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Proton-Coupled L-Lysine Uptake by Renal Brush Border Membrane Vesicles from Mullet *(Mugil cephalus)*

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Summary. The uptake of the basic amino acid, L-lysine, was studied in brush border membrane vesicles isolated from the kidney of the striped mullet (*Mugil cephalus*). The uptake of L-lysine was not significantly stimulated by a Na^+ gradient and no overshoot was observed. However, when a proton gradient ($pH_o = 5.5$; $pH_i = 8.3$) was imposed across the membrane in the absence of Na^+, uptake was transiently stimulated. When the proton gradient was short circuited by the proton ionophore, carbonylcyanide *p*-triflouromethoxyphenyl hydrazone, proton gradient-dependent uptake of lysine was inhibited. Kinetics of lysine uptake determined under equilibrium exchange conditions indicated that the V_{max} increased as available protons increased (2.1 nmol/min/mg protein at pH 7.5 to 3.7 nmol/min/mg at pH 5.5), whereas the apparent K_m (4.9 ± 0.6 mM) was not altered appreciably. When membrane potential (inside negative) was imposed by K^+ diffusion via valinomycin, a similar (but smaller) stimulation of lysine uptake was observed. When the membrane potential and the proton gradient were imposed simultaneously, a much higher stimulation in lysine uptake was shown, and the uptake of lysine was approximately the sum of the components measured separately. These results indicate that the uptake mechanism for basic amino acids is different from that of neutral or acidic amino acids and that the protonmotive force can provide the driving force for the uptake of L-lysine into the isolated brush border membrane vesicles.

Key Words renal brush border membrane · basic amino acid · H^+-coupled transport · protonmotive force · *Mugil cephalus*

Introduction

Renal transport of amino acids has been studied with a wide variety of physiological preparations, including intact animals, cortical slices and proximal tubules (Young & Freedman, 1971; Silbernagl, Foukes & Deetzen, 1975). These studies indicated that amino acids were reabsorbed in the proximal region of the nephron via Na^+-dependent transport mechanisms. The transport of neutral amino acids (Evers, Murer & Kinne, 1976; Fass, Hammerman & Sacktor, 1977; Hammerman & Sacktor, 1978), imino acids (Hammerman & Sacktor, 1977; McNamara, Pepe & Segal, 1979), sulfur-containing amino acids (Segal, McNamara & Pepe, 1977; McNamara, Pepe & Segal, 1981) and acidic amino acids (Burckhardt, Kinne, Stange & Murer, 1980; Schneider, Hammerman & Sacktor, 1980; Schneider & Sacktor, 1980) have recently been examined in renal brush border membrane vesicles. The driving force for tubular reabsorption of each of these amino acids was shown to be provided by the electrochemical gradient for Na^+ between lumen and cell. However, the picture for basic amino acids is still unclear. On the one hand, Na^+-dependent basic amino acid transport has been demonstrated in rat kidney by micropuncture (Ullrich, Rumrich & Klöss, 1974; Samarzija & Frömter, 1976) and in rabbit kidney by vesicle techniques (Busse, 1978; Hilden & Sacktor, 1981). On the other hand, Na^+-independent uptake of basic amino acids has also been observed in rat kidney slices (Fox, Thier, Rosenberg & Segal, 1964; Segal & Smith, 1969), in isolated renal tubules (Hillman & Rosenberg, 1970), and in brush border membrane (BBM) vesicles (Busse, 1978; Hilden & Sacktor, 1981; Hammerman, 1982). These results suggest that there may be multiple pathways for basic amino acids and/or that lumenal and contralumenal membranes may handle these amino acids differently.

In this paper, we describe the mechanism of basic amino acid (L-lysine) uptake by the kidney of a marine teleost, the striped mullet. L-lysine transport differed from that of neutral or acidic amino acids. It was not sodium dependent. Instead protonmotive force could provide the driving force for its uptake into isolated brush border membrane vesicles.

Materials and Methods

Materials

Striped mullet (*Mugil cephalus*) were collected in the vicinity of Matanzas Inlet, Florida. Valinomycin, carbonylcyanide *p*-

triflouromethoxyphenyl hydrazone (FCCP) and L-amino acids were purchased from Sigma Chemical Co., St. Louis, Mo. L-[^{3}H]-lysine, L-[^{3}H]-leucine and Aquasol were obtained from New England Nuclear Corp. All other chemicals were of reagent grade purity.

Preparation of Membrane Vesicles

The brush border membrane vesicles were prepared from mullet kidney by $CaCl_2$ precipitation followed by discontinuous sucrose density gradient centrifugation as described previously (Lee & Pritchard, 1983). As in our earlier studies, membrane purity was evaluated routinely by assay of alkaline phosphatase (a marker enzyme for brush border membrane) and (Na^+, K^+)-ATPase (a marker enzyme for basolateral membrane). Alkaline phosphatase was enriched approximately 10-fold in the final preparation compared to that found in the initial homogenate, whereas ouabain-sensitive (Na^+, K^+)-ATPase was enriched only twofold.

Assay of Transport

Transport of amino acids was measured by the Millipore filtration technique. Brush border membrane vesicles (50 to 100 μg of protein in 10 μl) were incubated at 25 °C in 50 μl of reaction mixture usually containing 1.0 mM Tris/HEPES, pH 7.5, 300 mM mannitol and 250 μM amino acids (containing L-[^{3}H]-amino acids, approximately 0.25 μCi). Unless indicated otherwise, the additions replaced mannitol isosmotically. Variation in pH from 5.5 to 8.3 was accomplished with 50 mM MES or 50 mM HEPES adjusted to the indicated pH with Tris. At indicated time intervals, samples were removed and immediately diluted in 2.0 ml of chilled 0.15 M NaCl solution supplemented with 10 mM Tris/HEPES, pH 7.5. Samples were rapidly filtered on a Millipore filter (0.45 μm) and washed with 2.0 ml of the same buffer. The radioactivity retained on filters was assayed in Aquasol on a liquid scintillation counter (Packard Prias). Values for the nonspecific retention of radioactivity on the filter were subtracted from the values of the incubated samples. Each experiment was repeated at least three times with different membrane preparations.

Protein concentration was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

Analysis of Intravesicular Content

Membranes were incubated with 250 μM L-[^{3}H]-lysine in an incubation medium as described above for 60 min and filtered on Millipore filters. After washing, the filters were extracted with 95% ethanol overnight at 4 °C. The extract was centrifuged and concentrated under nitrogen flow. The concentrated supernatant was subjected to thin-layer chromatography on Silica gel (Whatmann LK5D) using *n*-butanol/acetic acid/water (4:1:1, vol/vol) as the solvent (Busse, 1978). The chromatograms were then separated into 2-cm sections from the origin to the solvent front and the sections were counted. All of the isotope taken up chromatographed with lysine and no conversion to other compounds was detected.

Kinetics of Lysine Uptake

The initial rate of lysine uptake was estimated under equilibrium exchange conditions. Membrane vesicles (50 to 100 μg of protein) were incubated at 25 °C for 1 hr in a 50-μl reaction mixture containing 50 mM HEPES or 50 mM MES adjusted to the indicated pH with Tris, 300 mM mannitol and nonradioactive lysine at the specific concentrations. Lysine uptake was initiated by the addition of a trace amount of labeled lysine (approximately 0.25 μCi in 2.5 μl of the same reaction mixture). Uptake of L-[^{3}H]-lysine was assayed by filtration as described above.

Compartmental analysis was used to determine lysine uptake rates by the vesicles. Details of the assumptions and equations have been previously reported (Borle, 1969; Renfro, 1978). Uptake rates (ϕ_{ij}), rate constants (K_{ij}) and compartment sizes (S_j) were calculated with the assumption of a parallel model of exchange, where S_2 was the incubation medium and S_1 and S_3 vesicle lysine pools:

$$S_1 \underset{K_{21}}{\overset{}{\rightleftharpoons}} S_2 \overset{K_{23}}{\rightleftharpoons} S_3. \tag{1}$$

L-[^{3}H]-lysine uptake rates were determined from the uptake time course, plotted semilogarithmically, and graphically analyzed according to the following rate equation:

$$\frac{d\,(\text{lysine uptake})}{dt} = \phi_{23}\, e^{-k_{23}t} + \phi_{21}\, e^{-k_{21}t} \tag{2}$$

where ϕ_{23} and ϕ_{21} are uptake rate, respectively; k_{23} and k_{21} are the respective rate constants for lysine exchange by compartments S_3 and S_1. Compartment sizes were determined from the following relationship:

$$S_j = \phi_{ij}/k_{ij}. \tag{3}$$

Results

Lysine Uptake by Membrane Vesicles

When mullet kidney brush border membrane (BBM) vesicles were incubated with labeled amino acids, both lysine (basic amino acid) and leucine (neutral amino acid) were taken up. However, when the membrane vesicles (containing 1.0 mM Tris/HEPES, pH 7.5 and 300 mM mannitol) were incubated in media containing 1.0 mM Tris/HEPES, pH 7.5, 100 mM mannitol and 100 mM NaCl, as shown in Fig. 1 *B*, a typical Na^+ gradient-dependent transient overshoot in leucine uptake was produced. On the other hand, the uptake of lysine was not significantly stimulated by the Na^+ gradient and no overshoot was observed (Fig. 1 *A*), nor was uptake significantly reduced by Na^+ as observed in rabbit BBM (Busse, 1978; Hilden & Sacktor, 1981). Thus, it appeared that lysine uptake was not Na^+ dependent and, therefore, was very different from leucine uptake by BBM vesicles, not only from mullet kidney, but also from mammalian kidney (Young & Freedman, 1971; Busse, 1978; Hilden & Sacktor, 1981).

To ascertain that the uptake of lysine by BBM represented transport into the vesicles rather than membrane binding, the effect of changes in intravesicular volume on L-lysine uptake was determined.

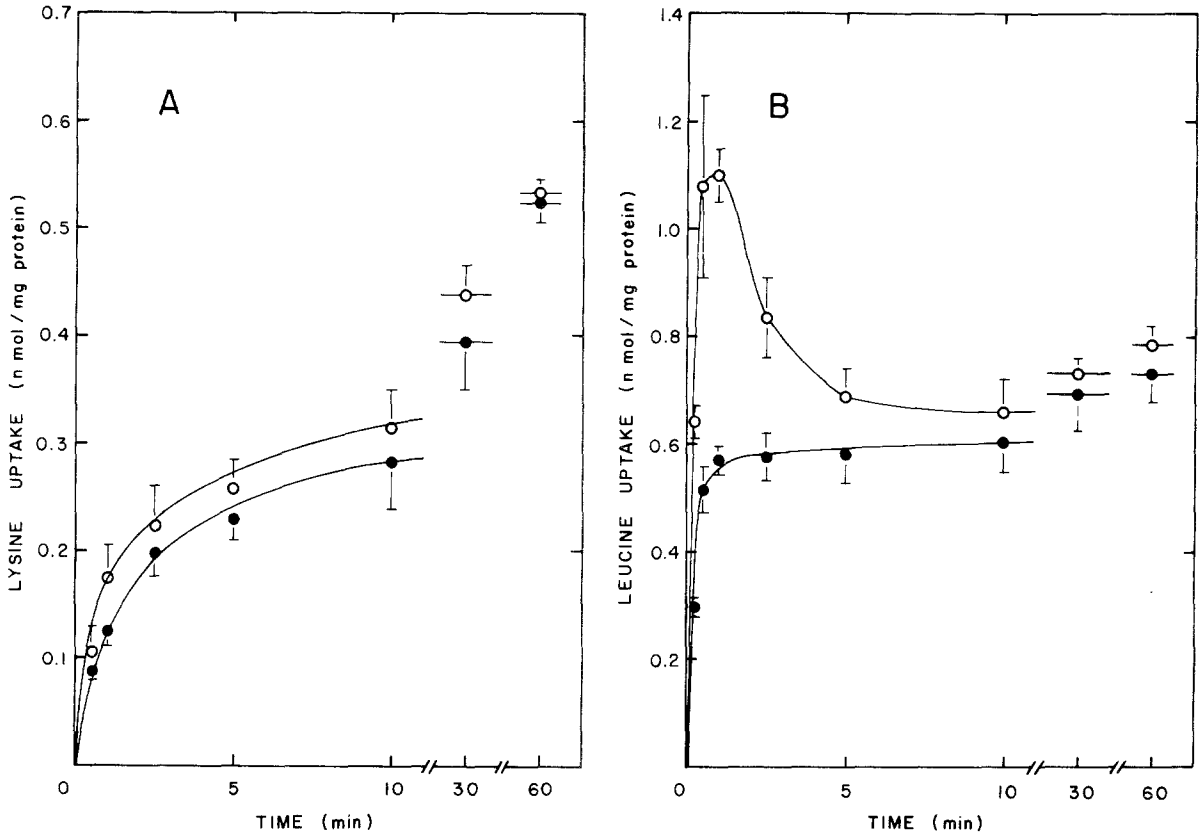

Fig. 1. Uptake of L-lysine and L-leucine by BBM vesicles. Membrane vesicles (50 to 100 μg of protein in 10 μl) contained 1.0 mM Tris/HEPES, pH 7.5 and 300 mM mannitol. Vesicles were suspended in media containing 1.0 mM Tris/HEPES, pH 7.5, 100 mM mannitol, 250 μM L-[^{3}H]-lysine (*A*) or 250 μM L-[^{3}H]-leucine (*B*) plus 100 mM NaCl (o—o) or 200 mM mannitol (•—•) in a final volume of 50 μl. Transport was carried out at 25 °C

As shown in Fig. 2, when intravesicular space was decreased by increasing the medium osmolality with sucrose, uptake of the amino acid by the vesicles after 60 min incubation was inversely proportional to medium osmolality from 0.35 to 0.8 M. Extrapolation to infinite medium osmolality indicated approximately 18% of the lysine at equilibrium was bound to the membrane and more than 80% was taken up into an osmotically reactive intravesicular space. These results are comparable to those of Hammerman (1982) for Na^+-independent L-arginine uptake by rabbit BBM (10% bound, 90% free), but show less binding than that observed by Hilden and Sacktor (1981) also in rabbit BBM. The data shown in this paper have not been corrected for the membrane-bound value.

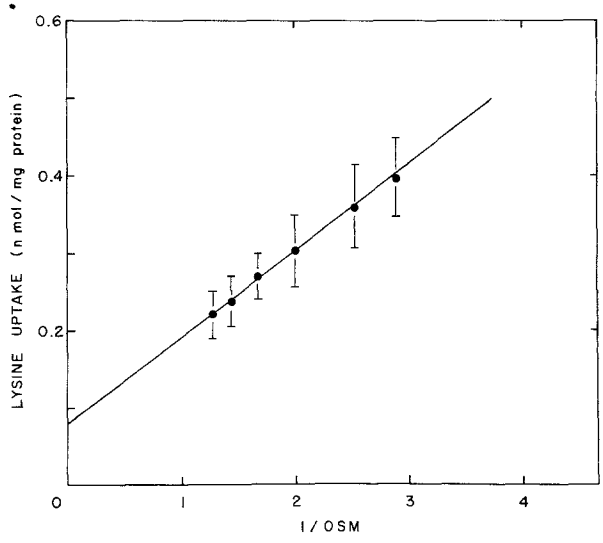

Fig. 2. Effect of medium osmolality on uptake of L-lysine by BBM vesicles. Membrane vesicles were incubated for 60 min with L-[^{3}H]-lysine in media containing various concentrations of sucrose in 300 mM mannitol buffered with 1.0 mM Tris/HEPES, pH 7.5

Effect of pH Gradient (ΔpH) on Lysine Uptake

Although a Na^+ gradient did not induce an overshoot in L-lysine uptake, when a pH gradient (ΔpH, i.e. $pH_o = 5.5$; $pH_i = 8.3$) was imposed

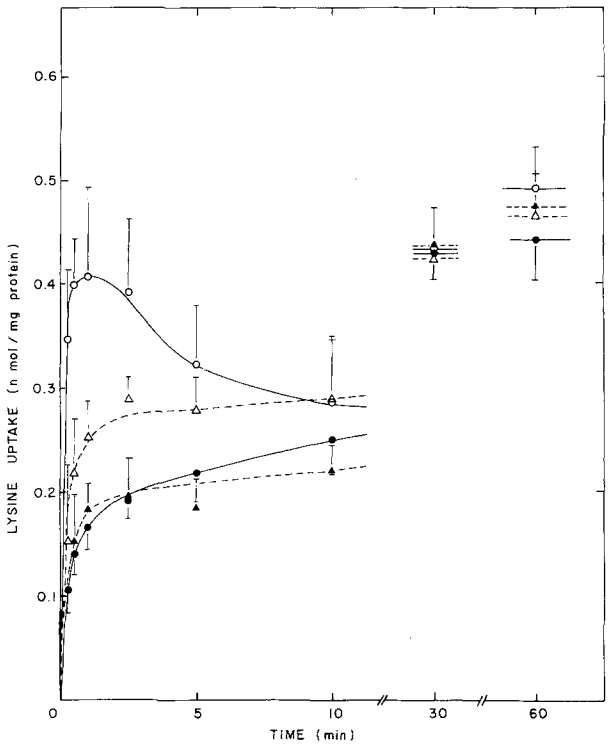

Fig. 3. Effect of pH gradient on L-lysine uptake. Membrane vesicles were preincubated at 25 °C for 60 min in media containing 300 mM mannitol plus 1.0 mM Tris/HEPES, pH 7.5 (●—●); 50 mM Tris/MES, pH 5.5 (▲---▲); 50 mM Tris/HEPES, pH 8.3 (○—○ or △---△). Vesicles were suspended in media containing 300 mM mannitol, 250 μM L-[^{3}H]-lysine plus 1.0 mM Tris/HEPES, pH 7.5 (●—●); 50 mM Tris/HEPES, pH 8.3 (▲---▲); 50 mM Tris/MES, pH 5.5 (○—○); 50 mM Tris/MES, pH 5.5 plus 10 μM FCCP (△---△)

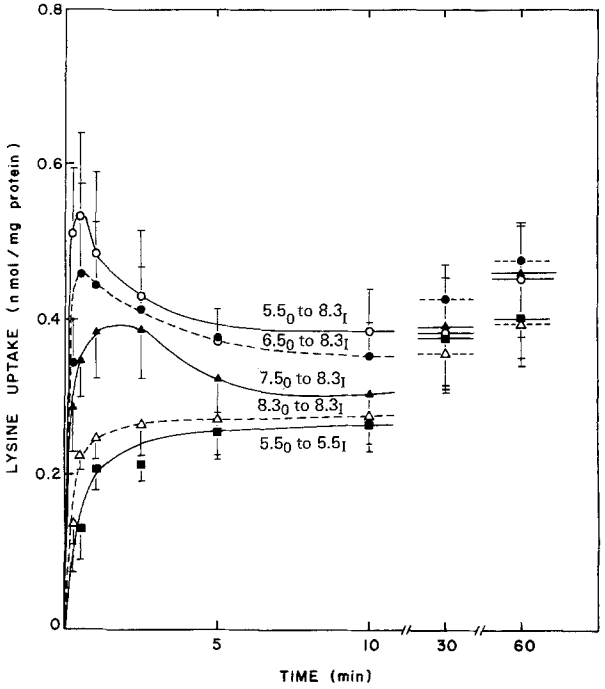

Fig. 4. Effect of the extravesicular pH on the ΔpH-dependent uptake of lysine. Membrane vesicles containing 300 mM mannitol buffered with 50 mM Tris/HEPES, pH 8.3 (except at pH 5.5 where 50 mM Tris/MES was used). Vesicles were suspended in media containing 300 mM mannitol, 250 μM L-[^{3}H]-lysine plus 50 mM HEPES or 50 mM MES adjusted to the indicated pH with Tris

across the membrane, a transient stimulation of uptake was produced in the absence of Na^+ gradient (Fig. 3). In these experiments BBM vesicles were incubated with 50 mM Tris/HEPES buffer at pH 8.3, and a ΔpH was induced by suspension of the vesicles in 50 mM Tris/MES buffer at pH 5.5. The ΔpH produced more rapid lysine uptake than the absence of a ΔpH. This rapid lysine uptake was inhibited by a proton ionophore, FCCP. The effect of FCCP may reflect either dissipation of the H^+ gradient or formation of inside positive membrane potential by FCCP-induced proton entry. The former explanation is more likely, however, since changes in $\Delta\psi$ alone (inside negative) were relatively ineffective in stimulating L-lysine uptake (*see below*). Furthermore, when the ΔpH was reversed (pH_o 8.3; $pH_i = 5.5$), no significant change in lysine uptake was observed (Fig. 3).

Effect of the Extravesicular pH on the ΔpH-Dependent Uptake of Lysine

The rate of H^+ gradient-dependent lysine uptake was found to be a function of the magnitude of ΔpH. As shown in Fig. 4, the initial stimulation of lysine uptake increased as the extravesicular pH was decreased from 8.3 to 5.5, while the intravesicular pH was maintained at 8.3. It should be noted that in the absence of a ΔpH, either at the high pH ($pH_o = 8.3$; $pH_i = 8.3$) or the low pH ($pH_o = 5.5$; $pH_i = 5.5$) no stimulation was seen and uptake was essentially identical at the two pH's. Like the FCCP effect (Fig. 3), these results indicate that it was the H^+ gradient, rather than the pH itself, which was responsible for the stimulated uptake of lysine into the BBM vesicles.

Kinetics of Lysine Uptake

Since the measurement of true initial rates for the kinetic studies is particularly difficult under gradient conditions where driving forces may change very rapidly (Hopfer & Groseclose, 1980), the initial rates of lysine uptake were estimated using equilibrium exchange conditions and compartmental analysis at different environmental pH as described under Materials and Methods. As shown in Fig. 5, uptake rates were determined from the

Table. Effect of pH on the initial rate of lysine uptake as determined under equilibrium exchange conditions

pH	Fast component			Slow component		
	ϕ (pmol/min/mg)	K (min^{-1})	S_3 (pmol/mg)	ϕ (pmol/min/mg)	K (min^{-1})	S_1 (pmol/mg)
8.3	90.8±13.6	0.39±0.06	235.7±30.3	8.4±4.1	0.034±0.010	234.9±72.0
7.5	113.5±42.9	0.41±0.11	269.1±37.7	13.3±1.6	0.052±0.014	266.7±51.2
5.5	174.0±14.9	0.49±0.02	355.2±36.7	8.0±5.7	0.061±0.024	140.4±81.8

ϕ = The initial rate of lysine uptake. K = The rate constant for that component. S_3 and S_1 = The size of the fast and slow component. Each value is the mean ± SE for three experiments at a lysine concentration of 0.25 mM.

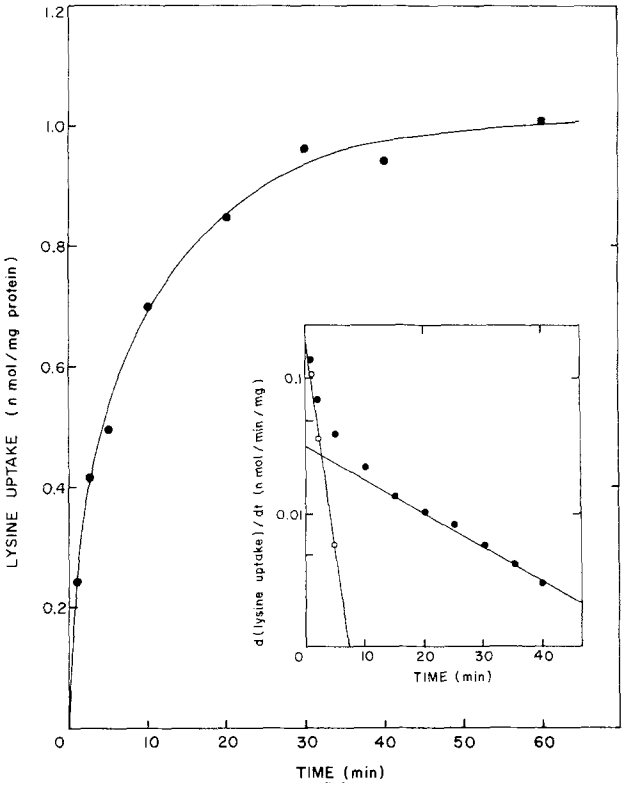

Fig. 5. Time course of L-lysine uptake by equilibrium exchange conditions. Uptake of lysine was measured under equilibrium exchange conditions as described under Materials and Methods. The reaction mixture contained 300 mM mannitol, 1.0 mM Tris/HEPES, pH 7.5 and 0.5 mM L-lysine. The inset shows semilogarithmic plot of rate of lysine uptake [d(lysine uptake)/dt] determined at numerous points along the line (solid circles). Graphical analysis yielded two components. The faster component (open circles reflect rates after subtraction of the slower component, ϕ_{21}) had an initial rate of 164.4 pmol/min/mg protein and a rate constant of 0.43 per min. The slower component (solid circle after 15 sec, ϕ_{23}) had an initial rate of 38.2 pmol/min/mg protein and a rate constant of 0.06 per min

hand-drawn uptake curve and plotted semilogarithmically. The resulting rate curve was multiexponential and could be described by two components according to Eq. (2) above. As shown in the Table, the initial rate of lysine uptake by the fast component increased markedly when different pH environments were imposed under equilibrium conditions, whereas the slow component did not respond. The rate constants for these two components differed from each other by at least one order of magnitude, but neither changed significantly with pH. The size of fast component (S_3) also increased somewhat with decreasing pH. However, the total exchangeable lysine (the sum of S_3 and S_1) in the vesicles was not altered (501±33 pmol/mg protein at lysine concentration of 0.25 mM).

The kinetics of L-lysine uptake were examined by varying the lysine concentration from 0.25 to 5.0 mM and observing changes in initial rates as determined above. As shown in Fig. 6, the initial rate of lysine uptake in the fast component appeared to be saturable. The reciprocal plots of lysine uptake under these conditions indicated that higher H^+ concentrations increased the V_{max} from 2.106 nmol/min/mg protein at pH 7.5 to 3.723 nmol/min/mg protein at pH 5.5. The apparent K_m value for lysine was not significantly altered (from 4.452 to 5.313 mM, 4.9±0.6 mM).

Stimulation of L-*Lysine Uptake by Membrane Potential ($\Delta\psi$) in the Absence of Na^+ Gradient*

According to Mitchell's hypothesis (1963), the movement of protons down their electrochemical gradient may be coupled to the uphill membrane transport of various substrates (substrate-proton symport). Since the protonmotive force is equal to the sum of the electrical potential across the membrane ($\Delta\psi$) and the chemical potential due to the pH gradient (ΔpH), the effect of electrical potential on lysine uptake was examined. $\Delta\psi$ was manipulated by loading the BBM vesicles with K^+ (100 mM KCl) and resuspending them in K^+-free media in the presence of 10 μM valinomycin. Under these conditions, as described previously (Lee & Pritchard, 1983), the vesicle interior will be transiently negative. As shown in Fig. 7, a small, but significant stimulation of L-lysine uptake was observed. In addition when the $\Delta\psi$ was imposed in

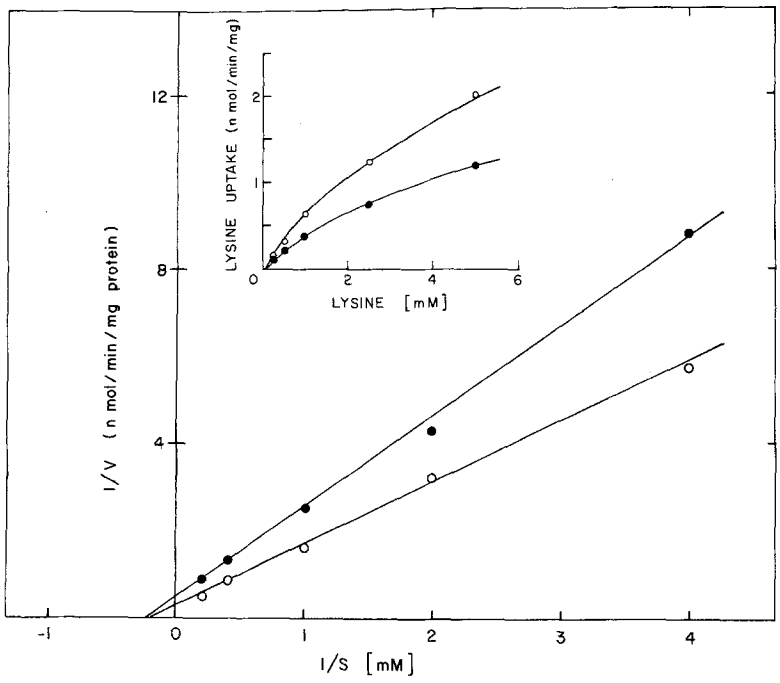

Fig. 6. Lineweaver-Burk plots for L-lysine uptake on fast component. The experimental conditions as described in Fig. 5, except that lysine concentrations varied as indicated. The inset shows the effect of various lysine concentrations on fast component. The lines were determined by linear regression analysis ($r^2 = 0.998$). o—o, pH 5.5; •—•, pH 7.5

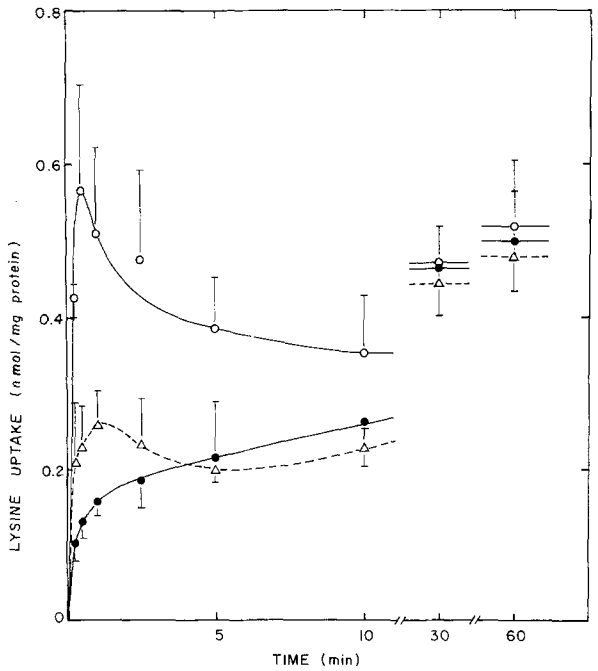

Fig. 7. Effect of $\Delta\psi$ and $\Delta\psi$ plus ΔpH on L-lysine uptake. Membrane vesicles were loaded with 300 mM mannitol buffered with 1.0 mM Tris/HEPES, pH 7.5 (•—•); 100 mM mannitol, 1.0 mM Tris/HEPES, pH 7.5 and 100 mM KCl (Δ---Δ); 100 mM mannitol, 50 mM Tris/HEPES, pH 8.3 and 100 mM KCl (o—o). Vesicles were suspended in media containing 300 mM mannitol, 0.25 mM L-[^{3}H]-lysine plus 1.0 mM Tris/HEPES, pH 7.5 (•—•); 1.0 mM Tris/HEPES, pH 7.5 and 10 μM valinomycin (Δ---Δ); 50 mM Tris/MES, pH 5.5 and 10 μM valinomycin (o—o)

the presence of the ΔpH ($pH_o = 5.5$; $pH_i = 8.3$), a much higher transient stimulation in lysine uptake was observed than that seen with the ΔpH or the $\Delta\psi$ alone. This stimulation of lysine uptake by ΔpH plus $\Delta\psi$ was approximately fourfold compared to the mannitol control, and appeared to approximate the sum of the ΔpH and the $\Delta\psi$-induced lysine uptake. These results indicate that the protonmotive force ($\Delta\psi$ plus ΔpH) can provide the driving force for the uptake of lysine into the BBM vesicles isolated from mullet kidney.

Discussion

The present study focuses upon the lumenal step in L-lysine reabsorption using BBM isolated from the kidney of the striped mullet, a marine teleost. This system was selected for two reasons. First, in contrast with other solutes, including sugars, urea and organic acids, data on the renal handling of amino acids by teleost kidney are almost completely lacking (Forster, 1967; Hickman & Trump, 1969). By analogy with sugar transport in the teleost kidney (Eveloff, Kinne & Kinter, 1979; Lee & Pritchard, 1983) and amino acid transport in the teleost gut (Cartier, Buclon & Robinson, 1979; Boge & Rigal, 1981), it would appear that Na^+ coupled cotransport may drive reabsorption of, at least, neutral amino acids. However, only in Burg and Weller's (1967) studies on isolated perfused

tubules from flounder kidney, has net lumen to bath (i.e. reabsorptive) amino acid (glycine) transport been demonstrated. Even in this system, Na^+ dependence was not shown. Other studies examining amino acid clearance *in vivo* have shown net secretion, rather than reabsorption, of phenylalanine, tyrosine and taurine (Williams, Chen & Huang, 1974; Schrock, Forster & Goldstein, 1982). Data on basic amino acids are unavailable. Second, even in mammals the roles of Na^+-dependent and Na^+-independent transport in the reabsorption of basic amino acids by the kidney remain unresolved (Busse, 1978; Hilden & Sacktor, 1981; Hammerman, 1982).

As shown in Fig. 1, whereas the transport of the neutral amino acid, L-leucine, does appear to be driven by the Na^+ gradient, the transport of L-lysine by mullet kidney BBM appears to be unique. L-lysine uptake was not significantly altered by imposition of a Na^+ gradient (out > in), neither highly stimulated, as would be expected for a Na^+ cotransport system, nor strongly inhibited, as seen for L-arginine uptake by rabbit BBM vesicles (Busse, 1978; Hilden & Sacktor, 1981). Furthermore, imposition of a pH gradient (inside acidic) did not stimulate uptake (Fig. 3) as observed by Hammerman (1982) for Na^+-independent uptake of arginine by rabbit BBM. Instead, imposition of an inside alkaline pH gradient greatly stimulated L-lysine uptake and produced a transient stimulation (Fig. 3), suggesting that L-lysine uptake by mullet kidney BBM may be driven by proton/lysine symport.

The additional properties of L-lysine uptake by mullet BBM examined here also appear to support this hypothesis. First, like Na^+-independent uptake of L-arginine by rabbit BBM vesicles (Hammerman, 1982), some of the L-lysine uptake resulted from binding to the membranes (Fig. 2), but the bulk of the accumulation (>80%) reflected uptake into the vesicle interior. Second, under gradient conditions L-lysine uptake increased as the magnitude of the imposed pH gradient (inside alkaline) increased (Fig. 4). Similarly, when the gradient was dissipated by the protonophore, FCCP, the magnitude of the uptake was markedly reduced (Fig. 3). FCCP inhibition under these conditions might arise either directly from dissipation of the proton gradient or through generation of increased inside positive membrane potential, i.e. from an FCCP-mediated H^+ diffusion potential. Such a potential would oppose passive entry of L-lysine itself (a cation) or mediated movement of a positively charged L-lysine/H^+-carrier complex. In view of 1) the relatively small effect of a K^+ diffusion potential alone (inside negative; Fig. 7), compared to that produced by a pH gradient alone (Fig. 3) and 2) the ability of increased proton availability per se to stimulate uptake under equilibrium conditions, i.e. in the absence of imposed pH gradients (Table), we suggest that FCCP inhibition was achieved primarily through dissipation of the proton gradient itself. Nevertheless, it is clear (Fig. 7) that imposition of an inside negative potential can stimulate L-lysine uptake either alone or in concert with an out > in proton gradient. Thus, both components ($\Delta\psi$ and ΔpH) of the protonmotive force may accelerate L-lysine uptake. Indeed, these two components appear to be additive (Fig. 7), as would be predicted for H^+/L-lysine cotransport (Mitchell, 1963). Finally, the kinetic studies summarized here demonstrate that the fast component of L-lysine uptake is pH sensitive under equilibrium exchange conditions (Table) and that this component may be saturated by L-lysine at both high (7.5) and low (5.5) pH (Fig. 6). Thus L-lysine uptake by mullet kidney BBM appears to be carrier mediated and driven by the electrochemical gradient for protons.

In summary, we have shown that the basic amino acid, L-lysine, was transported into the interior of mullet kidney BBM vesicles via a Na^+-independent mechanism. Transport was carrier mediated and could be driven by an imposed proton gradient (inside alkaline). Uptake could be stimulated by imposition of an inside negative membrane potential and inhibited by the protonophore, FCCP. Potential and proton-driven uptake were approximately additive. Therefore, L-lysine transport appears to be driven by proton/lysine symport at the lumenal membrane of the tubule.

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