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Electrogenic K^+-Basic Amino-Acid Cotransport in the Midgut of Lepidopteran Larvae

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Summary. Experiments performed on isolated midgut demonstrate that the model proposed for the absorption of neutral amino acids in the K^+-transporting intestinal epithelium of lepidopteran larvae applies also to the transport of the basic amino acids histidine and lysine. The characteristics of these K^+-basic amino-acid cotransports have been studied in brush-border membrane vesicles. Histidine and lysine are transported by different transport agencies, which share, to a different degree, a high sensitivity to transmembrane electrical potential difference. Kinetic analysis showed that K_m for histidine and lysine increased 10-fold and three-fold, respectively, whereas V_{max} was only slightly modified when the electrical potential difference was abolished. The relationship between potassium concentration and histidine uptake indicates a cooperative binding of more than one potassium to the transporter. Countertransport experiments with glutamine as elicitor show that histidine and glutamine are transported through the same system.

Key Words lepidopteran larvae midgut · basic amino-acid transport · K^+ cotransport · transstimulation · brush-border membrane vesicles

Introduction

Amino-acid absorption in the intestine of lepidopteran larvae is a secondary active transport which takes place by means of a K^+ amino-acid cotransport system (Hanozet et al., 1980), located at the luminal membrane of columnar cells. It has been suggested (Giordana et al., 1982) that the driving force for the transmucosal uptake is supplied by the potassium electrochemical gradient generated by the activity of the luminally directed K^+ pump (Dow et al., 1984). Amino acids play a key role in the metabolism of enterocytes (Parenti et al., 1985*b*) as well as in the osmoregulation of internal fluids (Florkin & Jeuniaux, 1974). In particular, basic amino acids show a vey high hemolymph-to-lumen accumulation ratio (Parenti et al., 1985*a*, *b*).

In brush-border membrane preparations from lepidopteran midgut, we have been able to show the presence of a symport between potassium and a number of neutral amino acids (Hanozet et al., 1980, 1984; Giordana et al., 1982; Sacchi et al., 1984). In the present work, the transepithelial absorption of the basic amino acids histidine and lysine as well as the transapical mechanisms involved in their transport have been studied. Experiments performed on the isolated midgut suggest that the transfer of these amino acids fits to the model proposed for neutral amino acid absorption. From kinetic measurements and transstimulation experiments in BBMV[1], it can be concluded that histidine and lysine are transported by different transport systems, with K_m values strongly influenced by $\Delta\psi$.

Part of this work was presented to the New York Academy of Sciences, at the Conference on Membrane Transport Driven by Ion Gradients (Capraro et al., 1984).

Materials and Methods

Experimental Animals

Larvae in the fifth instar of *Philosamia cynthia* were used. The larvae were fed on *Ailanthus glandulosa* leaves. The midgut was dissected from the larvae as a cylinder and the peritrophic membrane with the enclosed intestinal content was removed.

Flux Measurements, Intracellular Pool and Amino-Acid Metabolism Determinations

The midgut excised from the larvae was mounted as a cylinder on an apparatus similar to that described by Nedergaard and

[1] *Abbreviations:* BBMV, brush-border membrane vesicles; FCCP, carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone; HEPES, N-2-hydroxyethylpiperazine-N'2-ethansulfonic acid; MES, 2-(N-morpholino)-ethansulfonic acid; Tris, *tris*-(hydroxymethyl)aminomethane; TMA, tetramethylammonium; $\Delta\psi$, transmembrane electrical potential difference.

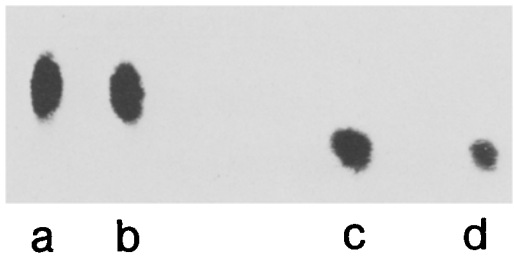

Fig. 1. Metabolism of histidine and lysine by isolated midgut. Midguts were exposed to labeled amino acids and tissue extracts were separated on thin-layer chromatography and autoradiographed as indicated in Materials and Methods. *a.* Standard L-^{3}H-histidine; *b.* extract of the midgut exposed to labeled histidine (0.75 μl deposition); *c.* standard L-^{3}H-lysine; *d.* extract of the midgut exposed to labeled lysine (0.75 μl deposition)

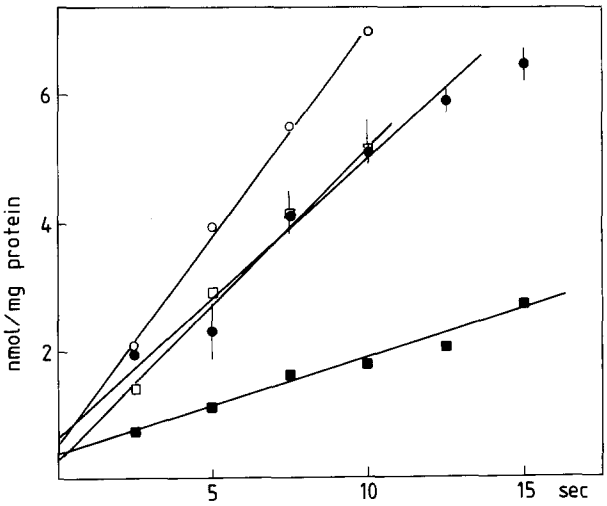

Fig. 2. Initial uptake rates of histidine and lysine. BBMV, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, were incubated in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 100 mM KSCN, 0.5 mM (●) or 5 mM (○) L-^{3}H-histidine; 0.5 mM (■) or 5 mM (□) L-^{3}H-lysine. Each point represents the mean ± SE of a typical experiment carried out in triplicate. When not given, SE bars were smaller than the symbols used

Harvey (1968). Flux measurements and intracellular pools were determined as previously described (Sacchi et al., 1984). To test amino-acid metabolism, midguts were prepared as previously reported (Hanozet et al., 1984). Analysis of tissue extracts (0.75 μl) was carried out by single-dimensional thin-layer chromatography on HPTLC cellulose plates (Merck no. 5787) with a solvent system composed of *n*-butanol-acetone-diethylamine-water (10:10:2:5). The labeled amino acids were used as standards. For autoradiography, the developed and dried plates were sprayed three times with Amplify (Amersham International plc, UK) and exposed to Kodak X-ray film (X-Omat Film SO-282) for 8 days at −80°C. As shown in Fig. 1, no metabolic products of histidine and lysine were present after incubation of the isolated midgut for 1 hr in the presence of the labeled amino acids.

BBMV Preparation and Transport Experiments

BBMV from *P. cynthia* midgut were prepared by means of Ca^{2+} precipitation following the procedure of Schmitz et al. (1973), modified by Kessler et al. (1978), as described in a previous paper (Giordana et al., 1982). When preloading of the vesicles was necessary, the pellet from the second centrifugation step and the final pellet were resuspended in an appropriate medium with the composition reported in the legends of the figures. Transport experiments were performed in triplicate or quadruplicate by the rapid filtration technique, as described by Hanozet et al. (1980). Incubation times shorter than 10 sec were performed with an automated device consisting of a timer assembled in our laboratory, which controls both a shaker (Vibrofix VF1, Janke & Kunkel Ika Werk, Staufen FRG) and an injector (Automatic Dispenser, Oxford, Athy, Ireland). The experimental procedure was similar to that reported by Kessler and Semenza (1983). The uptakes of histidine and lysine at 0.5 and 5 mM concentrations were linear up to 10 sec, as shown in Fig. 2. The nonzero positive intercepts on the ordinate in Fig. 2 indicate that a portion of the amino acid associated with BBMV was not taken up in the intravesicular space but it was bound to the membranes. In the concentration range used, the binding value appears to be independent of the amino-acid concentration. Correction for the zero-time binding of the initial rates in the kinetic experiments reported did not significantly change the kinetic parameters obtained. Radioactivity associated with the filters was measured with a Packard scintillation counter, Model 300 C. Ionophores, when present, were added from ethanol stocks, so that ethanol concentration in the incubation mixture did not exceed 0.5%. The final membrane pellet was resuspended at a protein concentration of 3 to 6 mg/ml as determined according to Bradford (1976) with a Bio-Rad kit, using bovine serum albumin as a standard.

Materials

L-(2,5-^{3}H)-histidine 50 Ci/mmol and L-(3,4-^{3}H)-lysine monohydrochloride 100 Ci/mmol were purchased from Amersham, International plc, UK; valinomycin from Boehringer (Mannheim, FRG); FCCP from Sigma (St. Louis, Mo.). All other reagents were analytical grade products from Merck (Darmstadt, FRG).

Results

The uptake of histidine and lysine in BBMV from *P. cynthia* midgut displays the potassium dependence which has already been observed for neutral amino acids: both basic amino acids showed a concentrative uptake driven by an inwardly directed potassium gradient, whereas the uptake became purely equilibrative with the addition of the potassium ionophore valinomycin (Fig. 3), indicating that the driving force for the intravesicular accumulation of the amino acid was supplied by the potassium gradient. Besides, the transport systems of both amino

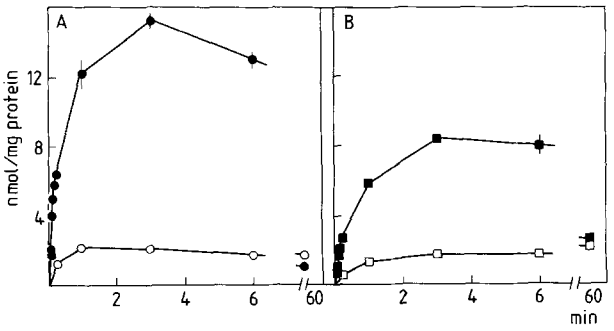

Fig. 3. Effect of valinomycin on histidine and lysine uptakes. BBMV, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, and preincubated 10 min in the absence (closed symbols) and in the presence (open symbols) of 3 μg/mg protein of valinomycin, were incubated in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 100 mM KSCN, 0.5 mM L-^{3}H-histidine (●), 0.5 mM L-^{3}H-histidine + 3 μg/mg protein of valinomycin (○), 0.5 mM L-^{3}H-lysine (■), 0.5 mM L-^{3}H-lysine + 3 μg/mg protein of valinomycin (□). Each point represents the mean ± SE of a typical experiment carried out in triplicate. When not given, SE bars were smaller than the symbols used

acids did not show a cation specificity, since lithium, sodium and potassium were all as effective (Table 1), the uptake in the presence of these cations being four- to sixfold higher than in the control conditions (i.e. in the presence of the impermeant cation TMA).

The effect of anions on histidine and lysine uptakes at 60 sec was studied in the presence of a 100 mM gradient of different potassium salts. With SCN^-, a 60% stimulation of the uptakes with respect to the impermeant anion gluconate was observed for both amino acids. Cl^- and SO_4^{2-} had the same effect of gluconate, as already observed for L- and D-alanine uptake (Hanozet et al., 1984), indicating a poor permeability of these anions across the vesicular membrane.

The electrogenicity of these transports was further studied imposing a Δ pH ($5.5_{in}/7.2_{out}$) and a potassium gradient across the vesicles. Sulfate was used as accompanying anion to avoid any interference in the genesis of the transmembrane potential. When a $\Delta\psi$ was generated by the addition of the proton ionophore FCCP, an accumulation ratio of 4.3 and 3.8 with respect to the equilibrium value for histidine and lysine, respectively, was found (Fig. 4). An overshoot for both amino acids was present also in the absence of FCCP, therefore an intrinsic proton conductance should be inferred, sulfate being almost impermeable across these membranes (Hanozet et al., 1984). In the same experimental conditions, but in the absence of potassium, the initial uptake rates were drastically reduced (Table 2;

Table 1. Effect of monovalent cations on histidine and lysine uptakes[a]

Salt added	Histidine uptake	Lysine uptake
TMACl	400 ± 13	427 ± 8
LiCl	2663 ± 143	2004 ± 41
NaCl	2178 ± 25	1644 ± 53
KCl	2129 ± 222	2447 ± 73
RbCl	644 ± 40	1054 ± 61
CsCl	529 ± 17	682 ± 15

[a] Membrane vesicles, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, were incubated in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 0.5 mM L-^{3}H-histidine or L-^{3}H-lysine, 100 mM of the indicated salt. Uptakes are expressed as pmol/60 sec/mg protein. Mean ± SE of three experiments.

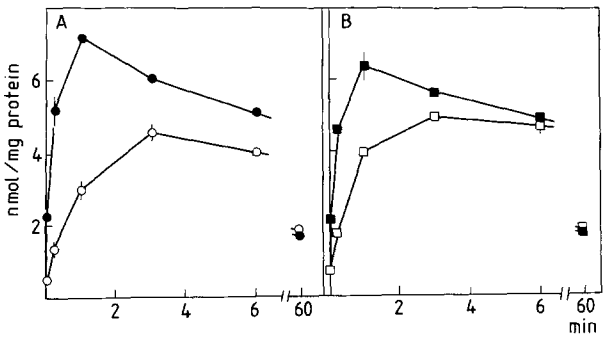

Fig. 4. Effect of FCCP and proton gradient on histidine and lysine uptakes. BBMV, resuspended in 193 mM mannitol, 90 mM MES and 17 mM Tris, pH 5.5, were incubated in a medium of the following final composition: 166 mM mannitol, 18 mM MES, 72 mM HEPES, 39 mM Tris, pH 7.21, 40 mM K_2SO_4, 0.5 mM L-^{3}H-histidine (○), 0.5 mM L-^{3}H-histidine + 80 μM FCCP (●), 0.5 mM L-^{3}H-lysine (□), 0.5 mM L-^{3}H-lysine + 80 μM FCCP (■). Each point represents the mean ± SE of a typical experiment carried out in triplicate. When not given, SE bars were smaller than the symbols used

Table 2. Effect of $\Delta\psi$ and of external potassium on histidine and lysine uptakes[a]

	Histidine uptake	Lysine uptake
Potassium	517 ± 22	656 ± 4
Potassium + FCCP	2281 ± 60	2083 ± 66
No potassium	388 ± 46	439 ± 19
No potassium + FCCP	651 ± 40	612 ± 8

[a] The uptakes were determined as described in Fig. 4, in the presence or in the absence of external K_2SO_4. Uptakes are expressed as pmol/3 sec/mg protein. Means ± SE of three experiments.

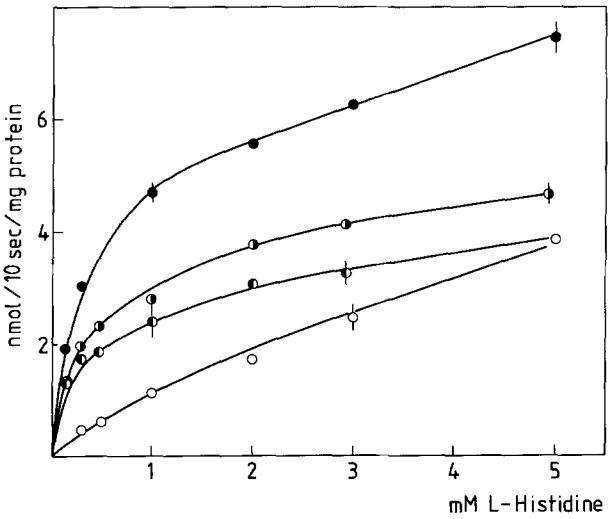

Fig. 5. Kinetics of histidine uptake as a function of $\Delta\psi$ and of internal potassium. BBMV, resuspended in 193 mM mannitol, 90 mM MES and 17 mM Tris, pH 5.5 (◑), or in the same buffer plus 40 mM K_2SO_4 (◐), were incubated in a medium of the following final composition: 166 mM mannitol, 18 mM MES, 72 mM HEPES, 39 mM Tris, pH 7.21, 40 mM K_2SO_4, 80 μM FCCP and 0.185 to 5.0 mM L-^{3}H-histidine. BBMV, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4 (●) or in the same buffer plus 100 mM KSCN (○), were incubated in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 100 mM KSCN and 0.185 to 5.0 mM L-^{3}H-histidine. Uptake was measured after 10-sec incubation. Each point represents the mean ± SE of a typical experiment carried out in quadruplicate. When not given, SE bars were smaller than the symbols used.

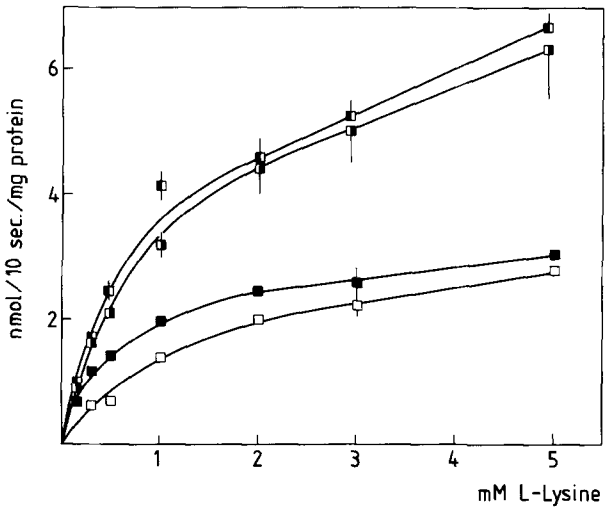

Fig. 6. Kinetics of lysine uptake as a function of $\Delta\psi$ and of internal potassium. BBMV, resuspended in 193 mM mannitol, 90 mM MES and 17 mM Tris, pH 5.5 (◨), or in the same buffer plus 40 mM K_2SO_4 (◧), were incubated in a medium of the following final composition: 166 mM mannitol, 18 mM MES, 72 mM HEPES, 39 mM Tris, pH 7.21, 40 mM K_2SO_4, 80 μM FCCP and 0.185 to 5.0 mM L-^{3}H-lysine. BBMV, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4 (■) or in the same buffer plus 100 mM KSCN (□), were incubated in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 100 mM KSCN and 0.185 to 5.0 mM L-^{3}H-lysine. Uptake was measured after 10-sec incubation. Each point represents the mean ± SE of a typical experiment carried out in quadruplicate. When not given, SE bars were smaller than the symbols used

see also Capraro et al., 1984). Further, also the potassium-independent amino-acid uptake was slightly increased by $\Delta\psi$.

In order to investigate the role of $\Delta\psi$ on the carrier mechanism, the kinetic parameters of the transport systems for histidine and lysine were determined at 100 mM external potassium, in the presence of a $\Delta\psi$ generated either by a proton diffusion potential, or by a SCN^- diffusion potential, and in the presence or in the absence of 100 mM internal potassium. In all conditions, the kinetics of histidine and lysine were consistent with a saturable component plus a linear one (Figs. 5 and 6). The diffusional constants were calculated from the linear part of the curve and their values varied from 2.0 to 6.1 $\times$ 10^{-8} liter mg protein^{-1} min^{-1} for histidine and from 1.8 to 6.0 $\times$ 10^{-8} liter mg protein^{-1} min^{-1} for lysine. Uptake values, corrected for the nonsaturable component, were plotted according to Eadie-Hofstee in order to obtain the kinetic parameters reported in Tables 3 and 4. For histidine (Table 3), a 10-fold increase of K_m was obtained when $\Delta\psi$ was abolished. The internal potassium in the presence of $\Delta\psi$ caused a decrease both of V_{max} and K_m (Table 3, lines 1 *vs.* 2). For lysine (Table 4), a $\Delta\psi \simeq 0$ caused an increase of K_m, although to a lesser extent compared to histidine. In the presence of $\Delta\psi$, internal potassium did not effect V_{max} for lysine (Table 4, lines 1 *vs.* 2). An acidic intravesicular pH seemed to exert no influence on the affinity of the transport systems of both amino acids, while it affected in opposite ways the V_{max} values, with a 30% decrease for histidine and a 37% increase for lysine (Tables 3 and 4, lines 1 *vs.* 3).

The dependence of histidine and lysine transport systems on the potassium concentration was investigated in the presence of $\Delta\psi$ (Fig. 7). The $\Delta\psi$ was generated by a pH gradient ($5.5_{in}/7.2_{out}$), since a constant SCN^- concentration gradient with counterions different from potassium was not feasible, because Na^+ is cotransported (Table 1) and choline or Tris at high concentrations behaved as inhibitors (*data not shown*). The uptakes of both amino acids displayed a saturation kinetics with respect to external potassium concentration, plus a potassium-independent component. From Fig. 7, a different

Table 3. Effect of $\Delta\psi$ and of internal potassium (K_{in}) on the kinetic parameters of histidine uptake[a]

Conditions				K_m	V_{max}
pH	salt	$\Delta\psi$	K_{in}		
1. $7.2_o/5.5_i$	K_2SO_4	+	−	0.25 ± 0.02	3.36 ± 0.09[b]
2. $7.2_o/5.5_i$	K_2SO_4	+	+	0.17 ± 0.03	2.70 ± 0.15[b]
3. $7.4_o/7.4_i$	KSCN	+	−	0.23 ± 0.02[c]	4.78 ± 0.15[d]
4. $7.4_o/7.4_i$	KSCN	−	+	2.68 ± 0.61[c]	3.54 ± 0.50[d]

[a] Lines 1 and 3: K^+ concentration was 100 $mM_o/0_i$; lines 2 and 4: K^+ concentration was 100 $mM_o/100$ mM_i. The constants were calculated from an Eadie-Hofstee plot of the uptake values corrected for the nonsaturable component. Kinetic parameters were obtained by linear regression analysis according to the least-squares method. K_m is expressed as mM ± SE, V_{max} as nmol/10 sec/mg protein ± SE; i = in; o = out.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P < 0.05$.

Table 4. Effect of $\Delta\psi$ and of internal potassium (K_{in}) on the kinetic parameters of lysine uptake[a]

Conditions				K_m	V_{max}
pH	salt	$\Delta\psi$	K_{in}		
1. $7.2_o/5.5_i$	K_2SO_4	+	−	0.41 ± 0.06	3.62 ± 0.23
2. $7.2_o/5.5_i$	K_2SO_4	+	+	0.48 ± 0.04	3.66 ± 0.13
3. $7.4_o/7.4_i$	KSCN	+	−	0.33 ± 0.03[b]	2.29 ± 0.07
4. $7.4_o/7.4_i$	KSCN	−	+	0.89 ± 0.15[b]	2.24 ± 0.18

[a] Lines 1 and 3: K^+ concentration was 100 $mM_o/0_i$; lines 2 and 4: K^+ concentration was 100 $mM_o/100$ mM_i. The constants were calculated from an Eadie-Hofstee plot of the uptake values corrected for the nonsaturable component. Kinetic parameters were obtained by linear regression analysis according to the least-squares method. K_m is expressed as mM ± SE, V_{max} as nmol/10 sec/mg protein ± SE; i = in; o = out.
[b] $P < 0.001$.

stoichiometry ratio between potassium and the two amino acids was to be expected, the relationship being clearly sigmoidal for histidine and essentially hyperbolic for lysine. The linear transformation (Hill plot, Fig. 7 inset) yields a slope = n, or apparent number of ligand binding sites, and a $K_{0.5}$ value, when $\log (v_o/V_{max} - v_o) = 0$. Hill coefficients and $K_{0.5}$ values, calculated after subtraction of the potassium-independent uptakes, were 2.50 ± 0.23 and 56.36 ± 7.32 mM, respectively, for histidine and 1.07 ± 0.18 and 14.38 ± 1.49 mM for lysine (means ± SE). To test the effect of pH on Hill coefficients, the experiment was repeated at higher pH values ($pH\ 7.5_{in}/8.8_{out}$). This condition is very similar to that occurring *in vivo* across the apical membrane of columnar cells. The obtained Hill coefficients and $K_{0.5}$ were 1.50 ± 0.10 and 31.62 ± 2.38 mM, respectively, for histidine and 1.14 ± 0.12 and 16.22 ± 3.08 mM for lysine.

Countertransport experiments can show if the same transport system is shared by two different substrates. In order to ascertain whether the two basic amino acids are transported by different agencies, vesicles were preloaded with 40 mM histidine or lysine and then diluted 20-fold in a medium containing the labeled amino acid. 50 mM K_2SO_4 was present on both sides of the vesicles and valinomycin was added to avoid any electrical coupling via transmembrane potential. Under these conditions, transstimulation and counterflow accumulation of labeled histidine was elicited by internal histidine, whereas lysine failed to behave as an elicitor (Fig. 8). The same pattern was obtained for lysine (Fig. 9): therefore the two amino acids cross the membrane via independent carrier-mediated transport systems. The same figures provide evidence that internal glutamine also elicited transstimulation and counterflow accumulation of histidine uptake, while it was ineffective for lysine: histidine and glutamine seem to share the same transport agency as

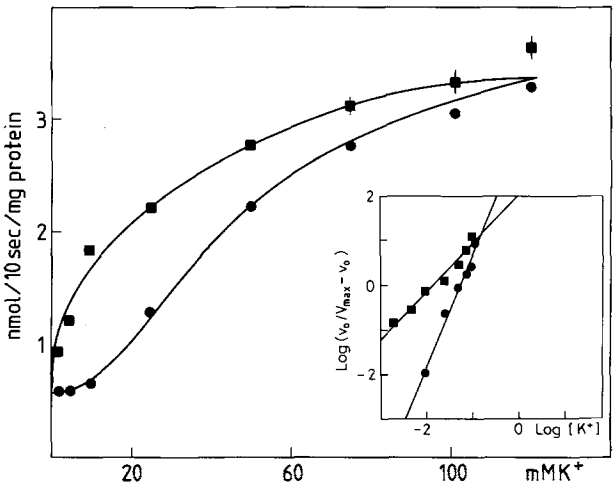

Fig. 7. Kinetics of histidine and lysine uptake as a function of external potassium concentration. BBMV resuspended in 193 mM mannitol, 90 mM MES, 17 mM Tris, pH 5.5, were incubated in a medium of the following final composition: 166 mM mannitol, 18 mM MES, 72 mM HEPES, 32 mM Tris, pH 7.21, 80 μM FCCP, 0.5 mM L-^{3}H-histidine (●) or L-^{3}H-lysine (■) and 0 to 60 mM K_2SO_4. Uptake was measured after 10-sec incubation. Each point represents the mean ± SE of a typical experiment carried out in quadruplicate. When not given, SE bars were smaller than the symbols used. In the inset, the Hill plot for both amino acids is reported. v_o = amino-acid uptake after subtraction of the potassium-independent uptake; V_{max} = uptake extrapolated to infinite potassium concentration in a double-reciprocal plot by means of a computer program

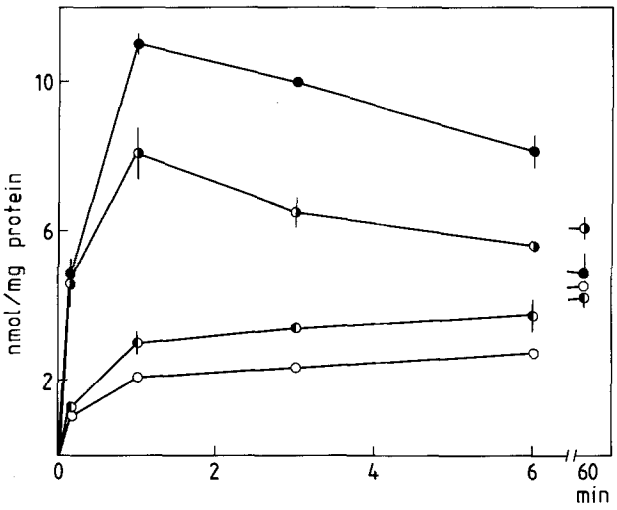

Fig. 8. Transstimulation of histidine uptake. BBMV, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 50 mM K_2SO_4 + 40 mM mannitol (○) + 40 mM histidine (●), + 40 mM lysine (◐), +40 mM glutamine (◑), and preincubated 10 min with 8 μg/mg protein of valinomycin, were diluted 1 : 20 in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 50 mM K_2SO_4, 8 μg/mg protein of valinomycin, 2 mM L-^{3}H-histidine. Each point represents the mean ± SE of a typical experiment carried out in triplicate. When not given, SE bars were smaller than the symbol used

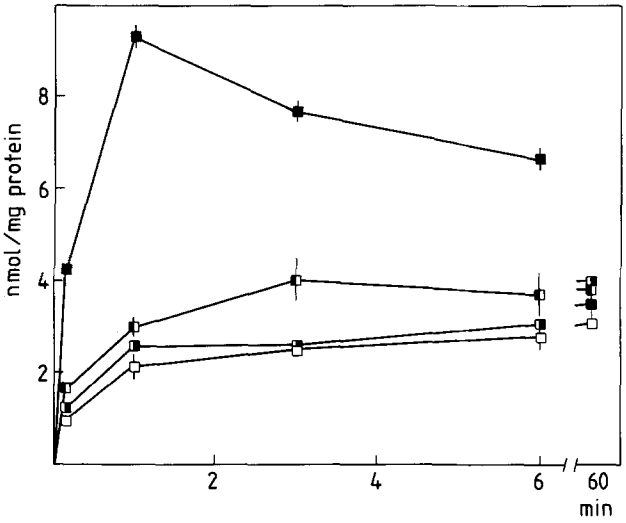

Fig. 9. Transstimulation of lysine uptake. BBMV, resuspended in 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 50 mM K_2SO_4 + 40 mM mannitol (□), + 40 mM lysine (■), + 40 mM histidine (◧), + 40 mM glutamine (◨), and preincubated 10 min with 8 μg/mg protein of valinomycin, were diluted 1 : 20 in a medium of the following final composition: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 50 mM K_2SO_4, 8 μg/mg protein of valinomycin, 2 mM L-^{3}H-lysine. Each point represents the mean ± SE of a typical experiment carried out in triplicate. When not given, SE bars were smaller than the symbol used

observed also in mammal hepatocytes (Kilberg et al., 1980).

The ability of the whole midgut to transport histidine *in vitro* was also investigated. Table 5 reports the unidirectional and net fluxes of the amino acid at 0.5 mM concentration in the presence and in the absence of potassium in the lumen. The spontaneous transepithelial electrical potential difference of each preparation was measured throughout the two sets of experiments, and the means ± SE were 91.6 ± 5.5 and 110 ± 1.9 mV, respectively; in the absence of luminal potassium the mean value of the potential difference was higher, as expected. The lumen to hemolymph flux (J_{l-h}) of histidine was 20-fold higher than the hemolymph to lumen flux (J_{h-l}), so that a relevant net absorption of the amino acid takes place across the midgut. Histidine net flux was 80% inhibited when potassium was removed from the lumen.

The luminal pool accounts for the amount of histidine that enters from the lumen into the cell: its value (Table 6) shows that a concentrative uptake of the amino acid occurs across the luminal barrier. This intracellular accumulation, up to 12-fold the extracellular histidine concentration, was drastically reduced but not abolished in the absence of luminal potassium. A small accumulation ratio was observed also for the amino acid entering the cell from the basolateral side.

Discussion

The transport of all the amino acids so far tested across the brush-border membrane of the midgut of lepidopteran larvae is, at least for the most part, strongly dependent on external potassium (Hanozet et al., 1980; Giordana et al., 1982; Hanozet et al., 1984; Sacchi et al, 1984). The basic amino acids histidine and lysine also share this feature (Fig. 3). The demand of the transport systems for the cotransported cation is not accomplished exclusively by potassium, since sodium and lithium are as effective as potassium for both amino acids (Table 1). Lithium is seldom effective in mammalian cotransports (Bihler & Adamic, 1967; Berteloot et al., 1982; Corcelli et al., 1982) and also in lepidoptera it is less efficient than sodium and potassium for neutral amino-acid cotransport (Giordana et al., 1982; Hanozet et al., 1984). In the lepidopteran midgut *in vivo* only potassium is present at high concentration in the lumen, thus it is the only cation which can be effectively used by the cotransport systems. Therefore, the amino-acid transport systems active in the

Table 5. Unidirectional and net fluxes of histidine in the isolated midgut.[a]

	J_{l-h}	J_{h-l}	J_{net}
Control	13.47 ± 2.82[b] (3)	0.67 ± 0.06 (4)	12.80 ± 0.68[c]
No potassium	3.11 ± 0.48[b] (7)	0.67 ± 0.06 (4)	2.44 ± 0.65[c]

[a] L-^{3}H-histidine concentration was 0.5 mM. J_{l-h} = lumen to hemolymph flux; J_{h-l} = hemolymph to lumen flux; $J_{net} = J_{l-h} - J_{h-l}$. In the experiments with no potassium, luminal $KHCO_3$ was replaced by choline HCO_3. Fluxes are expressed as μmol/g dry weight/hr. Means ± SE, number of experiments in parentheses.
[b,c] $P < 0.01$.

Table 6. Intracellular L-histidine pools in the isolated midgut[a]

	Luminal pool	Hemolymph pool
Control	6.30 ± 0.77[b] (6)	0.94 × 0.10 (4)
No potassium	2.95 ± 0.52[b] (7)	n.d.

[a] External L-histidine concentration was 0.5 mM. Luminal and hemolymph pools were measured by adding labeled histidine to either luminal or hemolymph solution. Pools are expressed as mmol/liter cell water. Means ± SE, number of experiments in parentheses; n.d., not determined.
[b] $P < 0.01$.

lepidopteran midgut can be called "K⁺-dependent" processes *in vivo*, although they are only "monovalent cation-dependent" processes *in vitro*. For the same reason, the electrogenic pump in the same tissue has been called "K⁺-pump" with regard to the ionic environment *in vivo*, although other cations can efficiently replace potassium *in vitro*.

Basic amino-acid transport has in common with the other amino acids studied a strong dependence on $\Delta\psi$ (Fig. 4 and Table 2), in agreement with the high transapical electrical potential difference recorded *in vivo* across midgut columnar cells (150 mV, lumen positive; *see* Giordana et al., 1982; Monticelli & Giordana, 1983). The effect of $\Delta\psi$ on kinetic parameters was carefully examined (Tables 3 and 4). The affinity of both carriers for basic amino acids strongly depended on $\Delta\psi$: about a 10-fold and a threefold increase of K_m was observed for histidine and lysine, respectively, when the $\Delta\psi$ was abolished. This cannot be ascribed to a potassium *trans* inhibition, since in the presence of internal potassium and of a $\Delta\psi$ generated by Δ pH, K_m values are similar to the control without internal potassium. A dependence from $\Delta\psi$ has also been found for L- and D-alanine carrier systems (Hanozet et al., 1984), suggesting a similar fitness of different transport agencies to the high electric field typical of the mucosal membrane of lepidopteran enterocyte. As suggested by Kessler and Semenza (1983) for Na⁺-D-glucose cotransporter in mammalian small intestine, a possible rationale for this result is that the unloaded carriers bear (a) negative charge(s), which is/are exposed to cytoplasmic side of the membrane when $\Delta\psi \simeq 0$. In the presence of an inside-negative $\Delta\psi$, the charged group(s) of the carriers are pushed outside and can bind the potassium ion and the amino acid. The effect of internal potassium in the presence of $\Delta\psi$ on the kinetic parameters can give some indications about the kinetic mechanism involved. A comparison between lines 1 *vs.* 2 of Tables 3 and 4 reveals that internal potassium affected the two systems in different ways. For histidine, a decrease of V_{max} was observed, whereas the ratio K_m/V_{max} was unaffected, as typical for uncompetitive inhibition. This pattern is compatible with an Iso Ordered Bi Bi mechanism (potassium first out, last in). For lysine, the lack of inhibition is compatible either with an Ordered Bi Bi or with a Rapid Equilibrium Random mechanism; in any case the concentration of the internal unloaded carrier form must be negligible.

The effect of an intravesicular acidic pH can be evaluated by comparing lines 1 and 3 of Tables 3 and 4: V_{max} was significantly increased for lysine and decreased for histidine. This indicates that the influence on V_{max} cannot be due to different values of the diffusion potentials generated by H^+ (line 1) or SCN^- (line 3). A stimulation of the initial uptake of lysine by outwardly directed proton gradient has been described in rat kidney (Stieger et al., 1983).

The dependence of the transport systems on the external potassium concentration was determined in the presence of a constant $\Delta\psi$ imposed across the vesicle membranes. Figure 7 indicates that a different number of binding sites for potassium to the transporters is involved in the translocation of the two amino acids. Lysine displayed a typical Michaelis-Menten curve, whereas sigmoidal curves were obtained for histidine at external pH values 7.2 and 8.8. The corresponding Hill coefficients were 2.5 and 1.5, indicating a cooperative binding of more than on potassium to the transporter at both pH values. This agrees with the higher sensitivity to $\Delta\psi$ showed by histidine carrier. The same figure clearly shows that a potassium-independent component occurs, being almost 12% of V_{max}. This component is also $\Delta\psi$ dependent (Table 2) and could be explained by the overall positive charge borne by

the two amino acids. Besides, the main part of the overall histidine and lysine transport is dependent on potassium. This is supported by the uptake values in the presence of a KSCN gradient with or without valinomycin (Fig. 3) and by the experiments with and without external potassium (Table 2). In this respect, lysine transport in lepidopteran midgut seems to be different from that operating in mammalian intestine, where the transport of this amino acid is mainly cation-independent (Stevens et al., 1982; Cassano et al., 1983).

The dependence of histidine transport on potassium was apparent also in the isolated gut. Histidine was actively absorbed by the midgut (Table 5) and intracellularly accumulated 12.6-fold from the lumen side (Table 6). The net flux and the luminal pool were strongly reduced by the removal of potassium from the lumen, results both consistent with the model proposed for amino acid absorption across the midgut of lepidopteran larvae (Giordana et al., 1982). The residual intracellular histidine pool, found when potassium was replaced by choline in the lumen, could be due to a recirculation of potassium from the hemolymph compartment through the potassium pump, as already pointed out (Sacchi et al., 1984). The ability of the midgut to actively absorb lysine has been documented in another *Saturnidae* larva (Nedergaard, 1973): in *Hyalophora cecropia,* with standard saline and in the presence of the transepithelial electrical potential difference, the ratio between J_{l-h} and J_{h-l} of lysine is 10.9. Active absorption of histidine and lysine seems to be operative also *in vivo*: the determination of the free amino-acid concentrations in the hemolymph and in the lumen content of *P. cynthia* larvae *in vivo* showed that the concentration of histidine and lysine in the hemolymph compartment is 4.8- and 7.5-fold higher than in the luminal one (Parenti et al., 1985*a*).

The different coupling ratio found between potassium and each amino acid, together with the different kinetic mechanism, suggest that different transport agencies should be involved in the transfer of histidine and lysine. Countertransport experiments (Figs. 8 and 9) demonstrate that only an outwardly directed gradient of the same amino acid elicits transstimulation and counterflow accumulation. These phenomena strongly suggest that the two amino acids do not share the same carrier. If this is true, there should be no cross-inhibition between the two amino acids, therefore the kinetic parameters of histidine were determined in the presence of lysine. The experiment was carried out with potassium-equilibrated vesicles, to avoid any indirect inhibition due to the dissipation of the potassium gradient by the potassium-lysine cotransport. In these conditions, lysine does not inhibit histidine uptake. Actually, at 0, 10 and 20 mM lysine concentrations, K_m values for histidine uptake were 2.68 ± 0.61, 3.21 ± 0.89 and 3.03 ± 0.57 mM, respectively; V_{max} values were 3.54 ± 0.50, 3.70 ± 0.67 and 3.79 ± 0.46 nmol/10 sec/mg protein, respectively. All these values were not statistically different. Moreover, in preliminary experiments, the uptake of histidine and lysine in BBMV displayed a different inhibition profile by the other amino acids. In particular, the histidine system has been proved to be more sensitive to inhibition by neutral amino acids. Countertransport experiments with glutamine as elicitor (Figs. 8 and 9) support the conclusion that histidine and glutamine share a common transporter, whereas lysine follows a different pathway. A transport system specific for histidine, asparagine and glutamine has been characterized in hepatocytes by Kilberg et al. (1980), known as system N.

Work is in progress in our laboratory to identify the amino-acid transport systems operating in the midgut of lepidopteran larvae.

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References

Berteloot, A., Khan, A.H., Ramaswamy, K. 1982. K^+ and Na^+ gradient dependent transport of L-phenylalanine by mouse intestinal brush border membrane vesicles. *Biochim. Biophys. Acta* **691**:320–331

Bihler, I., Adamic, S. 1967. The effect of lithium on intestinal sugar transport. *Biochim. Biophys. Acta* **135**:466–474

Bradford, M.M. 1976. A rapid sensitive method for the quantitation of protein utilizing the principle of dye-protein binding. *Anal. Biochem.* **72**:248–254

Capraro, V., Giordana, B., Hanozet, G.M., Parenti, P., Sacchi, V.F. 1984. K-dependent amino acid transport in lepidoptera. *The New York Academy of Sciences,* Conference on Membrane Transport Driven by Ion Gradients. New York, October 2–4

Cassano, G., Leszcynska, B., Murer, H. 1983. Transport of L-lysine by rat intestinal brush border membrane vesicles. *Pfluegers Arch.* **397**:114–120

Corcelli, A., Prezioso, G., Palmieri, F., Storelli, C. 1982. Electroneutral Na^+/dicarboxylic amino acid cotransport in rat intestinal brush border membrane vesicles. *Biochim. Biophys. Acta* **689**:97–105

Dow, J.A.T., Gupta, B.L., Hall, T.A., Harvey, W.R. 1984. X-ray microanalysis of elements in frozen-hydrated sections of an electrogenic K^+ transport system: The posterior midgut of tobacco hornworm (*Manduca sexta*) *in vivo* and *in vitro*. *J. Membrane Biol.* **77**:223–241

Florkin, M., Jeuniaux, C. 1974. Haemolymph composition. *In:*

The Physiology of Insecta. M. Rockstein, editor. Vol. V, pp. 255–307. Academic, New York

Giordana, B., Sacchi, V.F., Hanozet, G.M. 1982. Intestinal amino acid absorption in lepidopteran larvae. *Biochim. Biophys. Acta* **692:**81–88

Hanozet, G.M., Giordana, B., Parenti, P., Guerritore, A. 1984. L- and D-alanine transport in brush border membrane vesicles from Lepidopteran midgut: Evidence of two transport systems. *J. Membrane Biol.* **81:**233–240

Hanozet, G.M., Giordana, B., Sacchi, V.F. 1980. K^+-dependent phenylalanine uptake in membrane vesicles isolated from the midgut of *Philosamia cynthia* larvae. *Biochim. Biophys. Acta* **596:**481–486

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M., Semenza, G. 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. *Biochim. Biophys. Acta* **506:**136–154

Kessler, M., Semenza, G. 1983. The small-intestinal Na^+, D-glucose cotransporter: An asymmetric gated channel (or pore) responsive to $\Delta\psi$. *J. Membrane Biol.* **76:**27–56

Kilberg, M.S., Handlogten, M.E., Christensen, H.N. 1980. Characteristics of an amino acid transport system in rat liver for gluatamine, asparagine, histidine and closely related analogs. *J. Biol. Chem.* **255:**4011–4019

Monticelli, G., Giordana, B. 1983. Electrical profiles in the midgut of two larvae of Lepidoptera. *Atti Acad. Naz. Lincei* **73:**181–187

Nedergaard, S. 1973. Transport of amino acids in Cecropia midgut. *In:* Transport of Ions and Water in Animals. B.L. Gupta, R.B. Moreton, J.L. Oschman, and B.J. Wall, editors. pp. 239–264. Academic, New York

Nedergaard, S., Harvey, W.R. 1968. Active transport by the Cecropia midgut. IV. Specificity of the transport mechanism for potassium. *J. Exp. Biol.* **48:**13–24

Parenti, P., Cidaria, D., Hanozet, G.M., Giordana, B. 1985*a*. Free amino acid composition of the intestinal content, intestinal cells and haemolymph of Philosamia cynthia larvae. *Experienta (in press)*

Parenti, P., Giordana, B., Sacchi, V.F., Hanozet, G.M., Guerritore, A. 1985*b*. Metabolic activity related to the potassium pump in the midgut of *Bombyx mori* larvae. *J. Exp. Biol.* **116** *(in press)*

Sacchi, V.F., Hanozet, G.M., Giordana, B. 1984. α-aminoisobutyric acid transport in the midgut of two lepidopteran larvae. *J. Exp. Biol.* **108:**329–339

Schmitz, J., Presier, H., Maestracci, D., Ghosh, B.K., Cerda, J.J., Crane, R.K. 1973. Purification of the human intestinal brush border membrane. *Biochim. Biophys. Acta* **323:**98–112

Stevens, B.R., Ross, H.J., Wright, E.M. 1982. Multiple transport pathways for neutral amino acids in rabbit jejunal brush border vesicles. *J. Membrane Biol.* **66:**213–225

Stieger, B., Stange, G., Biber, J., Murer, H. 1983. Transport of L-lysine by rat renal brush border membrane vesicles. *Pfluegers Arch.* **397:**106–113

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