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CHARACTERISTICS OF CHANGES IN THE INTRACELLULAR POTENTIAL ASSOCIATED WITH TRANSPORT OF NEUTRAL, DIBASIC AND ACIDIC AMINO ACIDS IN *TRITURUS* PROXIMAL TUBULE

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SUMMARY

(1) Introduction of L-alanine and L-lysine into the lumen of the proximal tubule of *Triturus* kidney evoked an immediate and sustained depolarization of the peritubular membrane potential (E_{pm}) and a small increase in the transtubular potential (E_{tt}). L-Aspartate had no effect.

(2) The alanine-induced depolarization was absolutely dependent on the presence of Na^+, whereas the lysine-induced one was partially dependent on Na^+. In the absence of Na^+, alanine usually evoked a transient hyperpolarization of the E_{pm}, while lysine evoked a diffusion potential-like PD change.

(3) Addition of alanine or lysine to the peritubular fluid did not cause any immediate change in the E_{pm}, but the cells depolarized with a marked time delay. The delayed depolarization could be ascribed to the entrance of amino acids into the lumen through the nephrostomes and the paracellular pathways.

(4) Cellular uptake of alanine and lysine was partially dependent on Na^+, while that of aspartate was completely dependent on Na^+.

(5) Characteristics of the observed electrical events were explained in terms of the differences in the charge transfer associated with transport of these amino acids across the luminal membrane.

INTRODUCTION

Active transport of sugars and neutral amino acids across the small intestine and renal proximal tubule has been shown to be associated with an increase in the transmural potential difference (PD) [1–6]. For elucidating the genesis of the PD change, exact information is needed concerning the location of an electrogenic process and charactristics of the electrical events across the cell membranes. In an attempt to gain such information, microelectrode studies have been made by many investigators in various animals [7–12]. However, previous studies have mainly dealt with sugars and neutral amino acids, while little is known about the electrical phenomena associated with transport of dibasic and acidic amino acids.

In the present study, characteristics of changes in the intracellular potential caused by transport of both dibasic and acidic amino acids were compared with those induced by neutral amino acid transport in *Triturus* proximal tubule. Kinetic properties of cellular uptake of test amino acids were also studied in order for elucidating observed characteristics of the electrical events. Preliminary accounts of portions of this work were given elsewhere [13, 14].

METHODS

Tissue preparation

The pelvic part of the kidney of the newt (*Triturus pyrrhogaster*) was used. After decapitation, the kidneys were rapidly removed and transferred into normal Ringer's solution in a small petri dish. The collecting ducts, Wolffian or Mullerian ducts and connective tissues were carefully removed under a stereomicroscope. The tissue was then transferred into a small lucite chamber which had a small paraffin plate on its bottom. The tissue was fixed on the paraffin plate with fine syringe needles. Inside the chamber was continuously perfused with fresh oxygenated Ringer's solution at a constant rate of 0.5 ml/min. The fluid volume of the chamber was about 5 ml. Temperature of the bath was regulated constant at 25 °C.

Microperfusion and PD measurements

At first, one of Bowman's capsules was punctured by a glass capillary pipette filled with normal Ringer's solution containing 0.05 % Lissamine green. The dye at such a low concentration had no detectable effect on both the spontaneous intracellular potentials and amino acid-induced PD changes. By injecting the colored solution, portions of a single nephron to be examined were determined. In most experiments, cellular impalement with a Ling-Gerard type microelectrode was carried out at a distance of 1–2 mm from the beginning of the proximal tubule. Another glass pipette, filled with a test solution, was then inserted into the same Bowman's capsule in order to infuse the test solution. The transmembrane PD across the peritubular membrane (E_{pm}) and the transtubular PD (E_{tt}) were recorded during alternate perfusion with control (amino acid-free Ringer's) and a test solution. The methods of recording of the E_{pm} and the E_{tt} have been described in detail in our previous papers [12, 15]. The intraluminal perfusion was performed by applying pressure of 20–50 mmHg to the perfusion pipettes depending on their flow resistances. The tips of the perfusion pipettes were bevelled and sharpened according to the method described by Windhager [16].

The ionic composition of the standard Ringer's solution used in the present study was the same as that employed in our previous studies [12, 15]. Three different Na^+-free Ringer's solutions were prepared by replacing NaCl totally with LiCl, Tris · Cl or osmotically equivalent mannitol. Low Na^+ solutions were prepared by mixing the standard solution with the Tris- or the mannitol-substituted Ringer's solution at desired volume ratios. In the experiments with the Na^+-free solutions, the tissues were preincubated in Na^+-free media for at least 30 min before the start of potential measurements. Preliminary experiments revealed that, during the incubation in Na^+-free media, tissue Na^+ content rapidly decreased with time, showing a half time of 6 min. However, a small amount of Na^+, 2–3 mequiv./kg tissue H_2O, still

remained in the tissue even after 2 h. Before intraluminal application of amino acids, the tubular lumen was flushed and perfused for several min with a Na^+-free control solution.

L-Alanine, L-lysine-HCl and sodium L-aspartate were mainly examined in this study, but in some experiments, glycine, L-leucine, L-arginine and L-glutamic acid were also tested. These amino acids (all reagent grade) were dissolved in various Ringer's solutions to prepare the test solutions. In the subsequent description, L- is omitted since only L-type amino acids were examined in this study. When an acidic amino acid was dissolved in Na^+-free solutions, Tris (base) was added to readjust pH of the solutions to 7.3. When high concentrations (20–40 mM) of amino acids were tested, mannitol was added to control solutions at the same concentrations.

Uptake experiments

Tissue uptake of amino acids was studied by incubating isolated unilateral pelvic kidneys in various test media. Each test amino acid was added to the incubation medium at a desired concentration together with its ^{14}C-labeled tracer (0.2 μCi/ml, final concentration). The extracellular space was determined with D-[^{14}C]mannitol in a separate experiment. The estimated value of mannitol space was 44.2 ± 2.1 ml/100 g tissue wet wt. (mean$\pm$S.E.). Such a relatively large mannitol space may be ascribed to uncollapsed renal tubules and the entrance of mannitol into the lumen during a prolonged in vitro incubation. It was a usual finding that vivid ciliar movements around the nephrostomes persisted and most of Bowman's capsules remained expanded for many hours. Also it was frequently seen that a colored solution injected into the proximal tubule spontaneously moved toward the collecting duct. These findings imply that mannitol as well as test amino acids added to the peritubular fluid can enter the lumen through the nephrostomes and flow toward the distal portions. The entry through the paracellular pathways is also considered to take place since this paths of the proximal tubule of *Necturus*, an animal similar to *Triturus*, have been shown to have a measurable permeability to sucrose [17].

In the presence of Na^+, cellular uptake of amino acids linearly increased with time up to 90 min after the start of incubation at 25 °C. Based on this finding, the tissues were incubated for 60 min at 25 °C in order to determine the rate of uptake. At the end of incubation, the tissues were picked up from the incubation media and rinsed shortly (10 s) with a control solution. After blotted on filter paper and weighed on a torsion balance, each sample was placed in 1 ml extraction fluid (0.1 N HNO_3) for about 24 h. The radioactivities of the extraction fluids were counted in a liquid scintillation counter (Packard LSC-3324 or Aloka LSC-601). The identification of the amino acids taken up by the tissue was not carried out in the present study.

RESULTS

Effects of amino acids introduced into the tubular lumen

Experiments in the presence of Na^+. Fig. 1 shows typical examples of changes in the E_{pm} induced by infusions of 10 mM solutions of alanine, lysine and aspartate. Both alanine and lysine always evoked a marked depolarization of the E_{pm}, while aspartate did not cause any significant PD change. Alanine always evoked a larger depolarization than lysine.

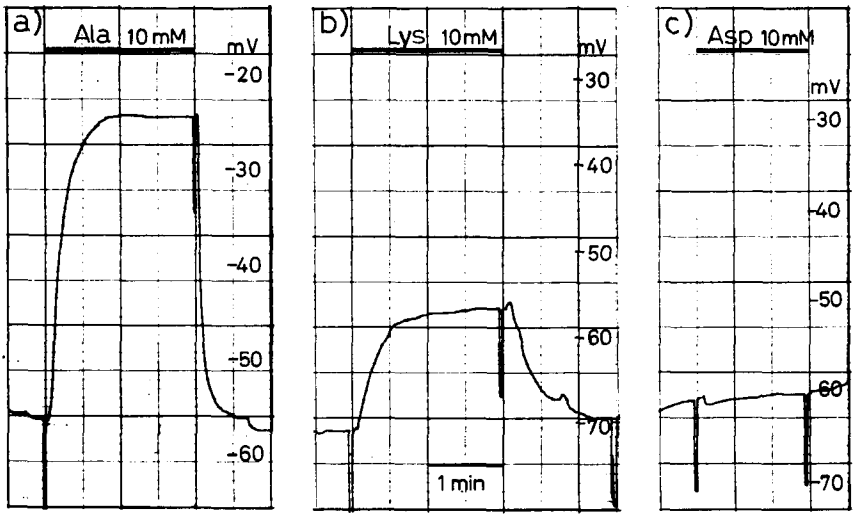

Fig. 1. Effects of alanine, lysine and aspartate on the transmembrane PD across the peritubular membrane (E_{pm}) of *Triturus* proximal tubule. Each amino acid was introduced into the tubular lumen by microperfusion techniques during a period indicated by a horizontal bar. Vertical bars indicate the time of switching from control to a test solution, or vice versa, and also the voltage calibration of 10 mV. Figures on the right side of each panel indicate the level of the E_{pm} with respect to the peritubular fluid.

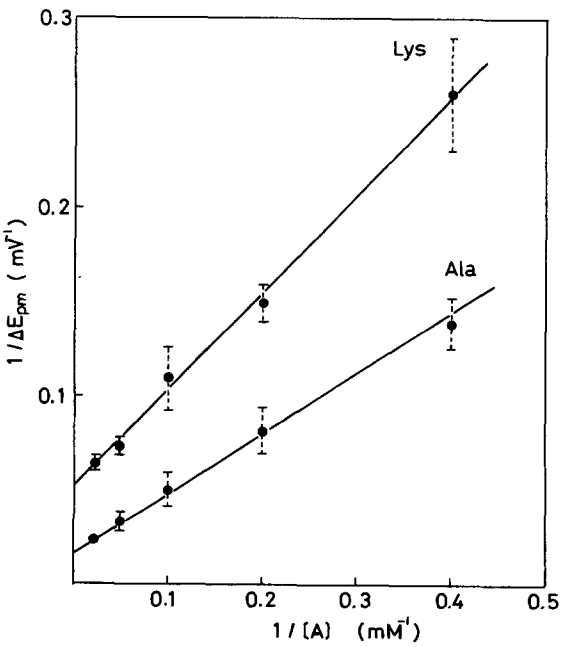

Fig. 2. Double-reciprocal plots of the amplitude of the amino acid-evoked depolarization (ΔE_{pm}) against intraluminal amino acid concentration ([A]).

The depolarization occurred immediately after the start of infusion of alanine or lysine. The PD change reached a maximum within about 1 min, thereafter the level of the E_{pm} was sustained for many minutes as long as the infusion was continued. On switching to control solution, the E_{pm} rapidly returned (repolarized) to the initial level. In the case of alanine, the repetition of alternate infusions of control and a test solutions caused a gradual increase in not only the amplitude of the depolarization but also the initial level of the E_{pm}. But both became stabilized after switching several times. The amplitude of the depolarization, therefore, was read after the stabilization. Lysine had no such initial augmenting effects.

The amplitude of the depolarization was dependent on the intraluminal concentration of respective amino acid. For both amino acids, a saturable (Michaelis-Menten like) relationship was seen between the amplitude and the concentration, as shown in Fig. 2. The maximum height of the depolarization which is corresponding to V was 62.5 mV for alanine and 18.5 mV for lysine. The values of the half saturation concentration (K_t) were 20 mM for alanine and 8.7 mM for lysine. Both alanine and lysine concurrently increased the E_{tt}, (the lumen became more negative with respect to the peritubular fluid). The ratios of changes in the E_{tt} to those in the E_{pm}, $\Delta E_{tt}/\Delta E_{pm}$, were about the same as that for glucose (0.12) which was estimated in a previous study [12].

Experiments in the absence of Na^+. Both the luminal and the peritubular sides were perfused with the LiCl-, Tris · Cl- or mannitol-substituted Ringer's solution, and one of test amino acids was introduced into the lumen.

The changes in the E_{pm} induced by alanine, lysine and aspartate were summarized in Table I. There were marked differences in effect among these amino acids. The characteristics of the observed responses can be summarized as follows: (1) Alanine induced a depolarization only in the presence of Na^+, while in the absence of Na^+, alanine induced a PD change in the opposite direction (hyperpolarization). (2) Lysine evoked a depolarization even in the absence of Na^+. (3) Aspartate did not cause any significant PD change also in the absence of Na^+.

There were characteristic features in both the hyperpolarization induced by alanine and the depolarization by lysine seen in Na^+-free media. These responses failed to form a plateau of the E_{pm} during the constant infusion of respective amino

TABLE I

AMPLITUDES OF CHANGES IN THE E_{pm} CAUSED BY ALANINE, LYSINE AND ASPARTATE. EFFECTS OF TOTAL REPLACEMENT OF NaCl BY LiCl, Tris · Cl OR MANNITOL

Amino acids were introduced into the tubular lumen by microperfusion. The concentration of amino acids was fixed at 10 mM throughout experiments. The sign + denotes depolarization and − hyperpolarization. The figures are means ±S.E. (number of experiments).

Medium	Amplitude of evoked potential change (mV)		
	Alanine	Lysine	Aspartate
Normal Ringer	+21.8±2.9 (5)	+ 8.60±1.57 (8)	+0.46±0.66 (7)
Li^+-Ringer	− 0.8±0.3 (8)	+ 4.98±0.63 (10)	−0.85±0.54 (6)
$Tris^+$-Ringer	− 6.1±1.6 (5)	+13.25±1.84 (7)	−0.64±0.31 (5)
Mannitol-Ringer	−10.3±2.8 (4)	+14.77±3.13 (4)	

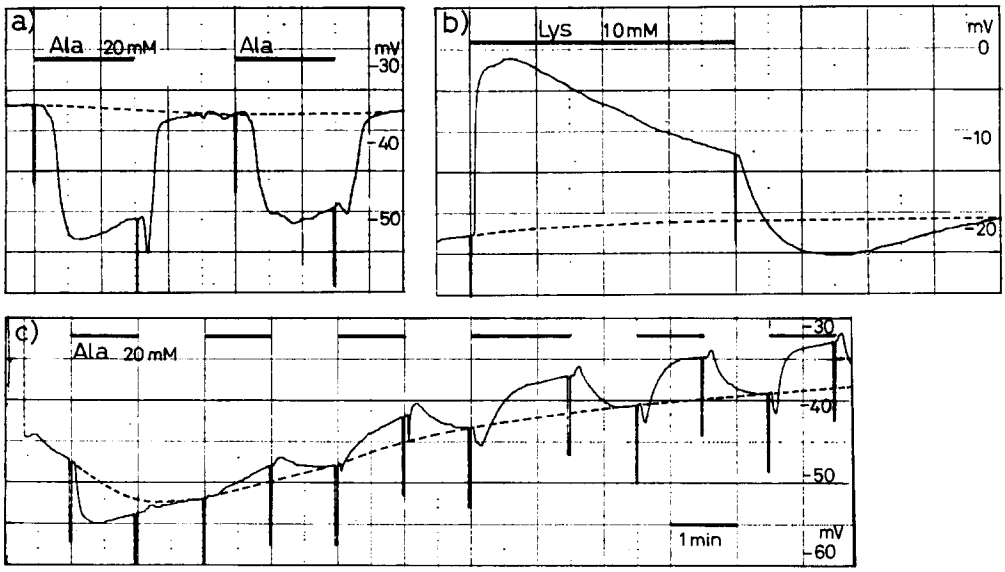

Fig. 3. Effects of alanine and lysine on the E_{pm} in the absence of Na^+ in the perfusion media. Each amino acid was infused during periods indicated by horizontal bars. During the other periods, control solutions were infused. (a) Alanine infusion test in the mannitol-media, (b) lysine infusion test in the mannitol media, (c) alanine infusion test in the Li^+ media.

acid, i.e., the E_{pm} spontaneously returned toward the initial level after reaching a maximum (Fig. 3). Furthermore, the amplitudes of the responses decreased when control and the test solutions were injected alternately and repeatedly. In these cases, therefore, the height of the responses had to be read at the first injection. In the Li^+ medium, the first injection of alanine usually caused a small hyperpolarization or no PD change, but subsequent repeated injections induced a small depolarization (Fig. 3C).

Effects of amino acids added to the peritubular fluid

Addition of the test amino acids into the peritubular fluid did not cause any immediate change in the E_{pm}. In the cases of alanine and lysine, however, the cells always depolarized after a some delay. The time course of the depolarization differed depending on the site of cellular impalement or from nephron to nephron. Three different types were observed: (1) The depolarization started within a few min and the E_{pm} rapidly attained to a maximally depolarized level (Fig. 4a). (2) The cells slowly and progressively depolarized to reach a maximum after several min (Fig. 4b). (3) Combination of the first and the second types, i.e. a slow progressive depolarization was followed by a rapid shift of E_{pm} to reach a maximum. The last type was usually seen in cells at distant portions beyond the usual sites of impalement.

The fully developed depolarization could not be abolished immediately by the rapid removal of the amino acids from the peritubular fluid. In contrast, flushing the lumen with control (amino acid-free) solution always resulted in a prompt and com-

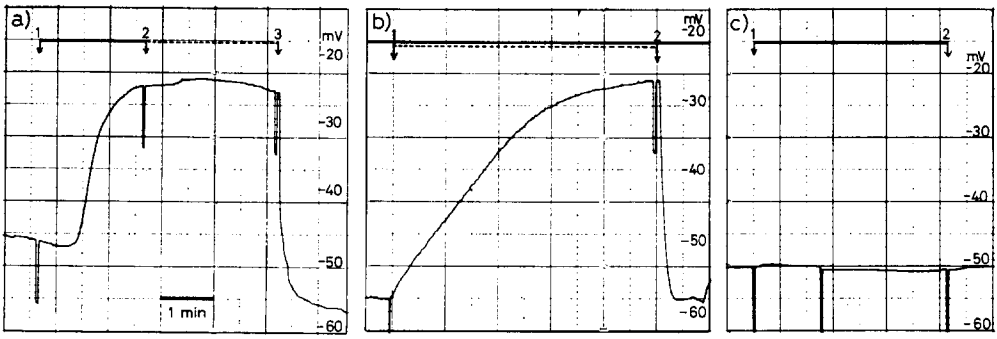

Fig. 4. Effects on the E_{pm} of alanine added to the peritubular fluid (normal Ringer's solution). (a) A typical example of tracings which showed a delayed onset but a rapid rising limb of depolarization. Alanine was added at the time indicated by arrow 1 and washed out at arrow 2. At the time of arrow 3, the lumen was flushed with control solution. (b) An example which showed slow progressive depolarization. The intraluminal perfusion with control solution was stopped at arrow 1 and resumed at arrow 2. (c) Effect on the E_{pm} of peritubularly applied alanine in an oil-filled proximal tubule. Alanine was added at arrow 1 and washed out at arrow 2.

plete repolarization of the E_{pm} to the initial level, as shown in Fig. 4. In the tubules, of which lumen was filled with mineral oil, such a delayed depolarization was never observed (Fig. 4c). These findings indicate that the depolarization caused by the amino acid added to the peritubular fluid is associated with the luminal membrane process of uptake of the same amino acid which have entered the lumen through the nephrostome and/or the paracellular pathways. The time course of the first type appears to be indicative of the dominant entrance through the nephrostome, whereas the second type is suggestive of the entrance of amino acids by diffusion mainly through the paracellular pathways. In either case, the maximum heights of the depolarization attained finally did not differ significantly from those obtained by direct introduction of the same amino acids into the lumen. These facts suggest that the main transport process responsible for cellular uptake of amino acids added to the peritubular fluid is the luminal membrane process.

There was no difference in effect between alanine and other neutral amino acids, e.g. glycine and leucine. Also no essential difference was noticed between lysine and arginine and between aspartate and glutamate, though the relevant data are not presented here.

Uptake experiments

Table II summarizes the data of uptake experiments carried out under normal ionic and Na^+-free conditions. Cellular uptake of alanine and lysine was partly dependent on the presence of Na^+, i.e., significant cellular uptake of these amino acids was seen in Na^+-free conditions. In contrast, aspartate uptake revealed a complete Na^+ dependence, namely, practically no cellular uptake was seen in the absence of Na^+.

Kinetic behaviour of cellular uptake of three amino acids at various external Na^+ concentrations was illustrated in Fig. 5, where the data were plotted double-reciprocally. Alanine uptake revealed the K-type kinetics (Fig. 5a), i.e. only the K_t

TABLE II

THE RATES OF CELLULAR UPTAKE OF ALANINE, LYSINE AND ASPARTATE. EFFECTS OF TOTAL REPLACEMENT OF NaCl BY LiCl, Tris · Cl OR MANNITOL

Tissue uptake of the amino acids from 5 mM solutions measured at 25 °C. Cellular uptake rates were obtained by correcting for the extracellular space (mannitol space). Values given are mean ±S.E. (number of kidneys examined).

Medium	Rate of cellular uptake (μmol · h^{-1} · g^{-1} wet wt.)		
	Alanine	Lysine	Aspartate
Normal Ringer	2.50±0.18 (11)	3.02±0.13 (6)	4.49±0.65 (7)
Li^+-Ringer	1.04±0.06 (4)	1.54±0.13 (4)	0.19±0.06 (5)
$Tris^+$-Ringer	1.05±0.03 (4)	1.80±0.16 (8)	0.01±0.01 (5)
Mannitol-Ringer	0.84±0.02 (4)	1.70±0.28 (4)	

was dependent on the Na^+ concentration, whereas aspartate showed the mixed-type kinetics, (Fig. 5b) i.e. both the V and K_t were dependent on the Na^+ concentration. As for lysine, the uptake in the presence of Na^+ could be described by a single carrier-mechanism. In contrast, the removal of Na^+ from the medium resulted in a complex change in kinetics, i.e., the double-reciprocal plots fell on a curve which crossed the zero origin (Fig. 5c).

The reciprocal of K_t for alanine was found to be a linear function of the external Na^+ concentration (Fig. 6). Thus, the kinetic behaviour of alanine uptake in the present preparations is apparently the same as that of alanine influx across the mucosal border of rabbit ileum [18]. When we use the kinetic model used by Curran et al [18] for rabbit ileum, the initial rate of cellular uptake of alanine (v) can be described by

$$v = V \cdot [A] \Big/ \left(\frac{k_1 k_2}{[Na]+k_2} + [A] \right) \qquad (1)$$

where V is the maximum rate of uptake which is independent of Na^+ concentration, k_1 and k_2 the dissociation constants of a carrier-alanine binary complex (X · A) and a

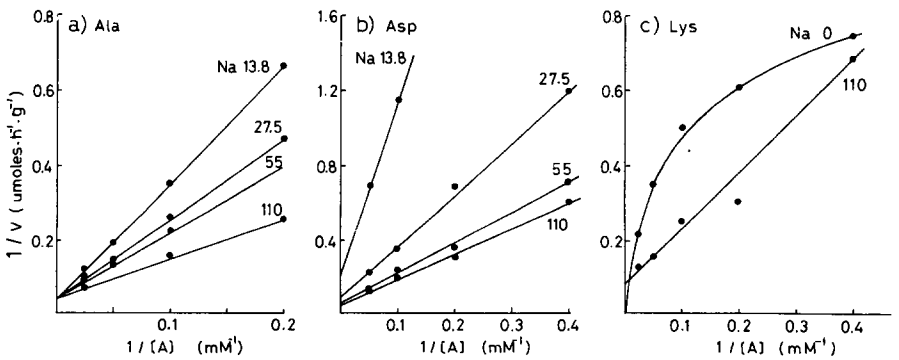

Fig. 5. Effects of medium Na^+ concentration on the rates of cellular uptake of alanine (a), aspartate (b) and lysine (c). Lineweaver-Burk plots. NaCl was replaced by Tris · Cl.

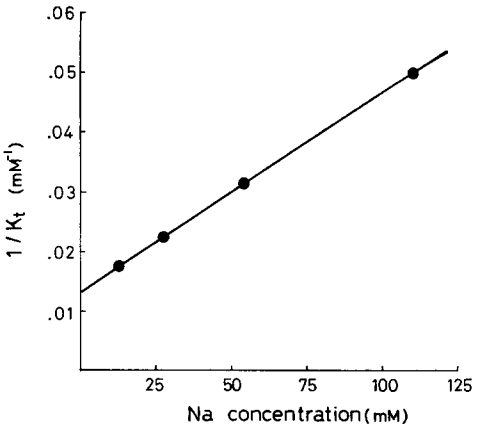

Fig. 5. Relationship between the reciprocal of K_t for alanine and medium Na^+ concentration.

carrier-alanine-Na^+ ternary complex ($X \cdot A \cdot Na$), [Na] and [A] the concentrations of Na and alanine, respectively. Calculated values of k_1 and k_2 were 74 and 42 mM, respectively. The value of k_1 is almost the same as that of the corresponding constant in rabbit ileum, whereas k_2 was about 2.5 times higher than that in rabbit ileum [20]. The value of K_t at normal Na^+ concentration (19 mM) was approximately the same as that determined by the electrical measurements (20 mM).

The kinetic behaviour of aspartate uptake in the present preparations resembled that of sugar influx across the mucosal border of rabbit ileum [19]. Fig. 7 shows the effect of Na^+ concentration on the V and K_t for aspartate. Based on the same kinetic model as that proposed by Goldner et al. [19] in order to describe sugar influx in rabbit ileum, the initial rate of uptake can be given by

$$v = \left(k' X_t \frac{[Na]}{k_3 + [Na]}\right) \cdot [A] \Big/ \left(\frac{k_1 k_2 + k_4 [Na]}{k_3 + [Na]} + [A]\right) \qquad (2)$$

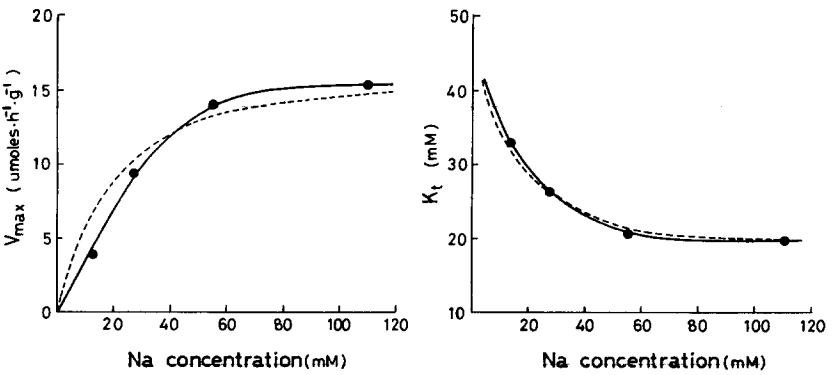

Fig. 7. Effects of medium Na^+ concentration on the V and K_t for aspartate. Closed circles indicate the actually estimated values. Dotted lines indicate the values calculated by using Eqn. 2 and values of 50, 10.3 and 17 mM for k_1, k_3 and k_4, respectively.

where k' is the rate constant of translocation of the ternary complex ($X \cdot A \cdot Na$) and the empty carrier, X_t the concentration of total carriers within the membrane, k_1 through k_4 the dissociation constants in four reactions; $X+A \rightleftharpoons X \cdot A$, $X+Na \rightleftharpoons X \cdot Na$, $X \cdot A+Na \rightleftharpoons X \cdot A \cdot Na$, $X \cdot Na+A \rightleftharpoons X \cdot A \cdot Na$. The values of k_1, k_3 and k_4 which gave the best fit of calculated values to actually obtained values of the V and K_t were 50 mM for k_1, 10.3 mM for k_3 and 17 mM for k_4. The obtained value of k_3 (10.3 mM), the dissociation constant of the reaction $X \cdot A+Na \rightleftharpoons X \cdot A \cdot Na$, was about the same as that of the corresponding constant for the same amino acid in rabbit ileum [20].

DISCUSSION

The results of the present study show that the transport of neutral and dibasic amino acids across the luminal membrane of *Triturus* proximal tubule is associated with marked changes in the intracellular potential, suggesting the involvement of an electrogenic process. In contrast, the transport of acidic amino acids across the same membrane was found to be entirely non-electrogenic.

In the presence of Na^+ in the extracellular fluid, neutral and dibasic amino acids introduced into the lumen induced a marked depolarization of the E_{pm} and a small increase in the E_{tt} (the lumen became more negative with respect to the peritubular fluid). The observed change in the potential profile across the tubule is essentially the same as that caused by glucose in the same tissue [12]. Such a profile change may be the result of current flow passing through the peritubular membrane in the outward direction which, in turn, flows through the paracellular shunt in the opposite direction. In view of the equivalent electrical circuit for *Triturus* proximal tubule [12], it is clear that such a flow of current is generated by an electromotive force (EMF) which is located at the luminal membrane and directed inwardly (from the lumen to inside the cell). The generation of such an EMF concurrently depolarizes the transmembrane potential across the luminal membrane (E_{1m}). As shown in a previous paper [12], the magnitude of the depolarization of the E_{1m} (ΔE_{1m}) is only slightly larger than that of E_{pm} (ΔE_{pm}). The ratio of ΔE_{1m} to ΔE_{pm} can be described as

$$\Delta E_{1m}/\Delta E_{pm} = (R_{pm}+R_s)/R_{pm} \tag{3}$$

where R_{pm} and R_s are the specific resistances of the peritubular membrane and the paracellular shunt, respectively. Under normal external ionic conditions, the ratio takes a value of 1.12 in *Triturus* proximal tubule [12] because of a considerably lower value of R_s than that of R_{pm}. Therefore, the change in the E_{pm}, as observed in the present study, can be taken to be the near equivalent for the change in the E_{1m}.

The depolarization of the E_{pm} induced by alanine was found to be absolutely dependent on the presence of Na^+ in the external medium, while cellular uptake of alanine was composed of both a Na^+-dependent and a Na^+-independent components. From these, the depolarization is considered to be a phenomenon related to the Na^+-dependent entry of alanine. All kinetic data obtained in this study can be explained on the basis of a transport model where alanine is assumed to be transported across the membrane via two types of carrier complexes, a binary ($X \cdot A$) and a ternary ($X \cdot A \cdot Na$) complexes. From these findings together with the observed pattern of the change in the potential profile, we can conclude that the translocation of the ternary complex

at the luminal membrane causes charge separation. In other words, the cotransport process at the luminal membrane is electrogenic.

In the absence of Na, alanine evoked a hyperpolarization of the E_{pm}. This response might be interpreted as a phenomenon related to the Na^+-independent entry of alanine into the cells. The foregoing discussion implies that the Na^+-independent entry of alanine itself is non-electrogenic. Therefore, this phenomenon needs additional explanation. Although a full explanation could not be provided in the present study, the following facts seem very suggestive. (1) The hyperpolarizing response is of transient nature. (2) In the Li^+-medium, the response is rapidly converted from hyperpolarization into depolarization by repeated infusions and washing out of alanine. (3) The level of the spontaneous E_{pm} remains unchanged before and after the generation of the hyperpolarization except in the Li^+ medium. All these facts suggest that some intracellular substances bearing a net positive charge go out into the lumen across the luminal membrane in association with Na^+-independent entry of alanine. The small depolarization evoked by alanine in the Li^+ medium can be explained in terms of the ability of Li^+ to partially substitute for Na^+ in the cotransport mechanism, as demonstrated in sugar-Na^+ cotransport [21, 22].

In contrast to alanine, lysine evoked a depolarization of the E_{pm} regardless of the presence of Na^+ in the external medium. Cellular uptake of lysine also showed a poor Na^+-dependence, as previously observed in rat cortical slices [23, 24]. As lysine carries a positive charge in the physiological range of pH, the Na^+-independent depolarization may be related to the Na^+-independent entry of this amino acid across the luminal membrane.

The configuration of the lysine-induced depolarization recorded in Na^+-free media differed from that recorded in the presence of Na^+. The distinct features are the failure of plateau formation during the constant infusion and a transient hyperpolarization upon removal of the amino acid from the luminal fluid. The overall configuration is very similar to that of the glucose-induced changes in the transmural PD of toad small intestine of which metabolism is inhibited [25]. In anoxia or in the presence of dinitrophenol, intestinal sugar transport system in toads fails to transport sugars actively, but the system behaves like a facilitated diffusion system for the coupled penetration of sugar and Na^+. The potential surges resembling the decaying depolarization followed by a transient hyperpolarization as observed in this study with lysine can be evoked by the addition of glucose and the removal of the added glucose from the mucosal solution. The second potential surge, corresponding to the hyperpolarization, was associated with simultaneous increase in outfluxes of sugar and Na^+ from the cells into the lumen. Therefore, it seems very likely that lysine transport without Na^+ is a kind of facilitated diffusion.

In the presence of Na^+, the lysine-induced depolarization forms a plateau, and the E_{pm} simply returns to the initial level when lysine is removed from the luminal fluid. The configuration of the PD tracing is very similar to that of the Na^+-dependent depolarization evoked by alanine. This fact and the foregoing discussion lead us to an interpretation that Na^+ plays an important role in the maintenance of a constant influx of lysine even in a situation of its increasing intracellular concentration. In other words, lysine movements across the luminal membrane undergo the rectification when Na^+ is present. Such an effect of Na^+ is also reflected in the cellular uptake of lysine as measured by 60 min incubation. As stated above, the uptake rate simply

obeyed Michaelis-Menten-like kinetics when Na^+ is present in the external medium. While, in the absence of Na, lysine uptake can not be described by a single carrier mechanism, and it exhibited complex kinetics involving a diffusional component. Such a role of Na^+ at the luminal membrane seems to be fundamentally important in the transcellular active transport of dibasic amino acids, which is known to absolutely require Na^+ in the external medium [26, 27].

The complete Na^+-dependence and entirely non-electrogenic nature of aspartate transport indicate that no net charge is transferred when this amino acid is transported across the luminal membrane. Kinetically, aspartate appears to be transported only when a ternary complex ($X \cdot A \cdot Na$) is formed within the membrane. This also explaines the non-electrogenic transport of this amino acid. Samarzija and Frömter [28] reported that, in rat proximal tubule, acidic amino acids introduced into the lumen caused a depolarization of E_{pm}. Their interpretation is that two Na^+ may be cotransported with each amino acid molecule across the luminal membrane.

The present study failed to demonstrate any electrogenic transport mechanism at the peritubular membrane for any of amino acids tested although at least four separate transport systems for amino acids are known to exist at this membrane [29]. This suggests that the process or processes of amino acid transport across this membrane are entirely different in mode from those across the luminal membrane, particularly in regard to charge transfer.

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