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THE K^+-DRIVEN AMINO ACID COTRANSPORTER OF THE LARVAL MIDGUT OF LEPIDOPTERA: IS Na^+ AN ALTERNATIVE SUBSTRATE?

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Summary

Amino acid accumulation within brush-border membrane vesicles (BBMV) from the larval midgut of Lepidoptera is driven by a K^+ gradient. However, it can also be driven by a Na^+ gradient, although with reduced efficiency. To examine the possibility that sodium and potassium ions are handled by the same amino acid transporter, glycine uptake into BBMV from *Philosamia cynthia* Drury was measured in the presence of a pH gradient and of a transmembrane electrical potential difference, i.e. in simulated 'physiological' conditions. The kinetics of glycine uptake at extravesicular saturating Na^+ or K^+ concentrations discloses a higher affinity of the cotransporter for the amino acid in the presence of Na^+ but a maximum transport rate with K^+. Glycine uptake at a fixed concentration as a function of external Na^+ or K^+ concentration yields curves that show saturation but do not fit a rectangular hyperbola, with Hill coefficients less than 1 with Na^+ and greater than 1 with K^+. These coefficients vary according to glycine concentration. Increasing the concentration of extravesicular Na^+ at a saturating external K^+ concentration reduced glycine uptake to 70% of the control value. This inhibition curve is compatible with competition between the two cations for the same cotransporter and with the presence of different kinetic constants with Na^+ or K^+. The data are consistent with a steady-state random two-substrate mechanism for glycine transport, with Na^+ and K^+ as alternative substrates.

Introduction

In recent years the midgut of lepidopteran larvae has been the subject of many studies because of the unusual physiological features of these insects (Dow, 1986). The major monovalent cation in the hemolymph and in the gut lumen is potassium (25–46 mmol l^{-1} in the hemolymph, depending on species, and 200 mmol l^{-1} in the

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gut) (Giordana and Sacchi, 1978) and, therefore, these insects have developed special adaptations to the high-potassium environment. At the intestinal level, the main secretory role of the midgut is the active extrusion of potassium from hemolymph to lumen (Harvey and Nedergaard, 1964; Harvey *et al.* 1983; Moffett and Koch, 1988). As a consequence, the enterocytes maintain large potassium and pH gradients, together with a high (up to 130 mV) lumen-positive transepithelial electrical potential difference (Dow, 1984; Nedergaard and Harvey, 1968). The electrochemical potassium gradient generated by the pump in turn allows a potassium-dependent amino acid uptake (Nedergaard, 1973; Giordana *et al.* 1982). This cotransport mechanism localized on the brush-border membranes of absorptive columnar cells, couples amino acid and potassium fluxes from the lumen to the cells, but it does not show a strict cation specificity since sodium, and in some case lithium, can activate the transport even if sodium concentration in the lumen, enterocytes and hemolymph is very low (below 5 mmol l^{-1}) (Giordana and Sacchi, 1978).

The ability of potassium to activate amino acid absorption seems to be a general feature of lepidopteran larvae (Wolfersberger *et al.* 1987; Henningan and Wolfersberger, 1989). It is not exclusive to this order (von Rutschke *et al.* 1976) and it is probably a specialization since, in other insects such as cockroaches (Blattoidea), amino acid absorption is exclusively sodium-dependent (Parenti *et al.* 1986).

The aim of this work is to shed light on whether sodium and potassium ions are handled by the same mechanism and to compare the effects of these cations on glycine uptake in brush-border membrane vesicles prepared from the midgut of *Philosamia cynthia* larvae.

Materials and methods

Preparation of brush-border membrane vesicles (BBMV) and transport experiments

Midguts from fifth-instar larvae of *Philosamia cynthia* were frozen in liquid nitrogen and stored at −80 °C for a period not exceeding 6 months. Samples of the frozen midguts (about 2 g) were rapidly thawed at 37 °C and then used to prepare BBMV by calcium precipitation following the procedure described in a previous paper (Giordana *et al.* 1982). The final membrane pellet was resuspended at a protein concentration of about 10 mg ml^{-1}, determined according to Bradford (1976) with a Bio-Rad kit, using bovine serum albumin as standard.

Transport experiments were performed in quadruplicate by a rapid filtration technique, as described previously (Sacchi *et al.* 1990). Incubation times shorter than 10 s were achieved using an automated apparatus consisting of a timer controlling a shaker, to mix 10 μl of BBMV with 10 or 40 μl of a cocktail solution, and an injector for the stop solution. The sample, diluted with 2 ml of ice-cold stop solution, was then filtered and radioactivity associated with the filters was measured with a Packard scintillation counter, Tri-Carb model 300 C.

The uptake of glycine was linear up to 12 s and incubations lasting 7 s were considered to be reliable for measuring initial uptake rates (data not reported).

Glycine uptake in the presence of a pH gradient was measured with an extravesicular buffer of the following composition: 18 mmol l^{-1} Hepes, 45 mmol l^{-1} Tris at pH 8.9 and sodium or potassium salts and unlabelled and ^{14}C-labelled glycine as indicated in the captions to figures. The intravesicular buffer had the following composition: 90 mmol l^{-1} Hepes, 45 mmol l^{-1} Tris at pH 7.5. Osmolarity was kept constant with mannitol so that at time zero no osmotic gradient was present across the membrane vesicles. The protonophore FCCP (carbonyl cyanide *p*-trifluorometoxyphenylhydrazone) was added from ethanol stock solutions, so that the ethanol concentration in the incubation medium did not exceed 0.5 %.

Transepithelial flux measurements

Midguts, excised from larvae of *Hyalophora cecropia* (L.) in the fifth instar were mounted in a modified Ussing chamber as a tube (Nedergaard and Harvey, 1968). Samples for lumen-to-hemolymph and hemolymph-to-lumen flux measurements were taken every fifth minute for 30 min (six determinations) using [U-^{14}C]glycine. The sample volume removed was replaced with non-radioactive bathing fluid, so that the volume of the bathing solution was kept constant during the experiment (Nedergaard, 1973). Serosal fluid consisted of (mmol l^{-1}): 30 KCl, 2 $KHCO_3$, 5 $CaCl_2$, 5 $MgCl_2$, 10 glycine, 166 sucrose, pH 8.1. Luminal fluid was (mmol l^{-1}): 5 $CaCl_2$, 5 $MgCl_2$, 10 glycine, 30 sucrose, and either 98 KCl and 2 $KHCO_3$, pH 8.1, or 98 NaCl and 2 $NaHCO_3$, pH 8.0. Steady-state fluxes were attained within 15 min. The average flux value for each experiment was the mean of the determinations at steady state (at 15, 20, 25 and 30 min).

Materials

[U-^{14}C]glycine and L-[U-^{3}H]leucine were obtained from Radiochemical Centre (Amersham International, Amersham, UK). FCCP was obtained from Sigma (St Louis, MO, USA). Hepes was obtained from Boehringer (Mannheim, FRG). All other reagents were analytical grade products from BDH (Chemicals Ltd Poole, England).

Results

At neutral pH values on both sides of the vesicle, sodium was more effective than potassium in driving glycine uptake into brush-border membrane vesicles (Giordana *et al.* 1989), while all neutral L-amino acids so far tested were more sensitive to a K^+ than to a Na^+ gradient. However, the first step for amino acid translocation occurs at the luminal border of the enterocytes, which in lepidopteran larvae has a steep pH gradient *in vivo* (pH 10–11 lumen, pH 7 in the cell) and an electrical potential gradient of up to 170 mV (cell interior negative). The effects of a neutral or an alkaline pH and of the transmembrane electrical potential on

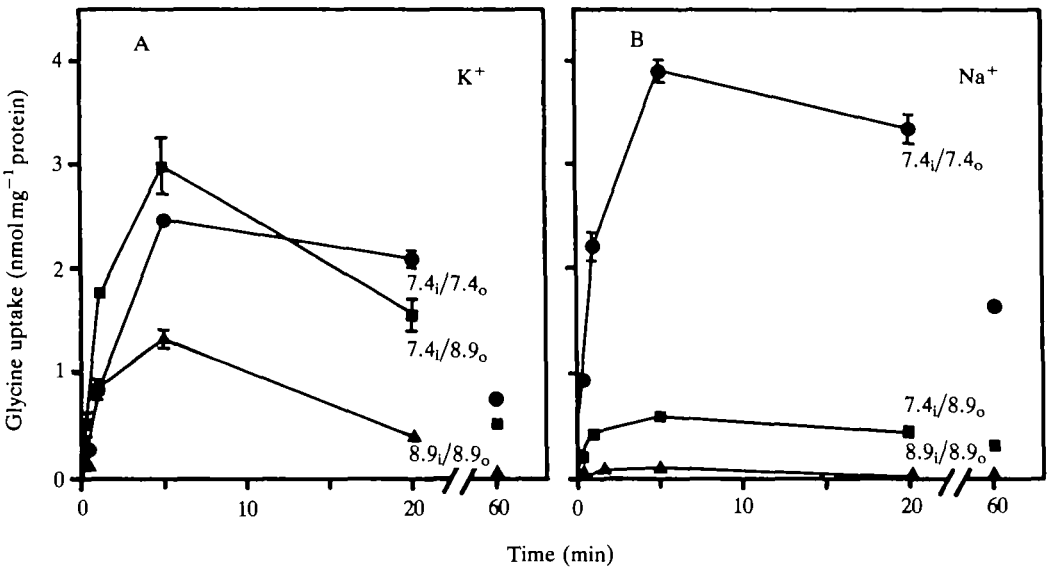

Fig. 1. Time course of glycine uptake into BBMV driven by a potassium (A) or a sodium (B) chemical gradient in the presence of different pH gradients across the vesicle membrane: (●) $7.4_i/7.4_o$; (■) $7.4_i/8.9_o$; (▲) $8.9_i/8.9_o$. Vesicle internal media were (mmol l^{-1}): ●, 268 mannitol, 90 Hepes, 45 Tris at pH 7.4; ■, 160 mannitol, 90 Hepes, 45 Tris at pH 7.4; and ▲, 268 mannitol, 45 Tris at pH 8.9. The vesicles were diluted 1:1 or 1:5 (pH gradient) in media of the following final composition (mmol l^{-1}): ●, 118 mannitol, 90 Hepes, 45 Tris at pH 7.4, 50 K_2SO_4 (A) or Na_2SO_4 (B), 0.73 [U-^{14}C]glycine (10 μC ml^{-1}); ■ and ▲, 118 mannitol, 18 or 0 Hepes, 45 Tris at pH 8.9, 50 K_2SO_4 (A) or Na_2SO_4 (B), 0.73 [U-^{14}C]glycine (10 μC ml^{-1}). Each point represents the mean±S.E. of a typical experiment performed in triplicate. When not present, error bars were smaller than the symbols.

glycine uptake were therefore tested. Fig. 1A,B shows that, in the absence of an electrical potential difference, the absolute values of glycine uptake were indeed higher in the presence of sodium when the internal and external solutions were buffered at pH 7.4. However, when the extravescicular pH was 8.9, irrespective of the internal pH value, Na^+-dependent glycine uptake was drastically reduced. In contrast, glycine uptake driven by potassium (Fig. 1A) required a neutral intravesicular pH. When a transmembrane electrical potential difference was induced by proton diffusion in the presence of the protonophore FCCP in vesicles preloaded with a neutral buffer and diluted in an alkaline medium (pH 8.9), potassium rather than sodium caused the highest values of glycine uptake and accumulation (Fig. 2).

Since a pH gradient ($7.5_i/8.9_o$) and an electrical gradient mimic the 'physiological' situation, Na^+-dependent and K^+-dependent uptake of glycine were studied under these experimental conditions.

The initial rate of glycine uptake (V_0, 7 s of incubation) as a function of external glycine concentration was determined in the presence of a saturating concen-

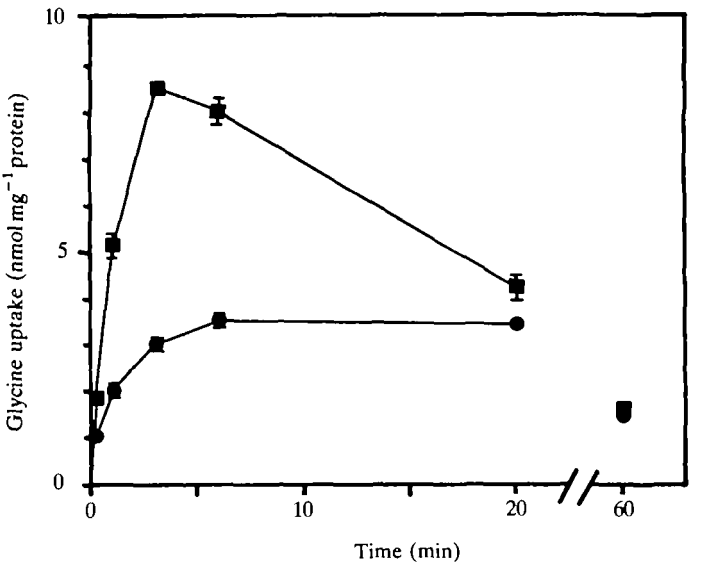

Fig. 2. Time course of glycine uptake in BBMV driven by an imposed potassium or sodium electrochemical gradient. Vesicle internal medium was (mmol l^{-1}): 160 mannitol, 90 Hepes, 45 Tris at pH 7.4. The vesicles were diluted 1:5 in media of the following final composition (mmol l^{-1}): 118 mannitol, 18 Hepes, 45 Tris at pH 8.9, 50 K_2SO_4 (■) or Na_2SO_4 (●), 0.1 FCCP and 0.73 [U-^{14}C]glycine (10 μC ml^{-1}). Each point represents the mean±S.E. of a typical experiment performed in triplicate. When not present, error bars were smaller than the symbols.

tration of sodium or potassium (Fig. 3A,B) and the kinetic constants were calculated from the Eadie–Hofstee plot of the data after subtraction of the linear component. The affinity of the cotransporter for glycine in the presence of sodium (K_m 0.65±0.03 mmol l^{-1}) was almost twice that measured in the presence of potassium (K_m 1.17±0.06 mmol l^{-1}). The maximal transport rate was 0.57± 0.02 nmol 7 s^{-1} mg^{-1} with sodium and 1.96±0.12 nmol 7 s^{-1} mg^{-1} with potassium. These data are in good agreement with the different abilities of the two cations to drive 'active' glycine absorption across the midgut epithelium isolated *in vitro*. Glycine is not metabolized by lepidopteran larval midgut (Bosquet, 1976; Nedergaard, 1977; Sacchi and Giordana, 1980; Parenti *et al.* 1985*b*; Chamberlin, 1989). Table 1 shows that the lumen-to-hemolymph net flux of the amino acid across the isolated gut of *Hyalophora cecropia* is threefold higher in the presence of luminal potassium than in the presence of sodium. Only slight differences are present in the two conditions as far as the transepithelial electrical potential (PD) is concerned, the potential decay during flux measurements being very small (5%), so that the different net fluxes and flux ratios cannot be ascribed to a difference in the electrical driving force.

A detailed analysis of the activation of glycine uptake by sodium and potassium in BBMV was then undertaken. Figs 4A and 5A illustrate a typical experiment showing the uptake of 0.73 mmol l^{-1} glycine as a function of external potassium

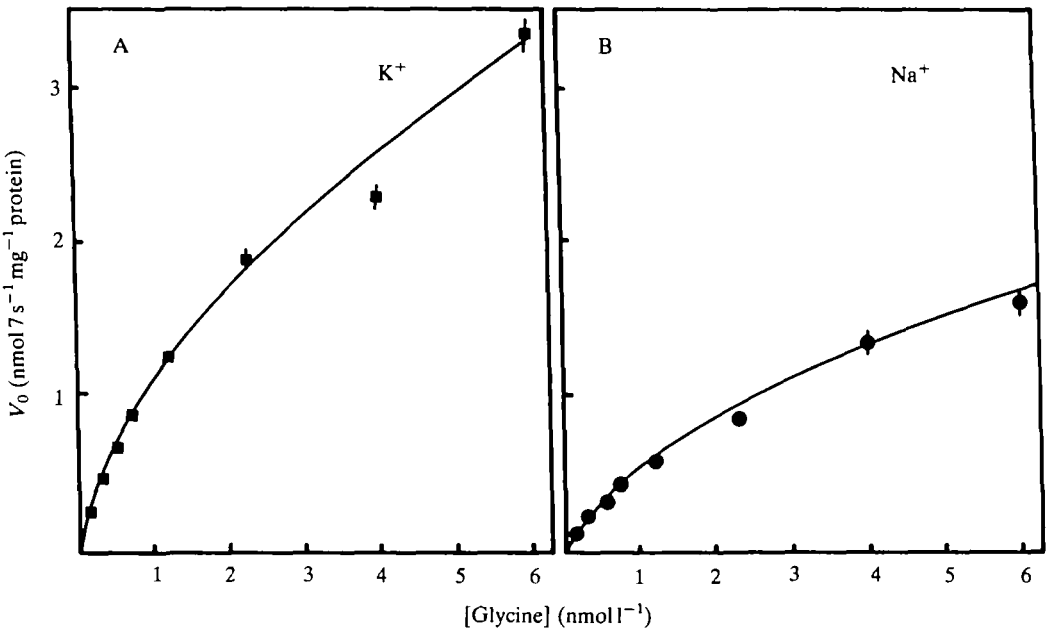

Fig. 3. Influx kinetics of glycine at saturating concentrations of extravesicular potassium (A) or sodium (B). Vesicle internal medium was (mmol l^{-1}): 160 mannitol, 90 Hepes, 45 Tris at pH 7.4. The vesicles were diluted 1:5 in media of the following final composition (mmol l^{-1}): 118 mannitol, 18 Hepes, 45 Tris at pH 8.9, 50 K_2SO_4 (A) or Na_2SO_4 (B), 0.1 FCCP and 0–6 [U-^{14}C]glycine (80 μC ml^{-1}). Incubations lasted 7 s. V_0, initial rate of uptake. Each point represents the mean±S.E. of a typical experiment performed in quadruplicate. When not present, error bars were smaller than the symbols.

(Fig. 4A) and sodium (Fig. 5A) concentrations. Both curves reach saturation but do not fit to a rectangular hyperbola. Neither the Lineweaver–Burk nor the Eadie–Hofstee plots (Figs 4B,C and 5B,C) are linear. They can be explained by Hill coefficients higher than 1 for potassium and lower than 1 for sodium. V_{max} values of 0.66 and 0.25 nmol 7 s^{-1} mg^{-1} protein for potassium and sodium, respectively, were estimated from the double reciprocal plots (Figs 4B and 5B). The Hill plot of the data ($\log V_0/(V_{max}-V_0)$ *vs* log[substrate]) allowed the calculation of cation concentrations giving the half-maximal rate of transport, $[K^+]_{50}$ and $[Na^+]_{50}$, and the Hill coefficients reported in Table 2. The table also shows that the Hill coefficients depend on glycine concentration, decreasing from 2.3 to 1.1 with potassium.

The effect of an increasing concentration of extravesicular sodium on the uptake of glycine at saturating external potassium concentration is reported in Fig. 6A. Sodium reduced glycine uptake to 70% of the control value. The same inhibition was also observed using leucine as the test amino acid (Fig. 6B). These results suggest competition between sodium and potassium for the same transporter. The inhibition patterns exerted by 14 amino acids on the uptake of glycine in the

Table 1. *Lumen-to-hemolymph (L–H) and hemolymph-to-lumen (H–L) unidirectional glycine fluxes across the isolated midgut of* Hyalophora cecropia *in the presence of luminal potassium or sodium*

	Potassium			Sodium		
	Flux	Potential difference	*N*	Flux	Potential difference	*N*
L–H flux	15.6±2.8	102±4	4	5.9±1.1	114±6	4
H–L flux	0.15±0.07	94±27	2	0.25±0.01	117±11	2
Net flux	15.4±1.0			5.6±2.0		
Flux ratio	104			24		

[U-^{14}C]glycine concentration was 10 mmol l^{-1}.

Fluxes are expressed as μmol h^{-1} 100 mg^{-1} dry mass.

Potential difference (mV): mean values of the transepithelial electrical potential difference during flux measurements.

Means±S.E.

Net fluxes and flux ratios are the difference and the ratio, respectively, between L–H and H–L flux values.

presence of potassium or sodium are very similar (Table 3) and a simple explanation of this result is the presence of a single transporter that can handle both potassium and sodium with different affinities and transport rates.

Discussion

The ability of the cotransport mechanism of lepidopteran enterocytes to accumulate amino acids exploiting both a sodium gradient and a potassium gradient as driving force was established by the initial experiments of Hanozet *et al.* (1980). The data reported in this paper indicate that the same cotransporter binds either sodium or potassium since an inhibition, rather than an additive effect, occurred when sodium was added at saturating potassium concentrations (Fig. 6). This conclusion is also supported by the same inhibition patterns (Table 3) exerted on glycine uptake by 14 different amino acids in the presence of potassium or sodium.

In addition, these inhibition patterns suggest that glycine is translocated by a transport system shared by all neutral amino acids (Giordana *et al.* 1989), although with a low affinity, as was also suggested by the high apparent inhibition constant ($K_{i,app}$) found for glycine in previous inhibition experiments (Hanozet *et al.* 1989). This idea is supported by the observation that the inhibition by sodium at a saturating potassium concentration is also exerted on the uptake of leucine (Fig. 6B), an amino acid that is typically transported by the neutral brush-border transport system.

Furthermore, the transporter is able to discriminate between the two cations and the inhibition can be explained by the different values of the kinetic constants

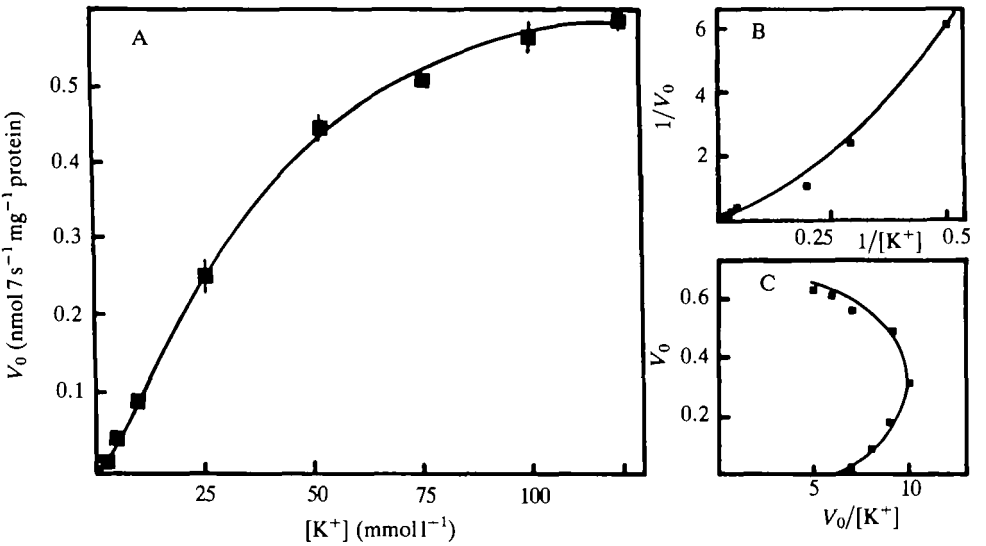

Fig. 4. Influx kinetics of glycine as a function of extravesicular potassium concentration. Vesicle internal medium was (mmol l^{-1}): 160 mannitol, 90 Hepes, 45 Tris at pH 7.4. The vesicles were diluted 1:5 in a medium of the following final composition (mmol l^{-1}): 118 mannitol, 18 Hepes, 45 Tris at pH 8.9, 0–50 K_2SO_4 plus 150–0 mannitol to compensate for osmolarity, 0.1 FCCP and 0.73 [U-^{14}C]glycine (40 μC ml^{-1}). Incubations lasted 7 s. V_0, initial rate of uptake. (A) Glycine uptake after subtraction of the K^+-independent component. (B) Lineweaver–Burk plot of the data. (C) Eadie–Hofstee plot of the data. Each point represents the mean±S.E. of a typical experiment performed in quadruplicate. When not present, error bars were smaller than the symbol.

in the presence of sodium and potassium (Fig. 3 and Table 2). The affinity of the cotransporter for sodium, when glycine concentration was 0.73 mmol l^{-1}, was almost fourfold higher than that for potassium (6.2 and 29 mmol l^{-1}, respectively) and, therefore, sodium ions displace potassium ions even at low concentrations. Consequently, glycine transport is reduced because the maximal transport rate is lower with sodium than with potassium (Fig. 3). This inhibition curve can be explained assuming (i) competition between sodium and potassium for the transporter and (ii) different kinetic constants for the transport process in the presence of sodium and potassium. In this case, glycine uptake can be described by the following equation (derived from the velocity equations for a competing alternative substrate, according to Segel, 1975, pp. 291–293):

$$V_0 = \frac{V_{max}(K^+) \times [Na^+]_{50} \times [K^+] + V_{max}(Na^+) \times [K^+]_{50} \times [Na^+]}{2 \times [K^+]_{50} \times [Na^+]_{50} + 2 \times [Na^+]_{50} \times [K^+] + 2 \times [K^+]_{50} \times [Na^+]}. \quad (1)$$

The computer-simulated curve (Fig. 7) is in good agreement with the experimental one (Fig. 6A).

Curves such as those reported in Figs 4A and 5A are usually explained as positive cooperative effects of different binding sites when the Hill coefficient is

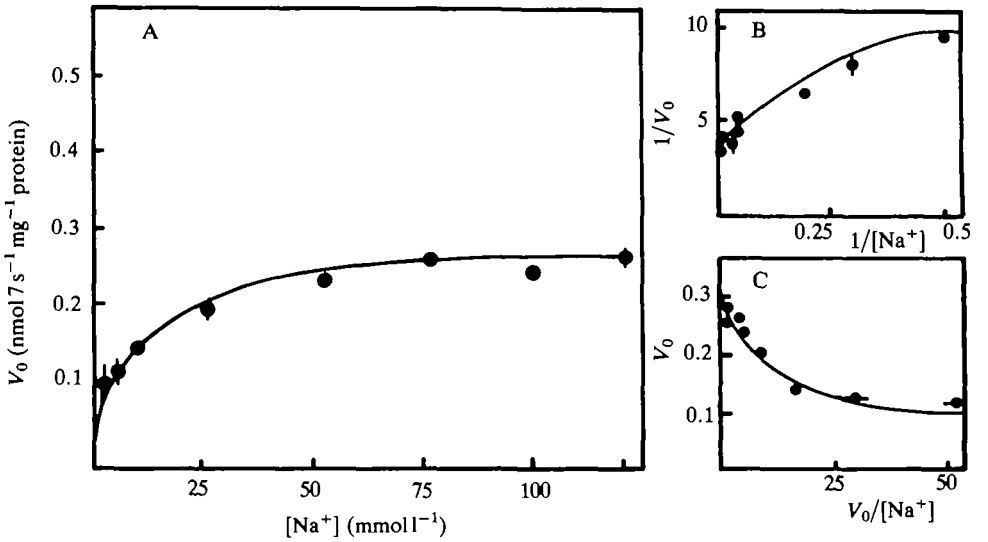

Fig. 5. Influx kinetics of glycine as a function of extravesicular sodium concentration. The compositions of the intravescicular and extravescicular media were identical to those reported in Fig. 4, except that Na_2SO_4 replaced K_2SO_4. Incubations lasted 7 s. V_0, initial rate of uptake. (A) Glycine uptake after subtraction of the Na^+-independent component. (B) Lineweaver–Burk plot of the data. (C) Eadie–Hofstee plot of the data. Each point represents the mean±S.E. of a typical experiment performed in quadruplicate. When not present, error bars were smaller than the symbol.

Table 2. *Kinetic constants of glycine uptake as a function of extravesicular potassium or sodium concentration in BBMV from* Philosamia cynthia

[Glycine] (mmol l^{-1})	Potassium			Sodium		
	$[K^+]_{50}$	V_{max}	n	$[Na^+]_{50}$	V_{max}	n
0.13	21±2 (2)	147±19 (2)	2.3±0.3 (2)	7.0±2.0 (2)	73±9 (2)	0.8±0.2 (2)
0.73	29±2 (3)	584±42 (3)	1.5±0.1 (3)	6.2±1.9 (4)	352±11 (4)	0.7±0.1 (4)
7.00	25±1 (2)	1253±48 (2)	1.1±0.1 (2)	–	–	–

Kinetic constants and Hill coefficients (n) were calculated, after subtraction of the cation-independent component, as described in Results, from experiments performed as reported in the legends of Figs 4 and 5, at the fixed glycine concentrations shown in the first column.

$[S]_{50}$ is expressed in mmol l^{-1}, V_{max} in pmol 7 s^{-1} mg^{-1} protein. Mean±S.E., number of experiments (each performed in quadruplicate for nine different cation concentrations) in parentheses.

$[K^+]_{50}$, $[Na^+]_{50}$, concentrations giving the half-maximal rate of glycine transport.

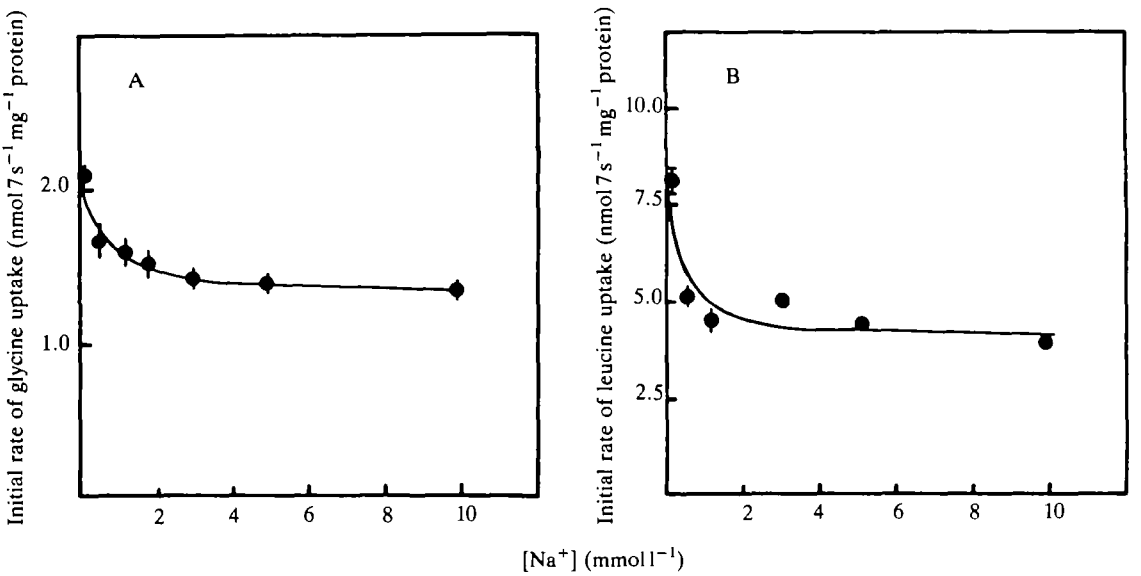

Fig. 6. The effect of increasing concentrations of sodium on (A) glycine or (B) leucine uptake in the presence of a saturating potassium concentration. The vesicle internal medium was (mmol l^{-1}): 470 mannitol, 90 Hepes, 45 Tris at pH 7.4. The vesicles were diluted 1:5 in a medium of the following final composition (mmol l^{-1}): 232 mannitol, 18 Hepes, 45 Tris at pH 8.9, 100 K_2SO_4, 0–5 Na_2SO_4, 0.1 FCCP and 0.97 [U-^{14}C]glycine (A) or 0.97 L-[^{3}H]leucine (B). Incubations lasted 7 s. Each point represents the mean±S.E. of a typical experiment performed in quadruplicate. When not present, error bars were smaller than the symbol.

greater than 1 (Fig. 4) and as negative cooperative effects when the Hill coefficient is less than 1 (Fig. 5). Alternatively, as stressed also by Restrepo and Kimmich (1985), the shape of these curves could be determined by the kinetic features of the transport mechanism. Curves similar to those found experimentally are predictable (Segel, 1975) assuming (i) a steady-state random two substrate (bi bi) mechanism, which means that glycine and the cation can both bind first to the cotransporter and that the binding is the rate-limiting step of the transport, and (ii) that one of the two pathways to the ternary complex is preferred (Fig. 8, kinetic pathways shown with K^+ as the cotransported cation). Sodium and potassium are alternative substrates and they induce a change in the pathway to the ternary complex. The variations in the Hill coefficients as a function of glycine concentration (Table 2) are to be expected according to this model and can be explained if glycine concentration can induce a variation in the order of substrate binding to the cotransporter. In our opinion, this kinetic interpretation of the results fits well with many of the data and it should, therefore, be preferred to that suggesting positive cooperativity for potassium ions and negative cooperativity for sodium ions.

If we now consider the absorption of glycine *in vivo* from an energetic point of view, the situation does not differ much in the presence of sodium or potassium.

Table 3. *Inhibition exerted by different amino acids on glycine uptake in the presence of extravesicular potassium or sodium in BBMV from* Philosamia cynthia

	Potassium		Sodium	
Amino acid	Glycine uptake	Percentage inhibition	Glycine uptake	Percentage inhibition
Control	1429±3	–	946±11	–
Glycine	433±6	77	287±16	81
Alanine	126±5	100	120±12	100
Leucine	100±8	102	94±8	104
Proline	1230±11	15	873±15	9
Phenylalanine	102±9	102	100±4	104
MeAIB	1556±30	0	1109±16	0
Serine	77±4	104	187±2	93
Cysteine	101±17	102	114±10	100
Asparagine	686±8	57	395±2	68
Glutamine	184±9	96	145±9	100
Aspartic acid	1228±50	15	800±4	18
Glutamic acid	1192±67	18	797±14	18
Lysine	950±19	37	746±15	25
Arginine	682±5	58	780±49	20
Histidine	145±12	100	164±55	100

Vesicle internal medium (in mmol l^{-1}) was: 160 mannitol, 90 Hepes, 45 Tris at pH 7.4. The vesicles were diluted 1:5 in a medium with the following final composition (mmol l^{-1}): 118 mannitol, 18 Hepes, 45 Tris at pH 8.9, 50 K_2SO_4 or Na_2SO_4, 0.1 FCCP, 0.63 [U-^{14}C]glycine (15 μC ml^{-1}) and 12 mmol l^{-1} inhibitor amino acid or mannitol. Incubation lasted 7 s.

Glycine uptake is expressed in pmol $7\,s^{-1}$ mg^{-1} protein.

Percentage inhibition was calculated with respect to the control condition after subtraction of the cation-independent component. Each value represents the mean±S.E. of an experiment performed in quadruplicate.

MeAIB: α-(methyl-amino)isobutyric acid.

The main source of the driving force is the large (up to 170 mV) electrical potential difference across the brush-border membrane; the chemical component is modest, though opposite for potassium and sodium, at least in *P. cynthia*. Their concentrations in the lumen, enterocytes and hemolymph are, respectively (in mmol l^{-1}) 197, 188 and 24 for potassium and 1, 3, and 5 for sodium (Giordana and Sacchi, 1978). In the same compartments, glycine concentrations are 2.4, 5.2 and 11.0 mmol l^{-1} (Parenti *et al.* 1985*a*) and, therefore, uphill transport should occur at both the luminal and basolateral membranes of absorptive cells. The luminal concentrations of glycine and cations indicate that *in vivo* the cotransporter should be almost saturated with potassium and that 1 mmol l^{-1} sodium would partially displace potassium (Fig. 6). From equation 1, it can be roughly estimated that the reduction of glycine influx caused by this concentration of sodium should be less than 5 % of the total flux.

In conclusion, on the basis of the data reported here we propose a steady-state

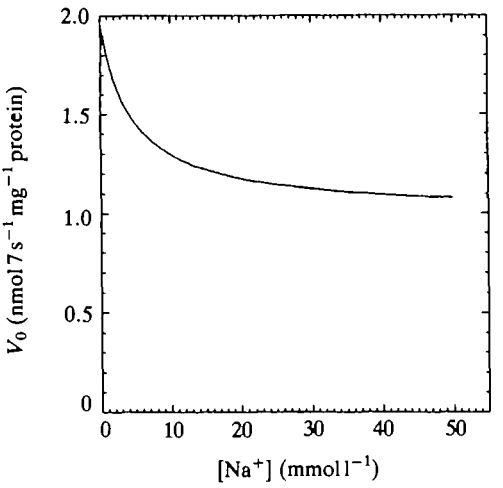

Fig. 7. Computer simulation of the inhibition of glycine uptake induced by increasing concentrations of extravesicular sodium in the presence of a saturating potassium concentration. See Discussion for an explanation.

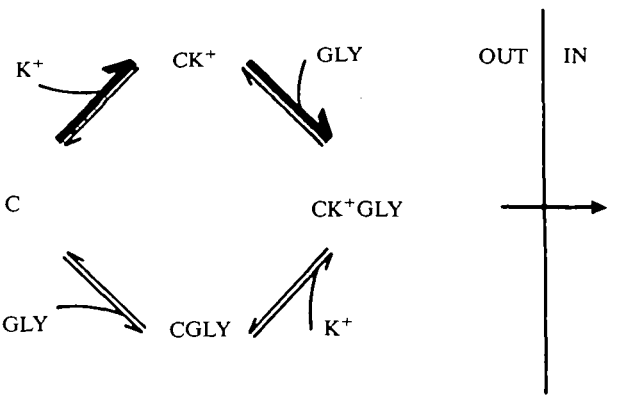

Fig. 8. Kinetic model for a random two-substrate (glycine and potassium) reaction with a preferred pathway to the ternary complex. See Discussion for details. C, cotransporter; GLY, glycine; K^+, potassium; CK^+, binary complex with potassium; CGLY, binary complex with glycine; CK^+GLY, ternary complex. Bold arrows indicate the preferred pathway to the ternary complex, which translocates across the membrane (out/in).

random bi bi mechanism for glycine transport with two alternative substrates, sodium and potassium, which modify the properties of the glycine transporter.

The nerve cord of lepidopteran larvae has a relatively high sodium concentration (Abbott and Treherne, 1977; Monticelli *et al.* 1985), whereas the passive permeability of the midgut to potassium and sodium ions is very low (S. Nedergaard, unpublished results). It is possible that the high affinity of the amino

acid cotransporter for sodium could be important for ensuring the uptake of this cation, which is necessary at high concentrations for the nervous system to function but is present only at low concentrations in the diet. Recently, Chamberlin (1990) has demonstrated net absorption of sodium across the isolated gut of *Manduca sexta* in the presence of several amino acids in the bathing solutions.

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