MEMBRANE-POTENTIAL-SENSITIVE, Na\(^+\)-INDEPENDENT LYSINE TRANSPORT BY LOBSTER HEPATOPANCREATIC BRUSH BORDER MEMBRANE VESICLES

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SUMMARY

Transport of L-\(^{3}\text{H}\)lysine by epithelial brush border membrane vesicles (BBMV) of lobster hepatopancreas, formed by a magnesium precipitation technique, was insensitive to transmembrane gradients of Na\(^+\), K\(^+\), TMA\(^+\) or H\(^+\). Apparent initial lysine entry rates (15 s uptake) and extent of amino acid accumulation against a concentration gradient (overshoot) were both stimulated by transmembrane anion gradients according to the following sequence: SCN\(^-\) > Cl\(^-\) > gluconate\(^-\). The magnitude of this anion-gradient-dependent transport was significantly increased by bilateral acidic pH. Lysine transport at acidic pH strongly responded to transmembrane potential developed by addition of valinomycin to K\(^+\)-loaded vesicles, or was markedly reduced if K\(^+\)-equilibrated vesicles were incubated with the ionophore in the presence of an inwardly directed SCN\(^-\) gradient. Lysine influx occurred by the combination of at least one carrier process and ‘apparent diffusion’. L-Arginine, L-alanine and L-leucine, added to the external medium, were all strong inhibitors of lysine influx. The first two were competitive inhibitors of lysine entry, while the latter was non-competitive in effect. These results suggest that lysine, arginine and alanine may share a common, Na\(^+\)-independent, membrane-potential-sensitive transport mechanism in lobster BBMV. Leucine transport may occur in these membranes by a separate agency.

INTRODUCTION

Epithelial transport of amino acids is performed by a heterogeneous assemblage of membrane proteins showing Na\(^+\)-dependent and Na\(^+\)-independent requirements. In vertebrate intestinal and kidney epithelial brush border membranes, multiple Na\(^+\)-dependent transport pathways for neutral amino acids are present and largely employ potential energy inherent in the transmembrane sodium gradient for cellular accumulation of the organic solutes (Mircheff, Kippen, Hirayama & Wright, 1982; Stevens, Ross & Wright, 1982). One or more Na\(^+\)-independent protein systems are also present in these epithelial membranes for neutral amino acids as well as for cationic or dibasic forms (Stevens et al. 1982), but the substrate specificities and

Key words: lysine transport, brush border membrane vesicles, Na\(^+\)-dependence, membrane potential, ion gradients, Homarus americanus, hepatopancreas.
driving forces associated with these transport mechanisms remain to be clarified (Hammerman, 1982; Cassano, Leszczynska & Murer, 1983; Jean, Ripoche & Poujeol, 1983; Lee & Pritchard, 1983; Stieger, Stange, Biber & Murer, 1983).

Some invertebrate gastrointestinal epithelial cells also show a combination of Na⁺-dependent and Na⁺-independent amino acid transport systems with similar properties to those displayed by vertebrate epithelia (Brick & Ahearn, 1978; Wyban, Ahearn & Maginniss, 1980; Gerencser, 1981, 1982; Ahearn, 1982). Other invertebrate tissues appear to diverge from the transport pattern established for mammalian cell types.

Recent studies with crustacean hepatopancreatic epithelial brush border membrane vesicles show that although Na⁺-dependent glucose transport could be illustrated by these preparations, a sodium requirement for alanine transport could not be detected (Ahearn et al. 1985a, b, 1986). In this tissue, brush border Na⁺-independent alanine transport was sensitive to membrane potential at acidic pH, and was strongly inhibited and trans-stimulated by lysine, while leucine was without effect. It was suggested that the bulk of carrier-mediated alanine transport across this membrane occurred by way of a classical y⁺ transport protein for dibasic amino acids that was Na⁺-independent. The present investigation describes some of the properties of lysine transport by lobster hepatopancreatic brush border and the driving forces responsible for cellular accumulation of this amino acid.

**MATERIALS AND METHODS**

Live Atlantic lobsters (*Homarus americanus*; 0.5 kg each) were purchased from commercial dealers in Hawaii and maintained unfed at 10°C for up to 1 week in filtered sea water. All animals used in this study were either in intermoult or early premoult, as assessed by the moult stage classification scheme introduced by Aiken (1973).

Hepatopancreatic brush border membrane vesicles (BBMV) were prepared from fresh tissue removed from individual lobsters. Each membrane batch was produced from a single organ (approx. 20 g fresh mass) using the magnesium-precipitation technique described in previous publications (Ahearn et al. 1985a, 1986). Purity of a final membrane sample was assessed by comparing enzyme activities of this pellet to those of a washed tissue homogenate (original homogenate was centrifuged at 27 000 g and resuspended twice in hypotonic buffer before an enzyme sample was taken, so that soluble enzymes, released by cell disruption and appearing in the first two supernatants, could be discarded). These comparisons showed final pellet enrichments of alkaline phosphatase, Na⁺/K⁺-ATPase and cytochrome c oxidase of 15.3-, 1.0- and 0.2-fold, respectively (Ahearn et al. 1985a), suggesting that the magnesium-precipitation method produced membrane preparations that were rich in brush borders and reduced in contamination from the basolateral membrane or membranes from cellular organelles such as mitochondria.
Transport studies using hepatopancreatic BBMV were generally conducted at 15°C using a temperature-controlled water bath and the Millipore filtration techniques developed by Hopfer, Nelson, Perrotto & Isselbacher (1973). Two types of transport experiments were performed. In long-term incubations, a volume of membrane vesicles (e.g. 20 μl) was added to a volume of radiolabelled medium (e.g. 160 μl) containing L-lysine (G-3H; ICN Radiochemicals). After incubation (15 s and 1, 2, 5, 10, 20 and 90 min) a known volume of this reaction mixture (20 μl) was withdrawn and plunged into 1-5 ml of ice-cold stop solution (composition varying according to experimental conditions) to stop the uptake process. The resulting suspensions were rapidly filtered through Millipore filters (0.65 μm), to retain the vesicles, and washed with another 10 ml of stop solution. Filters were then added to Beckman Ready-Solv HP scintillation cocktail and counted for radioactivity in a Beckman LS-8100 scintillation counter.

In short-term incubations, 5 μl of membrane suspension was mixed for predetermined times with 45 μl of buffer containing the isotope at 23°C using a rapid-exposure uptake apparatus (Inovativ Labor AG, Adliswil, Switzerland). Following isotope incubation, an ice-cold stop solution was injected into the membrane–isotope mixture. Vesicles in the stop solution were then treated as described above for long-term incubations.

The composition of the intravesicular medium was established by resuspending the penultimate membrane pellet in the appropriate internal solution with a Potter–Elvehjem homogenizer, and allowing this mixture to stand on ice for 90 min prior to the final 30-min high-speed centrifugation. Vesicles, therefore, had normally been incubated in internal media for at least 120 min before a transport experiment was initiated.

Lysine uptake values were expressed as pmol (using specific activity of lysine in medium) per milligram protein (Bio Rad protein assay) per filter. Each experiment was generally repeated two or three times using membranes prepared from different animals. Similar experimental findings were consistently obtained in the repetition of an experiment. Within a given experiment, 3–5 replicates were used exhibiting an experimental scatter generally around 10%. Throughout this study mean values and their standard errors are presented.

Tetramethylammonium hydroxide (TMA+-OH) was titrated against D-gluconic acid lactone to make TMA+-gluconate. Both were obtained from Sigma Chemical Co., as were valinomycin and other reagent grade chemicals.

**RESULTS**

*Effects of cation gradients on lysine uptake*

The effects of transmembrane cation gradients (directed inwards) on the time course of 5.5 μmolL⁻¹ L-[³H]lysine uptake by lobster BBMV were examined in two experiments. First, vesicles were loaded with 200 mmolL⁻¹ mannitol at pH 7.0 and were incubated for 15 s and 1, 2, 5, 10, 20 and 90 min in media containing
Fig. 1. Effects of cation gradients on the time course of 5.5 μmol l⁻¹ L-[³H]lysine uptake by lobster brush border membrane vesicles. (A) Vesicles were loaded with 200 mmol l⁻¹ mannitol at pH 7.0 and were incubated for the times indicated in media containing labelled lysine, 100 mmol l⁻¹ Na⁺-gluconate (○), K⁺-gluconate (△) or TMA⁺-gluconate (○) at pH 7.0. (B) Vesicles were loaded with 100 mmol l⁻¹ K⁺-gluconate and 50 μmol l⁻¹ valinomycin at pH 7.5 and were incubated for the times shown in media containing 100 mmol l⁻¹ K⁺-gluconate, 50 μmol l⁻¹ valinomycin and labelled lysine at pH 7.5 (○), 6.5 (△) or 5.5 (○). Buffers used in all media were either 12 mmol l⁻¹ Hepes-Tris or 12 mmol l⁻¹ Mes-Tris.

100 mmol l⁻¹ Na⁺-gluconate, K⁺-gluconate or TMA⁺-gluconate, and the radio-labelled amino acid at pH 7.0. Fig. 1A indicates that the time courses of lysine uptake in each medium were approximately similar, exhibiting apparent initial uptake rates (Na⁺ = 6.6 ± 1.0; K⁺ = 6.9 ± 0.9; TMA⁺ = 5.9 ± 0.7 pmol mg protein⁻¹ 15s⁻¹) and 90 min equilibrium values which were not significantly different from one another (P > 0.05). In no instance was there evidence of a cation-gradient-dependent uptake overshoot for lysine transfer as has been demonstrated for Na⁺-dependent amino acid transport by brush border vesicles of mammalian epithelia (Sigrist-Nelson, Murer & Hopfer, 1975). Similar experiments were conducted at pH 5.0 and yielded qualitatively similar results, that is, there were no stimulatory effects of Na⁺, K⁺ or TMA⁺ gradients on lysine uptake at this pH.

A second experiment was conducted to determine if direct coupling between the influxes of lysine and protons occurred in these vesicle preparations. Vesicles were loaded with 100 mmol l⁻¹ K⁺-gluconate and 50 μmol l⁻¹ valinomycin at pH 7.5 and were incubated for the same time intervals as in the first experiment in external media containing 100 mmol l⁻¹ K⁺-gluconate, 50 μmol l⁻¹ valinomycin and 5.5 μmol l⁻¹ L-[³H]lysine at pH 7.5, 6.5 or 5.5. Fig. 1B shows that the time courses of radio-labelled lysine uptake under these three conditions were not significantly different from each other (P > 0.05), suggesting that a transmembrane proton gradient directed inwards, was not stimulatory for transport of this amino acid. A similar
experiment comparing $L-[{}^3\text{H}]$lysine uptake into vesicles having identical pH values on both sides (pH = 7.5) with vesicles containing an outwardly-directed proton gradient (pH$_i$ = 5.5, pH$_o$ = 7.5) did not lead to amino acid accumulation rates that were significantly different ($P > 0.05$) from each other. The results of these two experiments clearly indicate the lack of dependency of lysine transfer on transmembrane cation gradients and suggest the coupling of cellular lysine uptake to other sources of potential energy.

**Effects of anion gradients on lysine uptake**

In order to establish whether anions in the external medium could influence lysine uptake rates, BBMV were loaded with 200 mmol l$^{-1}$ mannitol at pH 5.5 and were incubated in media at pH 5.5 containing 100 mmol l$^{-1}$ K$^+$-gluconate, KCl or KSCN, and 5.5 mmol l$^{-1}$ $L-[{}^3\text{H}]$lysine. Fig. 2 indicates that the species of external anion had a significant effect on the time course of amino acid uptake by these membrane preparations. Apparent lysine influxes displayed the following responses to differential anion gradients: SCN$^->Cl^->$gluconate (15.3 ± 0.3, 10.6 ± 0.3, 6.3 ± 0.5 pmol mg protein$^{-1}$ 15 s$^{-1}$, respectively). This sequence of lysine influx stimulation by

![Fig. 2. Effects of anion gradients on time course of 5.5 mmol l$^{-1}$ $L-[{}^3\text{H}]$lysine uptake by lobster brush border membrane vesicles. Vesicles were loaded with 200 mmol l$^{-1}$ mannitol at pH 5.5 and were incubated in media at pH 5.5 containing 100 mmol l$^{-1}$ K$^+$-gluconate (A), KCl (O) or KSCN (●) and the labelled amino acid. Mes-Tris (12 mmol l$^{-1}$) was used as the buffer in each medium.](image-url)
Fig. 3. Effects of pH on anion-gradient-dependent uptake of 5-5 μmol l⁻¹ L-[³H]lysine by lobster brush border membrane vesicles. Vesicles were loaded with 100 mmol l⁻¹ Na⁺-gluconate at pH 8.0 (Δ), 7.0 (○) or 5.0 (●) and were incubated in media at the same pH values with labelled lysine and 100 mmol l⁻¹ NaSCN.

Anions corresponds with reported anion permeabilities of a variety of epithelial brush border membranes (P_{SCN} > P_{Cl} > P_{gluconate}). In addition, while a SCN⁻ gradient resulted in a lysine uptake overshoot with a maximal value at 2 min, neither Cl⁻ nor gluconate media produced this effect. Amino acid uptake in the presence of a Cl⁻ gradient was, however, significantly greater than that exhibited by vesicles incubated in gluconate. Vesicles exposed to all three media yielded similar equilibrium uptake values at 90 min. These results suggest that lysine uptake from an acidic external medium responds to variations in transmembrane potential induced by differential anion fluxes.

A second experiment was conducted to ascertain whether anion-gradient-stimulated lysine uptake was influenced by transmembrane pH. Vesicles were loaded with 100 mmol l⁻¹ Na⁺-gluconate at pH 8.0, 7.0 or 5.0 and were incubated in external media at the same pH values with 100 mmol l⁻¹ NaSCN and 5.5 μmol l⁻¹ L-[³H]lysine. As indicated in Fig. 3, transmembrane pH, in the presence of a transmembrane anion gradient, had a marked effect on the uptake of this amino acid. Apparent lysine influxes were stimulated in decreasing order according to the following relationship with pH: pH 5.0 > pH 7.0 > pH 8.0 (18.8 ± 0.7, 13.7 ± 1.3, 11.8 ± 0.3 pmol mg protein⁻¹ 15 s⁻¹, respectively). Lysine uptake overshoot occurred at 2 min of incubation in pH 5.0 medium, but were delayed until 5 min for
solutions at pH 7.0 and 8.0, suggesting a considerably slower transfer rate of the amino acid across the membrane under the latter conditions. All media yielded similar equilibrium uptake values at 90 min. These two experiments suggest that lysine transport across lobster BBMV is stimulated by transmembrane anion gradients and that the magnitude of this stimulation is enhanced by an acidic pH.

Electrogenic nature of lysine uptake

Results from Figs 2 and 3 suggest that anion-gradient-dependent lysine uptake by lobster BBMV is electrogenic and that the apparent transfer rate of the external anion largely determines the extent of amino acid accumulation. Two additional experiments were conducted to confirm the electrogenic nature of lysine transport. In the first, vesicles were loaded with 100 mmol l⁻¹ K⁺-gluconate at pH 5.5 and were incubated in a medium containing 100 mmol l⁻¹ TMA⁺-gluconate at pH 5.5 and 5.5 μmol l⁻¹ L-[³H]lysine. One group of vesicles had 50 μmol l⁻¹ valinomycin added to internal and external media, while the other group lacked the K⁺-specific ionophore. Fig. 4A shows that, in the presence of valinomycin, an outwardly directed potassium diffusion potential (inside negative) accelerates the uptake of

![Graph A and B](image-url)
lysine at this pH compared to that illustrated by vesicles lacking the ionophore. Both groups had similar 90 min equilibrium values.

In the second experiment, vesicles were loaded with 100 mmol l\(^{-1}\) K\(^+\)-gluconate at pH 5.3 and were incubated in a medium containing 100 mmol l\(^{-1}\) KSCN at pH 5.3 and 5.5 μmol l\(^{-1}\) L-[\(^{3}\)H]lysine. One group of these vesicles was incubated with 50 μmol l\(^{-1}\) valinomycin (both sides), while the other lacked the ionophore. Fig. 4B indicates that a transmembrane SCN\(^-\) gradient, in the absence of valinomycin, stimulated lysine uptake compared to that displayed by vesicles short-circuited by the ionophore. These two experiments indicate that lysine transport in an acidic medium responds to an electrical potential gradient established either by a potassium diffusion potential or by the transmembrane flow of a permeable anion.

**Kinetic characteristics of lysine influx**

The time course of L-[\(^{3}\)H]lysine uptake by lobster BBMV was examined at very short intervals (2, 4, 6, 8 s), using a rapid uptake apparatus, to establish an accurate assessment of initial entry rates of the amino acid at external concentrations of 5.5, 10, 20, 40, 60, 100, 150 and 250 μmol l\(^{-1}\) lysine. Vesicles were loaded with 100 mmol l\(^{-1}\) K\(^+\)-gluconate at pH 5.0 and were incubated in media containing 100 mmol l\(^{-1}\) KCl at pH 5.0 and each of the above lysine concentrations. At each time interval four or five replicate uptake values were obtained for all amino acid concentrations.

Uptake of L-[\(^{3}\)H]lysine by BBMV was a linear function of time from 2 to 8 s of incubation at each lysine concentration. The time courses of amino acid entry at two representative concentrations, 10 and 150 μmol l\(^{-1}\) lysine, are displayed in Fig. 5. The six other concentrations gave similar patterns. Straight lines are drawn through the uptake data in Fig. 5 based on linear regression analyses, providing an estimate of unidirectional lysine entry (slope) and lysine binding (extrapolated vertical intercept). Lysine binding values, determined from calculated vertical intercepts at each external amino acid concentration, were not significantly different (\(P > 0.05\)) from those obtained using vesicles injected into ice-cold (0°C) uptake medium at time zero and then rapidly filtered ('blank uptake values'). Lysine influxes (\(J_{oi}\)) at these two concentrations were 0.6 ± 0.07 pmol mg protein\(^{-1}\) s\(^{-1}\) (10 μmol l\(^{-1}\)) and 6.8 ± 0.9 pmol mg protein\(^{-1}\) s\(^{-1}\) (150 μmol l\(^{-1}\)). Significant (\(P < 0.05\)) lysine binding to vesicles occurred for all eight lysine concentrations and increased in a linear fashion as external amino acid concentration was elevated. Plotting lysine binding (vertical intercepts) against external lysine concentration resulted in an estimate of bound amino acid for each incubation condition. Lysine binding determined in this manner was 0.14 ± 0.02 pmol mg protein\(^{-1}\) (μmol l\(^{-1}\))\(^{-1}\) lysine (25.9 ± 1.5 % uptake at 8 s) and was used in subsequent estimations of 8-s lysine influx in the presence and absence of inhibitory amino acids (Fig. 8).

Fig. 6 illustrates lysine influx from incubation medium to vesicular interior determined in the manner described in Fig. 5 for external lysine concentrations ranging from 5.5 to 250 μmol l\(^{-1}\). Over this concentration range, \(J_{oi}\) was a curvilinear function of external amino acid concentration and can be described as the sum of at
least two independent processes operating simultaneously: (1) a Michaelis–Menten carrier mechanism illustrating saturation kinetics and (2) a linear entry system with a rate that is proportional to the external amino acid concentration and probably represents simple diffusion. These two processes operating together can be described by the equation:

\[ J_{oi} = \frac{J_M[L]}{K_i + [L]} + P[L], \]

where \( J_{oi} \) is total alanine influx in pmol mg protein\(^{-1}\) s\(^{-1}\); \( J_M \) is maximal carrier-mediated influx, \( K_i \) is the lysine concentration resulting in half-maximal influx, \([L]\) is the external lysine concentration, and \( P \) is the rate constant of the linear process which can be defined as an ‘apparent diffusional permeability constant’. Non-saturable lysine influx (Fig. 6, dotted line) was subtracted from total influx at each amino acid concentration yielding an estimate of the carrier transport component. Calculated carrier-mediated lysine influxes were drawn in an Eadie–Hofstee plot (Fig. 6, inset) to provide estimates of the transport constants \( K_i \) (60.8 ± 3.4 \( \mu \)mol l\(^{-1}\)) and \( J_M \) (2.3 ± 0.2 pmol mg protein\(^{-1}\) s\(^{-1}\)).

**Inhibitory effects of external amino acids on lysine uptake**

Three amino acids known to be transported by several distinct mechanisms in other tissues were examined for their potential inhibitory effects on lysine transport in lobster BBMV. The time course of 5.5 \( \mu \)mol l\(^{-1}\) L-[\(^3\)H]lysine uptake into vesicles...
loaded with 100 mmol l\(^{-1}\) K\(^{+}\)-gluconate at pH 5.0 and incubated in media containing the labelled amino acid, 100 mmol l\(^{-1}\) KSCN at pH 5.0, and 5 mmol l\(^{-1}\) L-leucine, L-alanine or L-arginine was followed. Fig. 7 shows that the control condition, that is, 5.5 μmol l\(^{-1}\) L-[\(^{3}\)H]lysine uptake in the absence of other amino acids, resulted in the most rapid apparent influx rate (37.6 ± 2.6 pmol mg protein\(^{-1}\) 15 s\(^{-1}\)) and greatest accumulation of label against a concentration gradient (overshoot). Addition of 5 mmol l\(^{-1}\) L-arginine strongly reduced the apparent rate of lysine influx (9.0 ± 0.9 pmol mg protein\(^{-1}\) 15 s\(^{-1}\)) and abolished the overshoot properties of lysine accumulation. Adding 5 mmol l\(^{-1}\) L-leucine or L-alanine to the external media led to apparent lysine influxes that were intermediate between the two extremes (leucine = 22.4 ± 1.0, alanine = 19.0 ± 1.3 pmol mg protein\(^{-1}\) 15 s\(^{-1}\)). While lysine uptake in the presence of L-alanine was hyperbolic in character, a small and delayed overshoot of lysine accumulation occurred when L-leucine was used as the inhibitor.

\[ K_i = 60.8 \mu\text{mol} \text{l}^{-1} \]

\[ J_M = 2.3 \text{ pmol mg} \text{ protein}^{-1} \text{s}^{-1} \]

\[ J_{oi} = 3.0 \text{ pmol mg} \text{ protein}^{-1} \text{s}^{-1} \]

\[ J_{oi}/[\text{Lysine}] \]

Fig. 6. Effects of external lysine concentration on L-[\(^{3}\)H]lysine influx into lobster brush border membrane vesicles. Vesicles were loaded with 100 mmol l\(^{-1}\) K\(^{+}\)-gluconate at pH 5.0 and were incubated for time intervals ranging from 2 to 8 s in media containing different concentrations of labelled lysine and 100 mmol l\(^{-1}\) KCl at pH 5.0. Symbols in the main body of the graph are mean influx values and their standard errors (sample size was 5) determined as the slope of a time course experiment as described in Fig. 5 for each external lysine concentration. The dotted line is an estimate of non-saturable lysine influx over the concentration range examined. Carrier-mediated lysine entry was determined by subtraction of calculated non-saturable influx from total influx. The inset is an Eadie–Hofstee plot of estimated carrier-mediated lysine influx. Kinetic constants displayed on the graph were derived from the slope (\(K_i\)) and vertical intercept (\(J_M\)) of this plot using linear regression analysis. \(J_{oi}\), lysine influx.
These results suggest that all three amino acids may have inhibitory effects on lysine uptake by BBMV, but their mode of action is unclear.

In order to determine the mechanism of inhibition of lysine influx by external amino acids, 8-s uptakes of L-[3H]lysine (5.5, 10, 20, 40, 60, 100, 150 and 250 μmol l⁻¹) were measured in the presence of 500 μmol l⁻¹ L-alanine, L-arginine or L-leucine. Lysine influx was determined under these conditions at each external lysine concentration by first subtracting a 25.9% binding component (see Fig. 5) from total uptake. Each resulting plot of lysine influx versus lysine concentration in the presence of the inhibitors was curvilinear, resembling the results in Fig. 6. From each biphasic curve was subtracted the non-saturable transport component, yielding an estimate of carrier-mediated lysine influx in the presence of each inhibitory amino acid. These data are displayed in Eadie–Hofstee plots in Fig. 8. Kinetic constants obtained from the control experiment (Fig. 8A) were similar to those presented previously (Fig. 6), except that maximal lysine influx in this measurement was only about one-third that of the earlier determination and may reflect minor differences in membrane preparations. Both L-alanine and L-arginine significantly (P < 0.01) increased lysine influx Kᵣ (reduced apparent binding affinity), without having significant (P > 0.05) effects on maximal lysine influx (Jₓ). In contrast, L-leucine did not have a significant (P > 0.05) influence on lysine influx Kᵣ, but did significantly
Fig. 8. Mechanisms of amino acid inhibition of L-[3H]lysine influx into lobster brush border membrane vesicles. Vesicles were loaded with 100 mmol l\(^{-1}\) K\(^+\)-gluconate at pH 5.0 and were incubated for 8 s in media containing 100 mmol l\(^{-1}\) KCl at pH 5.0, the labelled amino acid at concentrations of 5.5, 10, 20, 40, 60, 100, 150 and 250 μmol l\(^{-1}\), and 500 μmol l\(^{-1}\) L-leucine (D), L-alanine (B) or L-arginine (C). (A) Control incubation with lysine alone. Lysine influx was corrected for binding and non-saturable entry as described in the text. Calculated carrier-mediated lysine influx under control and inhibited conditions are displayed in Eadie-Hofstee plots. Kinetic constants were derived from these data as described in Fig. 6. Each point is the mean of triplicate samples. \(K_i\), lysine concentration resulting in half-maximal influx; \(J_M\), maximal carrier-mediated influx.

\(P < 0.05\) lower maximal lysine entry rate. These results suggest that alanine and arginine are competitive inhibitors of lysine influx, arginine being the most potent, while leucine is a non-competitive inhibitor of lysine entry.

DISCUSSION

Results of the present investigation suggest that in lobster hepatopancreatic brush border membrane vesicles lysine transport occurs by at least one carrier process that is cation-gradient independent, but electrogenic, responding strongly to transmembrane electrical potential. Similar results were recently obtained with this
preparation for L-alanine transport (Ahearn et al. 1986). However, in another study, lobster BBMV exhibited Na⁺-gradient-dependent and membrane-potential-dependent D-glucose transport (Ahearn et al. 1985a). In all three instances a reduction in pH enhanced organic solute transport, but the nature of the proton effect appeared to differ for sugars and amino acids. Na⁺-dependent, D-glucose transport was stimulated at acidic pH due to a significant enhancement of apparent sugar binding affinity ($K_t$) and apparent diffusional permeability ($P$) of the membrane to the sugar. In contrast, an increase in proton concentration (decreased pH from 6·0 to 4·0) led to a 10-fold elevation of apparent maximal alanine influx rate ($J_M$). The major influence of hydrogen ions on alanine transfer appeared to relate to relative protonation of the amino acid at each pH and the subsequent effect of the membrane potential on the cationic alanine produced. In the present study, membrane-potential-sensitive lysine transport increased with a reduction in bilateral pH (Fig. 3), but was not affected by a proton gradient directed inwards in the absence of a membrane potential (Fig. 1B), suggesting that protonation of this cationic amino acid also enhances its electrogenicity.

In crustaceans, hepatopancreatic luminal pH is acidic (Gibson & Barker, 1979), leading to protonation of amino acids to a greater extent than is likely in vertebrate gut where luminal pH is near neutrality. It might be expected, therefore, that in these invertebrates a larger fraction of the luminal dietary amino acid load would carry a net positive charge and be responsive to transmembrane potential across the hepatopancreatic apical pole. Organic solutes such as sugars, and possibly 'acidic' amino acids, such as glutamic acid, may largely be electrically neutral at in vivo luminal pH and be unresponsive to transmembrane potential. In this latter instance, coupling of nutrient flow with that of a cation, such as sodium, could provide the necessary electrochemical driving forces for organic solute transfer.

In mammalian gastrointestinal and renal epithelia, alanine and several other neutral amino acids use a number of Na⁺-dependent carrier proteins for transfer across the brush border (Mircheff et al. 1982; Stevens et al. 1982). In contrast, while a portion of lysine or arginine transport across this membrane in kidney cells occurs by a Na⁺-dependent process (Hilden & Sacktor, 1981; Mircheff et al. 1982; Stieger et al. 1983), the bulk of the transmembrane fluxes of these cationic amino acids in renal and intestinal tissues is mediated by Na⁺-independent transfer proteins which have unique specificities for substrates (Hammerman, 1982; Stevens et al. 1982; Cassano et al. 1983; Jean et al. 1983). Generally, the cationic amino acids have reduced inhibitory effects on Na⁺-dependent neutral amino acid uptake and Na⁺-dependent neutral amino acids exert minimal inhibition on Na⁺-independent lysine or arginine transport (Stevens et al. 1982).

Crustacean intestinal brush border exhibits high- and low-affinity lysine transport mechanisms, both being inhibited by arginine, but only the former being Na⁺-dependent (Brick & Ahearn, 1978). The present study indicates that the hepatopancreas, where the bulk of nutrient absorption is believed to occur (Dall & Moriarty, 1983), possesses Na⁺-independent carrier-mediated lysine transport (Figs 1, 6) which is inhibited by L-arginine, L-alanine and L-leucine (Figs 7, 8). The kinetic
analysis of this interaction (Fig. 8) indicates that arginine and alanine are competitive inhibitors of lysine influx, while leucine is a non-competitive inhibitor. These results support previous work with the same preparation showing that 5 mmol L\(^{-1}\) L-leucine has little effect on 0.05 mmol L\(^{-1}\) L-[\(^3\)H]alanine influx, while 5 mmol L\(^{-1}\) L-lysine was strongly inhibitory (Ahearn et al. 1986). That study also shows that trans-stimulation of alanine influx occurs in vesicles preloaded with lysine, but not in vesicles containing leucine. Taken together, these data suggest that lysine, alanine and arginine appear to share the same Na\(^+\)-independent, membrane-potential-sensitive carrier mechanism. This carrier may be analogous to the classical y\(^+\) system described for mammalian cells (Christensen, 1964, 1975), but have a broad substrate range in crustaceans because of the acidic nature of their nutrient absorption site. The nature of the non-competitive effect of L-leucine on L-lysine influx (Fig. 8) is presently unclear, but may either be the result of amino acid interactions at a single membrane transport protein (e.g. y\(^+\) system), or be due to competition for the membrane potential between protonated amino acids using two distinct Na\(^+\)-independent, membrane-potential-sensitive carrier proteins (e.g. y\(^+\) and LEU systems). Future studies are planned to ascertain the nature of L-leucine transport by these vesicles and whether a separate transport agency is responsible for its uptake by hepatopancreatic brush border membranes.

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REFERENCES


