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Fisiologia. — *Amino acid transport systems in Brush Border Membranes of* Philosamia Cynthia *Midgut* (*). Nota di ENZINA LOCATELLI, PAOLO PARENTI, BARBARA GIORDANA, FRANCA V. SACCHI (**) e GIORGIO M. HANOZET, presentata (***) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Le vescicole di orletto a spazzola isolate dall'intestino medio della larva del lepidottero *Philosamia cynthia*, sono state utilizzate per individuare la presenza di diversi sistemi di trasporto degli aminoacidi. Valutando l'accumulo intravescicolare di aminoacido marcato, in vescicole precaricate con un aminoacido freddo, è possibile discriminare quali aminoacidi possono essere trasportati da un medesimo sistema di trasporto. I risultati ottenuti suggeriscono la presenza di quattro diversi sistemi responsabili del trasporto di aminoacidi neutri, di un sistema per la lisina e di un sistema per l'acido glutammico.

INTRODUCTION

Amino acid transport in mammalian cells occurs via different well characterized transport systems [1]. Some of these systems have been recognized also in epithelial membranes, in which different categories of amino acid transport agencies have been found [2, 3]. In rat jejunal brush border membrane vesicles five different amino acid transport systems have been described and the following nomenclature was given: the sodium dependent NBB (neutral amino acids), IMINO, PHE and the sodium independent y^+ (cationic amino acids) and L (leucine and branched amino acids) [3].

The midgut of lepidopteran larvae can actively absorb amino acids [4], but the mechanism of the transepithelial and luminal transport of several amino acids has been proved to involve potassium rather than sodium ion. Actually, on the brush border of midgut columnar cells, an electrogenic co-transport mechanism which couples potassium and amino acid influx is present [5-7]. *In vivo*, the driving force for the uphill uptake of these amino acids is mostly due to the high transmucosal electrical potential difference (cell interior negative to the lumen side) generated by a K-pump extruding this cation towards the lumen [6].

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To investigate the presence of different transport agencies in this invertebrate model epithelium, we pre-loaded purified brush border membrane vesicles with 15 different amino acids and tested their ability to elicit a counterflow accumulation of a labelled amino acid present in the medium outside the vesicles. This experimental procedure allows us to discriminate the amino acids which share the same transport agency, since only these can produce a transient accumulation of the label inside the vesicles.

Materials and Methods

Larvae of *Philosamia cynthia* (Saturnidae) in the fifth instar were used. The larvae were fed on *Ailanthus glandulosa* leaves. The midgut was dissected from the larvae and the peritrophic membrane with enclosed intestinal contents was removed.

Brush border membrane vesicles (BBMV) were prepared by means of Ca^{++} precipitation, following the procedure of Schmitz *et al.*, 1973 [8], modified by Kessler *et al.*, 1978 [9] as described in a previous paper [6]. The pellet obtained from the second centrifugation step and the final pellet were resuspended in a medium containing 100 mM mannitol, 10 mM HEPES-Tris pH 7.4, 50 mM K_2SO_4 and either 40 mM elicitor amino acid or 40 mM mannitol. This was assumed to be the intravesicular medium.

The final membrane pellet was resuspended at a protein concentration of 10-15 mg/ml, as determined according to Bradford, 1976 [10] with a Bio-Rad kit, using bovine serum albumine as standard.

Transport experiments were performed in quadruplicate by the rapid filtration technique, as described by Hanozet *et al.*, 1980 [5], with the following modifications: 10 µl of BBMV, pre-incubated 10 min with valinomycin (8 µg/mg protein), were mixed with 190 µl of a cocktail containing the labelled amino acid. Therefore, the final concentrations of the extravesicular medium were: 100 mM mannitol, 10 mM HEPES-Tris, pH 7.4, 50 mM K_2SO_4 and 2 mM (10 µCi/ml) of the labelled amino acid. At selected times, 70 µl samples were withdrawn from the incubation mixture, diluted with 5 ml of ice cold stop solution (150 mM NaCl, 2 mM HEPES-Tris, pH 7.4), filtered through a cellulose nitrate filter (0.65 µm pores) and rapidly rinsed with 2 × 5 ml of ice cold stop solution. Valinomycin was added from ethanol stock, so that the ethanol concentration in the incubation mixture did not exceed 0.5%.

L-(U-^{14}C)alanine, L-(4,5-^{3}H)leucine, L-Phenyl(2,3-^{3}H)alanine, L-(6-^{3}H)glutamic acid, L-(4,5-^{3}H)lysine monohydrochloride and L-(2,5-^{3}H)histidine were purchased from Amersham, International plc, U.K.

Results and Discussion

Counterflow accumulation has been defined as " the accumulation of a substrate *a* beyond its equilibrium concentration, driven by the imposition of a second substrate *b*, which shares the same transporter and binding site » [11].

In the experiments reported in this paper, substrate *b* was the amino acid pre-loaded in the vesicles and it is called elicitor. Fig. 1 reports a typical countertransport. experiment. Histidine and glutamine, but not mannitol, elicited a transient intravesicular accumulation of labelled histidine in the absence of any salt gradient but in the presence of potassium. Any electrical coupling via transmembrane potential was avoided by the addition of the potassium ionophore valinomycin. The intravesicular accumulation of a substrate means that a net flux occurs from the outside medium into the vesicles even when the intra-

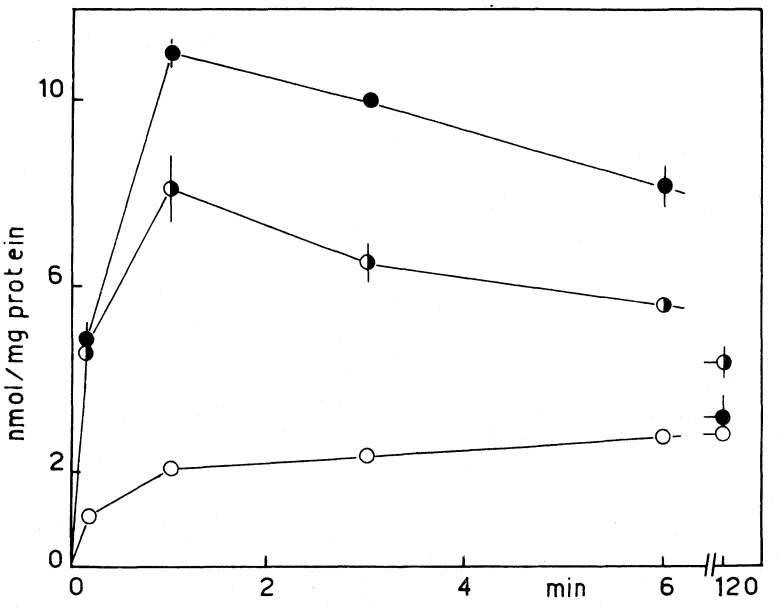

Fig. 1. – Countertransport of histidine elicited by histidine and glutamine in BBMV from *P. cynthia* midgut. BBMV, re-suspended in 100 mM mannitol 10 mM HEPES-Tris, pH 7.4, 50 mM K_2SO_4 + 40 mM mannitol (○) + 40 mM histidine (●) + 40 mM glutamine (◑) and pre-incubated 10 min with 8 μg/mg protein of 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 means ± SE of the typical experiment carried out in quadruplicate. When not given, SE bars were smaller than the symbol used.

vesicular concentration of the labelled amino acid is higher than in the external medium. This happeans because the outflux of the labelled amino acid, once entered into the vesicles, is inhibited by the presence of the high concentration of the cold substrate. Of course this inhibition can take place only if the labelled amino acid and the cold form inside the vesicles share the same transporter.

In order to have a first screening of the transport systems involved in amino acid transport, the vesicles were pre-loaded with fifteen different elicitor amino

acids and the uptakes of six labelled amino acids into BBMV were measured. The data are reported in Table I.

The counterflow accumulation due to the elicitor was evaluated as the ratio between the peak of the overshoot curve (at 1 min, in our conditions, see fig. 1) and the equilibrium value measured after an incubation time of 120 min. The statistical significance of the difference between these two uptake values was tested with *t*-test. Only when P was < 0.05 was the ratio considered significantly different from 1, and the elicitor was therefore considered effective in causing a counterflow accumulation of the tested amino acid.

Lysine and glutamic acid seem to have very specific transport systems since none of the elicitors tested could stimulate their uptakes and since they failed to behave as elicitors for the other amino acids. The same is true for glycine,

TABLE I.

Counterflow accumulation of labelled alanine, leucine, phenylalanine, glutamic acid, lysine and histidine in BBMV *of* P. cynthia *midgut preloaded with elicitor amino acids.*

	ALA	LEU	PHE	GLU	LYS	HIS
ELICITOR						
ALA	1.55(*)	1.79(*)	1.28(*)	0.24	0.81	1.41(*)
LEU	1.67(*)	2.52(*)	2.09(*)	0.33	0.74	1.90(*)
PRO	0.92	0.93	1.42(*)	0.31	0.30	0.93
PHE	1.73(*)	3.68(*)	2.85(*)	0.63	0.74	2.37(*)
MeAIB . .	1.96(*)	1.58(*)	1.33(*)	0.34	0.70	1.63(*)
GLY . . .	1.27	0.87	1.18	0.17	0.32	0.58
SER	1.43(*)	1.15	1.31(*)	0.57	0.72	1.45
CYS	0.55	2.83(*)	2.16(*)	0.44	0.66	1.61(*)
ASN	1.89(*)	1.79(*)	1.29(*)	0.31	0.49	1.54(*)
GLN	1.81(*)	1.66(*)	1.57(*)	0.25	0.57	2.11(*)
ASP	0.55	0.79	0.93	0.53	0.38	0.73
GLU	0.87	0.98	0.55	1.46(*)	0.41	0.68
LYS	0.89	0.67	1.10	0.34	2.72(*)	0.97
ARG . . .	0.34	1.57	0.79	0.23	0.14	0.50
HIS	1.68(*)	2.56(*)	1.13	0.48	0.70	4.23(*)
MANNITOL	0.73	0.82	0.86	0.28	0.47	0.82

The accumulation is expressed as the ratio between the uptake value of the labelled amino acid at 1 min and the uptake value at 120 min.

* Ratio significantly different from 1 (see text).

MeAIB = methyl amino iso-butyric acid.

which appears to be transferred by a specific transport system as observed in vertebrate plasma membranes [3]. This possibility is also supported by inhibition experiments which showed that glycine did not inhibit the uptake of alanine, histidine and lysine (unpublished result).

From the pattern shown by the neutral amino acids other than glycine and by histidine it can be inferred that at least three different transport systems for neutral amino acids exist in BBMV of *P. cynthia*. Two of these systems also transport histidine. Most neutral amino acids are not discriminated by these systems, but each of them excludes a small number of amino acids. The characteristics of the proposed amino acid transport systems are resumed in Table II.

In a previous work [12], the analysis of histidine and lysine transport mechanisms indicated a potassium: amino acid stoichiometry higher than one for the former and about one for the latter. The data here reported confirm that these two amino acids are transported by different systems; besides histidine can be transported by system 1 and 2 (Table II) possibly with different transport mechanisms and stoichiometry.

TABLE II.

Amino acid transport systems in BBMV *from* P. cynthia *midgut.*

System	Transported amino acids	Excluded amino acids
1	ALA, LEU, PRO, PHE, MeAIB, SER, CYS, ASN, GLN	GLY, HIS, LYS, GLU
2	ALA, LEU, PHE, MeAIB, CYS, ASN, GLN, HIS	GLY, PRO, SER, LYS, GLU
3	ALA, LEU, PHE, MeAIB, SER, ASN, GLN, HIS	GLY, PRO, CYS, LYS, GLU
4	GLY	All the other tested amino acids
5	LYS	All the other tested amino acids
6	GLU	All the other tested amino acids

Thus it is apparent that the transport of amino acids in the midgut of lepidopteran larvae is performed, as in mammalian cells, by different transport agencies, some of which show a wide range of overlapping substrate specificities. Nonetheless, these transport systems seem to have little or nothing in common with those well characterized and conventionally designated in mammalian cells [13].

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