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Neutral amino acid symport in larval *Manduca sexta* midgut brush-border membrane vesicles deduced from cation-dependent uptake of leucine, alanine, and phenylalanine

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Uptake of tritiated leucine, alanine, and phenylalanine was measured at the physiological pH of 10 by rapid filtration in brush-border membrane vesicles from the midgut of the larval tobacco hornworm, *Manduca sexta*. A 20-fold excess of unlabeled leucine, isoleucine, methionine, valine, alanine, lysine, histidine, phenylalanine, and glutamine inhibited uptake of leucine and phenylalanine, and six of these amino acids inhibited uptake of alanine, by more than 50% both in the presence and absence of a potassium ion gradient. These inhibitory amino acids also drove countertransport of leucine, alanine, and phenylalanine with accumulation ratios exceeding 2. These results are consistent with the hypothesis that leucine, alanine, and phenylalanine share a common uptake system – a broad scope B type symporter – which interacts strongly with half of the commonly occurring amino acids, interacts moderately with an additional quarter of them, but does not interact with cysteine, arginine, glutamate, aspartate, or proline.

Introduction

Among the most metabolically active tissues known, lepidopteran midgut takes up massive amounts of amino acids as energy substrates [1] and for incorporation into proteins while the larval fresh weight increases 1000-fold during the four weeks when the egg matures to a 5th instar larva at 25°C. Several cation-dependent symport systems have been described earlier in brush-border membrane vesicles (BBMV) from midgut epithelia of other Lepidopteran species, e.g., *Philosamia cynthia, Bombyx mori*, and *Pieris brassicae* [2]. In particular, a broad spectrum, B-type, neutral amino acid system has been described in *Philosamia cynthia* at pH 7.5 [2,3]. In *M. sexta* BBMV the uptake of leucine, alanine, and phenylalanine has been shown to be optimal at the physiological pH of 10 and ion specificities of uptake at this pH have been described in the previous paper by Hennigan et al. [15]. Here, uptake inhibition and countertransport accumulation of these three amino acids at pH 10 show that this *M. sexta* system is a B-type amino acid symporter.

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Materials and Methods

Rearing of insects. Fifth instar *M. sexta* larvae, weighing 5.5 ± 0.5 g, were used in all experiments. *M. sexta* eggs and larval diet were purchased from Carolina Biological Supply Company (Burlington, NC). The larvae were reared at 27 °C under constant light.

Midgut isolation and BBMV preparation. Midguts were isolated by the method of Harvey et al. [4]. They were rinsed with ice cold 300 mM mannitol, 5 mM ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid (EGTA), and 17 mM tris(hydroxymethyl)aminomethane at pH 7.5, gently blotted, weighed, and used immediately for BBMV preparation.

BBMV were prepared by the differential magnesium precipitation method of Biber et al. [5] as modified by Wolfersberger et al. [6]. BBMV from larval *M. sexta* midgut yield mean enrichment factors (specific activity of BBMV/specific activity of homogenate) for aminopeptidase (EC 3.4.11.2) and cytochrome-*c* oxidase (EC 1.9.3.1) of 12.84 and 0.42, respectively [7]. The vast majority of BBMV prepared by differential precipitation methods are in a right-side-out orientation, i.e., the membrane in the vesicles has the same orientation as in the intact epithelium [8].

Amino acid uptake experiments. Uptake experiments were performed at 25 ± 1°C using the rapid filtration

technique [6,9]. Unless otherwise noted, BBMV were resuspended to a protein concentration of 0.5 mg/ml in 100 mM mannitol, 50 mM aminomethylpropanediol (AMPD), adjusted to pH 10 with HCl and allowed to equilibrate for one hour on ice. The BBMV were centrifuged (16000 rpm, 30 min, Sorval RC-5B, SS-34 rotor) and again resuspended in the same buffer at a protein concentration of approx. 5 mg/ml. Final resuspension was accomplished with a syringe equipped with a 25 gauge × 5/8 inch needle. Incubations were started by mixing 10 μl aliquots of BBMV with 10 μl aliquots of 1 mM or 2 mM radioactively labeled amino acid in transport buffer (100 mM mannitol, 50 mM AMPD, 100 mM KSCN) and other components as described in the tables, adjusted to pH 10. They were stopped by the addition of 2 ml of ice-cold 100 mM mannitol, 50 mM AMPD, 100 mM KSCN, pH 10 (stop solution); the diluted mixtures were filtered immediately through a prewetted cellulose nitrate filter (0.65 μm pore size, Sartorius No. 11305, Haywood, CA). All filters were immediately washed twice with 4 ml of ice-cold stop solution, placed into a vial with 10 ml of scintillation cocktail (ScintiVerse E or BD, Fisher Scientific, Pittsburgh, PA), and counted in a liquid scintillation spectrometer (Model 2000CA, Packard Instruments, Downers Grove, IL). 10 μl aliquots of labeled amino acid solution were spotted on filters, counted and used to convert radioactivity in cpm into moles of amino acid [6].

Inhibition of amino acid uptake. Determinations of inhibition of labeled amino acid uptake by excess unlabeled amino acid were started by mixing 10 μl BBMV with 10 μl transport buffer containing 20 mM inhibiting amino acid and 1 mM radiolabeled amino acid; they were stopped at 9 s when control experiments showed that uptake was still linear (Fig. 1). Determinations of inhibition of uptake in the absence of a K^+ gradient were started by mixing 10 μl BBMV (preloaded with stop solution and incubated with valinomycin, 8 μg/mg protein (40 μg/ml) with 10 μl transport buffer containing 20 mM inhibiting amino acid and 1 mM radiolabeled amino acid; they were stopped at 6 s.

Countertransport experiments. BBMV were resuspended in transport buffer containing 20 mM elicitor amino acid and allowed to equilibrate for one hour on ice. After equilibration the BBMV were collected by centrifugation and resuspended at a protein concentration of 5 mg/ml in transport buffer containing 20 mM of the same elicitor amino acid. Valinomycin was added to the BBMV suspension to yield a concentration of 40 μg/ml. The suspensions were incubated for 30 min on ice before use.

Countertransport incubations were initiated by mixing 5 μl of the BBMV suspension with 95 μl of transport buffer containing 1 mM radiolabeled amino acid. Duplicate incubation mixtures were stopped at 40 s and 60 min. 60 min was determined to provide the equilibrium uptake value which remained unchanged up to 120 min. The vesicles were filtered as described above. Accumulations were calculated by dividing the uptake of labeled amino acid at 40 s by the equilibrium uptake at 60 min.

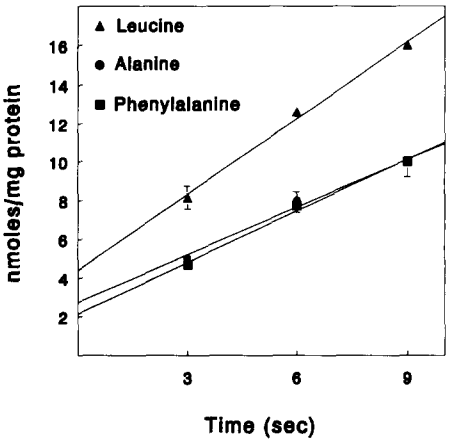

Fig. 1. Time course of K^+ driven initial uptake of leucine, alanine, and phenylalanine. BBMV in 100 mM mannitol, 50 mM aminomethylpropanediol (AMPD) at pH 10 were resuspended to yield a reaction mixture with the following composition: 100 mM mannitol, 50 mM AMPD, 50 mM KSCN, and 0.5 mM radiolabeled amino acid at pH 10. Mean uptake values with their standard deviations are from triplicate determinations of a BBMV preparation.

Protein determinations. The protein concentration of homogenates and BBMV preparations was determined by the method of Bradford [10] using a Bio-Rad kit (Richmond, CA) with bovine serum albumin as standard.

Statistics. Statistically significant differences (P value < 0.05) were calculated using Student's t-test. For correlation analysis, uptake and/or countertransport values were plotted against each other, a least-squares line was drawn, and R, the square root of the coefficient of determination, was calculated, using Slide Write Plus (Advanced Graphics Software, Sunnyvale, CA).

Chemicals and reagents. L-[3,4,5-^{3}H]Leucine, L-[2,3-^{3}H]alanine, and L-[*side chain*-^{3}H]phenylalanine were from ICN Biochemicals (Costa Mesa, CA). All non-radioactive amino acids were from Sigma (St. Louis, MO). Aminomethylpropanediol was from Eastman Kodak (Rochester, NY). All other chemicals were analytical grade products from either Fisher (Pittsburgh, PA) or Mallinckrodt (St. Louis, MO).

Results

Inhibition of K^+ gradient driven uptake of radiolabeled leucine, alanine, or phenylalanine by the twenty commonly occurring amino acids, aminoisobutyrate

(AIB) and methylaminoisobutyrate (MeAIB) is shown in Table I. The monoamino monocarboxylic aliphatic amino acids plus histidine, glutamine, and methionine are the best inhibitors. Cysteine, proline, arginine, aspartate, and glutamate are the worst inhibitors. Lysine is the most inconsistent inhibitor; it is a much more potent inhibitor of leucine uptake than of either alanine or phenylalanine uptake.

To measure amino acid uptake in the absence of both a potassium gradient and an osmotic gradient, the BBMV were preloaded with resuspension buffer augmented with KSCN equal in concentration to that in the radioactive amino acid transport buffer and then treated with 40 μg/ml valinomycin before testing for the inhibitory ability of the amino acid. The only driving force for uptake was the gradient of the labeled amino acid. Table II shows the results for the inhibition of leucine, alanine, and phenylalanine uptake by the twenty commonly occurring amino acids plus AIB and MeAIB in the absence of a K^+ gradient. Inhibition without a gradient is similar to inhibition with a gradient (compare Tables I and II). Leucine again is the strongest inhibitor of the uptake of all three labeled amino acids; histidine and methionine are again very strong inhibitors. Lysine again is a stronger inhibitor of leucine uptake than of alanine or phenylalanine uptake. Again aspartate, glutamate, arginine, and proline are the poorest inhibitors.

In a countertransport experiment the time course of phenylalanine accumulation elicited by intravesicular leucine rose gradually with a broad peak at 40 s (Fig. 2); a similar time course was observed when alanine or phenylalanine was the elicitor. The results of countertransport accumulation at 40 s, elicited by the twenty common amino acids, are summarized in Table III. Maximum values with leucine are approx. 50% higher than those with alanine or phenylalanine. Alanine has only slightly lower countertransport accumulation values than phenylalanine. Histidine and methionine plus

TABLE I

Inhibition of leucine, alanine, and phenylalanine uptake by common amino acids in the presence of a K^+ gradient

BBMV in 100 mM mannitol, 50 mM AMPD, at pH 10 were resuspended to give a final reaction mixture containing the following: 100 mM mannitol, 50 mM AMPD, 50 mM KSCN, 10 mM inhibiting amino acid, 0.5 mM radiolabeled amino acid (pH 10). Uptake was stopped at 9 s. Mean uptake values (nmol amino acid/mg protein) with their standard errors are from triplicate BBMV preparations.

Inhibitor		Transported amino acid		
		[^{3}H]leucine	[^{3}H]alanine	[^{3}H]phenylalanine
Leu	L	4.80±0.48	1.78±0.06	1.57±0.07
Lys	K	5.39±0.12	7.34±0.07	2.87±0.07
Ile	I	5.57±0.02	1.97±0.11	2.29±0.04
Ala	A	5.61±0.06	2.74±0.06	2.46±0.09
His	H	5.65±0.21	5.56±0.11	2.57±0.01
Met	M	6.18±0.07	2.85±0.08	2.02±0.05
Phe	F	6.27±0.18	6.63±0.10	3.48±0.10
Val	V	6.58±0.04	2.54±0.10	2.52±0.07
Gln	Q	7.68±0.20	5.95±0.04	3.02±0.12
Trp	W	8.43±0.09	6.72±0.32	4.40±0.04
Tyr	Y	9.23±0.05	6.45±0.54	3.86±0.12
Ser	S	10.88±0.05	4.72±0.14	3.68±0.14
Asn	N	10.88±0.25	8.26±0.14	5.63±0.12
Thr	T	12.31±0.16	5.66±0.24	4.02±0.08
Asp	D	13.72±0.24	9.47±0.22	7.94±0.32
Gly	G	13.96±0.46	7.40±0.28	5.36±0.12
Glu	E	14.58±0.26	10.17±0.70	8.83±0.40
Pro	P	14.91±0.27	8.33±0.09	5.91±0.18
Cys	C	15.02±0.25	8.63±0.20	9.44±0.18
Arg	R	15.55±0.09	8.72±0.38	7.54±0.10
AIB	J	8.89±0.19	4.89±0.06	4.46±0.05
MeAIB	X	14.63±0.44	9.76±0.12	11.39±0.22
Control	O	17.07±0.30	10.46±0.41	10.85±0.18

TABLE II

Inhibition of leucine, alanine, and phenylalanine uptake by common amino acids in the absence of a K^+ gradient

BBMV in 100 mM mannitol, 50 mM AMPD, 100 mM KSCN at pH 10 were preincubated with valinomycin (8 μg/mg protein = 40 μg/ml) and resuspended to give a final reaction mixture containing the following 100 mM mannitol, 50 mM AMPD, 100 mM KSCN, 10 mM inhibiting amino acid, 0.5 mM radiolabeled amino acid (pH 10). Mannitol was substituted for inhibiting amino acid in the control experiment. Uptake was stopped at 6 s. Mean uptake values (nmol/mg protein at 6 s) with their standard errors are from triplicate BBMV preparations.

Inhibitor	Transported amino acid		
	[^{3}H]leucine	[^{3}H]alanine	[^{3}H]phenylalanine
Leu	0.45±0.07	0.43±0.01	0.42±0.01
Lys	0.45±0.01	0.64±0.05	0.61±0.06
Ile	0.58±0.03	0.52±0.04	0.49±0.02
Met	0.59±0.01	0.52±0.03	0.49±0.04
His	0.60±0.03	0.47±0.02	0.52±0.02
Val	0.62±0.01	0.49±0.01	0.54±0.01
Gln	0.64±0.05	0.60±0.02	0.66±0.04
Phe	0.70±0.08	0.55±0.02	0.57±0.05
Ala	0.72±0.01	0.50±0.01	0.64±0.01
Cys	0.74±0.03	0.58±0.04	0.72±0.06
Asn	0.78±0.03	0.67±0.01	0.76±0.04
Thr	0.83±0.06	0.52±0.02	0.80±0.01
Ser	0.88±0.01	0.64±0.03	0.80±0.05
Tyr	0.88±0.02	0.67±0.07	0.72±0.03
Gly	0.92±0.06	0.52±0.05	0.84±0.05
Trp	1.00±0.02	0.78±0.03	0.79±0.05
Pro	1.14±0.01	0.71±0.06	0.95±0.03
Glu	1.29±0.06	0.85±0.04	1.23±0.04
Arg	1.28±0.01	0.81±0.03	1.13±0.05
Asp	1.41±0.03	1.00±0.10	1.18±0.11
AIB	0.80±0.01	0.50±0.03	0.67±0.01
MeAIB	1.29±0.03	0.90±0.06	1.10±0.02
Control	1.61±0.06	1.33±0.04	1.47±0.01

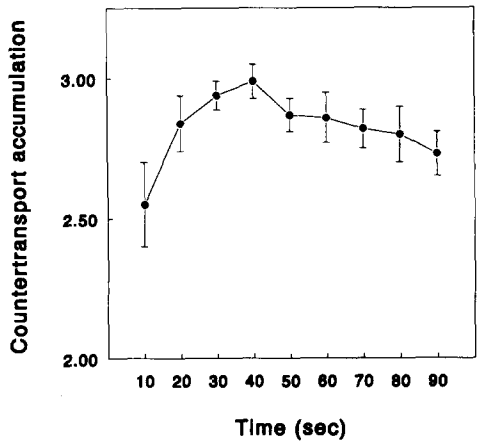

Fig. 2. Typical time course of phenylalanine uptake elicited by leucine. Conditions for countertransport are specified in the legend of Table III. Countertransport accumulations are calculated by dividing the uptake at 40 s by the equilibrium value at 60 min. Mean countertransport accumulation values with their respective standard deviations are from triplicate determinations of a BBMV preparation.

the monoamino monocarboxylic aliphatic amino acids are the best elicitors of countertransport, whereas glutamate, aspartate, arginine, and cysteine are least effective. Lysine elicits strong countertransport accumulation of leucine but only slight accumulation of phenylalanine and no accumulation of alanine. MeAIB, a System A substrate analog, fails to elicit countertransport accumulation of labeled leucine, alanine, and phenylalanine whereas AIB elicits countertransport accumulation significantly above equilibrium (Table III).

Discussion

Inhibition in presence of K^+ gradient

Each of the twenty naturally occurring amino acids depressed uptakes below that of the control in the presence of a K^+ gradient (Table I). Leucine was most effective, inhibiting leucine, alanine, and phenylalanine uptake by 72%, 83%, and 86%, respectively. Arginine, glutamate, and cysteine were least effective, inhibiting leucine, alanine, and phenylalanine uptake by 9%, 3%, and 13%, respectively. Ten amino acids inhibited leucine uptake by more than 50% – Leu, Lys, Ile, Ala, His, Met, Phe, Val, Gln, and Trp. Only six amino acids inhibited alanine uptake by more than 50% – five strong inhibitors of leucine uptake – Leu, Ile, Val, Ala, and Met, plus Ser. Thirteen amino acids inhibited phenylalanine uptake by more than 50% – the ten which inhibited leucine uptake, plus Ser, Tyr, and Thr. Amino acids which depress uptake could be competing with the labeled amino acids for the K^+ gradient, inhibiting competitively, inhibiting noncompetitively or acting by a combination of all three mechanisms. Moreover, the initial osmotic gradient might be influencing the results in an unexpected way. Nevertheless, the similar ordering of amino acids as inhibitors of leucine, alanine, and phenylalanine uptake suggests that they may share a common symport system (Table IV, left columns).

Inhibition in absence of K^+ gradient

Without the K^+ gradient all uptake values were reduced to approx. 10% of the values with the gradient. Cys, Asn, and nine of the ten amino acids which inhibited leucine uptake by more than 50% with the gradient did so without the gradient. His, Thr, Gly, Phe, Cys, Gln, Lys, and all six amino acids which inhibited alanine uptake by more than 50% with the K^+ gradient did so without the gradient. Cys and ten of the thirteen amino acids which had inhibited phenyl-

TABLE III

Countertransport accumulation of leucine, alanine, and phenylalanine by common amino acids

BBMV were preloaded with 20 mM of elicitor amino acid in 100 mM mannitol, 50 mM AMPD, 100 mM KSCN (pH 10) and preincubated with valinomycin (8 μg/mg protein = 40 μg/ml fc). The vesicles were resuspended in a reaction mixture containing the following final concentrations: 100 mM mannitol, 50 mM AMPD, 100 mM KSCN, 1 mM radiolabeled amino acid (pH 10). Reaction mixtures were stopped at 40 s. Mean countertransport accumulation with their standard errors are determined by dividing uptake of labeled amino acid at 40 s by equilibrium uptake value at 60 min for triplicate BBMV preparations. Countertransport accumulations significantly greater than 1 are indicated with *. The numbers in parentheses represent BBMV preparations greater than three.

Elicitor	Transported amino acid		
	[^{3}H]leucine	[^{3}H]alanine	[^{3}H]phenylalanine
His	4.06±0.31 *	1.86±0.18 *(4)	2.36±0.16 *(6)
Met	3.68±0.25 *	1.47±0.10 *(4)	1.71±0.09 *(5)
Ala	3.56±0.26 *	2.16±0.13 *(4)	2.41±0.17 *(5)
Val	3.43±0.21 *	1.82±0.11 *(4)	2.24±0.17 *(5)
Leu	3.39±0.18 *	2.04±0.11 *(4)	3.01±0.17 *(6)
Ile	3.27±0.35 *	1.89±0.10 *(4)	2.48±0.18 *(5)
Gln	3.21±0.32 *	1.43±0.16 *(4)	1.48±0.12 *(4)
Phe	3.19±0.22 *	1.45±0.09 *(4)	1.68±0.13 *(5)
Lys	2.78±0.22 *	0.99±0.05 (4)	1.43±0.06 *(4)
Ser	2.33±0.26 *	1.39±0.10 *(4)	1.66±0.19 *(4)
Asn	2.23±0.25 *	1.19±0.09	1.48±0.13 *
Tyr	1.83±0.06 *	1.34±0.12 *(4)	1.40±0.14 *(4)
Thr	1.81±0.22 *	1.37±0.09 *(4)	1.29±0.09 *(6)
Trp	1.79±0.09 *	1.42±0.09 *(4)	1.45±0.11 *(4)
Gly	1.77±0.24 *	1.29±0.06 *	1.41±0.11 *(4)
Pro	1.45±0.06 *	1.25±0.11	1.29±0.09
Cys	1.45±0.05 *	0.84±0.08	0.76±0.06
Arg	1.01±0.10	0.63±0.11	0.79±0.06
Asp	0.93±0.10	0.71±0.12	0.75±0.08
Glu	0.92±0.07	0.67±0.17	0.88±0.15
AIB	3.76±0.21 *	1.93±0.36 *	1.89±0.18 *
MeAIB	0.87±0.05	0.60±0.10	0.69±0.04

TABLE IV

Rank order of common amino acids as inhibitors of amino acid uptake in the presence and absence of a K^+ gradient and as countertransport elicitors

Rank	Transported amino acid								
	with K^+ gradient			without K^+ gradient			countertransport		
	Leu (inhibitor)	Ala (inhibitor)	Phe	Leu (inhibitor)	Ala (inhibitor)	Phe	Leu (elicitor)	Ala (elicitor)	Phe
1	Leu	Leu	Leu	Leu	Leu	Leu	His	Ala	Leu
2	Lys	Ile	Met	Lys	His	Ile	Met	Leu	Ile
3	Ile	Val	Ile	Ile	Val	Met	Ala	Ile	Ala
4	Ala	Ala	Ala	Met	Ala	His	Val	His	His
5	His	Met	Val	His	Met	Val	Leu	Val	Val
6	Met	Ser	His	Val	Thr	Phe	Ile	Met	Met
7	Phe	His	Lys	Gly	Ile	Lys	Gln	Phe	Phe
8	Val	Thr	Gln	Phe	Gly	Ala	Phe	Gln	Ser
9	Gln	Gln	Phe	Ala	Phe	Gln	Lys	Trp	Asn
10	Trp	Tyr	Ser	Cys	Cys	Tyr	Ser	Ser	Gln
11	Tyr	Phe	Tyr	Asn	Gln	Cys	Asn	Thr	Trp
12	Ser	Trp	Thr	Thr	Lys	Asn	Tyr	Tyr	Lys
13	Asn	Lys	Trp	Ser	Ser	Trp	Thr	Gly	Gly
14	Thr	Gly	Gly	Tyr	Asn	Thr	Trp	Pro	Tyr
15	Asp	Asn	Asn	Gly	Tyr	Ser	Gly	Asn	Thr
16	Gly	Pro	Pro	Trp	Pro	Gly	Pro	Lys	Pro
17	Glu	Cys	Arg	Pro	Trp	Pro	Cys	Cys	Glu
18	Pro	Arg	Asp	Glu	Arg	Arg	Arg	Asp	Arg
19	Cys	Asp	Glu	Arg	Glu	Asp	Asp	Glu	Cys
20	Arg	Glu	Cys	Asp	Asp	Glu	Glu	Arg	Asp

alanine uptake by more than 50% with the K^+ gradient did so without the gradient. Since the same amino acids inhibit uptake with and without the gradient, the postulated broad scope symporter cannot be an artifact, attributable to competition for a K^+ gradient. Cysteine is the only amino acid whose order of effectiveness changed in the presence of a K^+ gradient, being a weaker inhibitor in the presence than in the absence of the gradient (Tables I and II).

Countertransport

Eleven amino acids – Ser, Asn, and nine of the ten which inhibited leucine uptake with a gradient – yielded leucine countertransport accumlulation ratios greater than 2. Only two amino acids, Ala and Leu, gave accumulation ratios greater than 2 for alanine, both being in the group which had inhibited K^+ driven alanine uptake by more than 50%. Five amino acids – Leu, Ile, Ala, His, and Val – yielded phenylalanine accumulation ratios greater than 2, all five being near the top of the $> 50\%$ inhibition groups. Since the same amino acids which inhibit uptake with and without a K^+ gradient also elicit countertransport accumulation, the postulated broad scope symporter cannot be attributed to noncompetititive inhibition.

Rank orders of amino acids as inhibitors and elicitors

The rank orders of the twenty naturally occurring amino acids as inhibitors of leucine, alanine, and phenylalanine uptake driven with a K^+ gradient (Table IV, left columns) as inhibitors of uptake without a gradient (Table IV, center columns) and as elicitors of countertransport (Table IV, right columns) are all very similar to each other. The similar ordering again supports the hypothesis that these three neutral amino acids share a common symporter.

Indifference of uptake to initial osmotic balance

For uptake with a K^+ gradient the osmolarity of the solutions was 160 mosM greater outside than inside initially whereas for uptake without a gradient and for countertranport accumulation there was no osmotic gradient at any time. The similar rank order of inhibition and elicitation in all cases (Table IV) and the high correlation coefficients of uptake with and without gradients discussed below ($R > 0.75$ in all cases) confirms the earlier finding [15] that System B symport is virtually independent of initial osmotic pressure in *M. sexta* BBMV.

Correlation analysis

Inhibition of leucine uptake without and with the ion gradient is well correlated (Fig. 3A; $R = 0.85$). The correlation is not quite as good for alanine uptake (Fig. 3B; $R = 0.76$) but is nearly as good for phenylalanine uptake (Fig. 3C; $R = 0.84$), although the K^+ gradient appears to weaken the inhibition by competing amino acids. These high correlation coefficients, along with their clustering near the origin in Fig. 3 support the hypothesis that leucine, alanine, and phenylalanine uptake are all mediated by the same symporter.

The high correlation between countertransport vs. uptake inhibition with a K^+ gradient of leucine ($R = 0.89$), alanine ($R = 0.89$) and phenylanine ($R = 0.83$) and between countertransport vs. uptake inhibition without a K^+ gradient of leucine ($R = 0.84$), alanine ($R = 0.82$) and phenylalanine ($R = 0.82$) is consistent with a common symport system. Correlation plots of countertransport of leucine vs. alanine (Fig. 4A), leucine vs. phenylalanine (Fig. 4B) and phenylalanine vs. alanine (Fig. 4C) yield correlation coefficients of 0.86, 0.85, and 0.94, respectively. The same amino acids cluster at high accumulation values in all three plots in Fig. 4. The ability of leucine, alanine, and phenylalanine to induce countertransport of each other so effectively is consistent with their use of a common symporter.

Amino acids which do not use System B of M. sexta BBMV

Aspartate, glutamate, arginine, and proline do not appear to be using System B because they are the least

effective inhibitors of uptake and do not elicit countertransport accumulation of leucine, alanine, and phenylalanine. Cysteine is a poor inhibitor of K^+ driven leucine, alanine, and phenylalanine uptake. Moreover, it barely elicits leucine uptake and fails to elicit alanine or phenylalanine uptake.

The inhibitory value of lysine varies; it is a strong inhibitor of leucine uptake, slightly weaker for phenylalanine, and weaker still for alanine. This same pattern for lysine inhibition was found in both the presence and the absence of a K^+ gradient. Lysine also elicits strong countertransport accumulation of leucine but minimal accumulation of phenylalanine and no accumulation of alanine in these measurements at pH 10. However, in experiments on BBMV from *P. cynthia* at pH 8.8, lysine does not inhibit leucine uptake in the presence or absence of a K^+ gradient [11], nor does it elicit significant countertransport of leucine or phenylalanine [3]. Lysine may have its own symporter at pH 10 through which it elicits the uptake of leucine and some phenylalanine but not alanine. A K^+-dependent lysine symporter is present in *P. cynthia* [12].

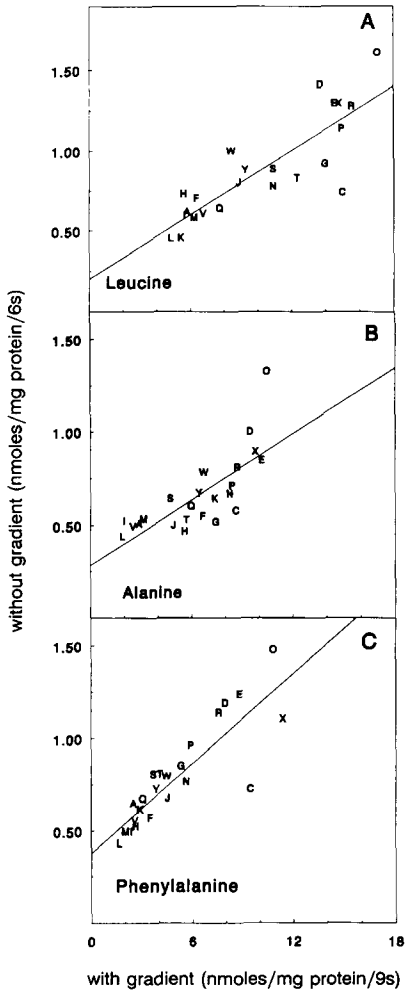

Fig. 3. Uptake without vs. with K^+ gradient. The inhibitory effect of each of the 20 common amino acids as well as AIB and MeAIB (see Table I for symbols used) on the uptake of labeled (A) leucine (B) alanine and (C) phenylalanine is plotted in the absence vs. presence of a K^+ gradient.

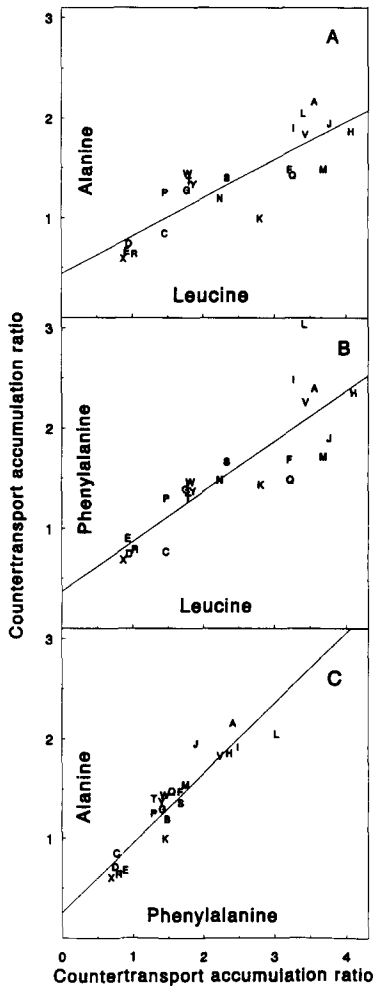

Fig. 4. Comparison of abilities of 20 common amino acids to elicit countertransport of (A) alanine vs. leucine (B) phenylalanine vs. leucine and (C) alanine vs. phenylalanine.

Relationships between the Manduca System B and other systems

The broad scope symport system in *M. sexta* BBMV is similar to System NBB (renamed System B) in rabbit jejunal BBMV [13] in that both systems transport most of the neutral amino acids and exclude MeAIB. However, it is unlike 'neutral' amino acid System A, in vesicles from rat basolateral membranes, which can transport MeAIB [14]. The *M. sexta* System B is also unlike the ASC system in rabbit jejunum BBMV because it does not transport cysteine but does transport phenylalanine [13]. The B type transport system described here for *M. sexta* at pH 10 is very similar to the transport system described at lower pH in BBMV from *P. cynthia* [2,3]. Our findings support the hypothesis that a broad spectrum, B type transport system operates in the midgut of all Lepidopteran larvae.

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References

1 Parenti, P., Giordana, B., Sacchi, V.F., Hanozet, G.M. and Guerritore, A. (1985) J. Exp. Biol. 116, 69–78.
2 Giordana, B., Sacchi, V.F., Parenti, P. and Hanozet, G.M. (1989) Am. J. Physiol. 257, R494–R500.
3 Hanozet, G.M., Giordana, B., Sacchi, V.F. and Parenti, P. (1989) J. Exp. Biol. 143, 87–100.
4 Harvey, W.R., Crawford, D.N. and Spaeth, D. (1990) Methods Enzymol. 192, 599–608.
5 Biber, J., Steiger, B., Haase, W. and Murer, H. (1981) Biochim. Biophys. Acta. 647, 169–176.
6 Wolfersberger, M., Luethy, P., Maurer, A., Parenti, P., Sacchi, F.V., Giordana, B. and Hanozet, G.M. (1987) Comp. Biochem. Physiol. 86A, 301–308.
7 Eisen, N.S., Fernandes, V.F., Harvey, W.R., Spaeth, D.D. and Wolfersberger, M.G. (1989) Insect Biochem. 19, 337–342.
8 Haase, W., Schafer, A., Murer, H. and Kinne, R. (1978) Biochem. J. 172, 57–62.
9 Kessler, M., Tannenbaum, V. and Tannenbaum, C. (1978) Biochim. Biophys. Acta 509, 348–359.
10 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
11 Sacchi, V.F., Giordana, B., Campanini, F., Bonfanti, P. and Hanozet, G.M. (1990) J. Exp. Biol. 149, 207–221.
12 Giordana, B., Parenti, P., Hanozet, G.M. and Sacchi, V.F. (1985) J. Membr. Biol. 88, 45–53.
13 Stevens, B.R., Ross, H.J. and Wright, E.M. (1982) J. Membr. Biol. 66, 213–225.
14 Mircheff, A.K., Van Os, C.H. and Wright, E.M. (1980) J. Membr. Biol. 52, 83–92.
15 Hennigan, B.B., Wolfersberger, M.G., Parthasarathy, R. and Harvey, W.R. (1993) Biochim. Biophys. Acta 1148, 209–215.