following: The mechanism illustrated in Fig. 3A is in all essentials, similar to accepted models for Na^+-dependent entry of neutral amino acids into the absorptive cells of the small intestine [2]. Therefore, it may be anticipated that the driving force for lysine transport in this mode will be the sum of the electrochemical potential gradients for Na^+ and for lysine across the brush-border membrane, i.e. in the presence of high external [Na^+] rheogenic lysine entry into the absorptive cell should be, in principle, accumulative. In other words, under these conditions, steady-state intracellular [Lys] may be anticipated that are above the level corresponding to a passive distribution of lysine across the brush-border membrane. On the other hand, Fig. 3B implies that the driving force for lysine entry in the absence of external Na^+ is its own electrochemical potential gradient across the brush-border membrane. Therefore, in the limit, the maximum intracellular [Lys] achievable through this mode of entry should correspond to passive distribution of lysine across the brush-border membrane as defined by the Nernst equation. Finally, in the absence of Na^+, a direct inhibitory effect of lysine on its influx across the brush-border membrane of the absorptive cell should be demonstable under appropriate conditions.

Acknowledgment

These studies were supported by USPHS grant AM 12715.

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