REVISTA ESPAÑOLA DE FISIOLOGÍA, 45 (4), 373-376, 1989

Kinetics of Glycylsarcosine Transport by Isolated Chicken Intestinal Epithelial Cells

M. L. Calonge, A. Ilundain and J. Bolufer*

Departamento de Fisiología y Biología Animal
Facultad de Farmacia
41012 Sevilla (Spain)

(Received on May 3, 1989)

M. L. CALONGE, A. ILUNDAIN and J. BOLUFER. *Kinetics of Glycylsarcosine Transport by Isolated Chicken Intestinal Epithelial Cells.* Rev. esp. Fisiol., 45 (4), 373-376, 1989.

Kinetics of Glycylsarcosine (Gly-Sar) uptake by isolated chicken enterocytes was studied by measuring its intracellular concentration, and by discriminating between the saturable and the diffusive components of the total uptake. The diffusive component was greater at pH 6.0 than at pH 7.4, and the Jmax was also increased by lowering external pH, whereas the Km remained in the same order of magnitude. Carnosine competitively inhibits Gly-Sar uptake, indicating that both share a common transport system.

Key words: Dipeptide transport, Isolated cells, Chicken intestine, Kinetics.

Early studies demonstrated that peptides are transported across the small intestine by systems different from those for free amino acids (7, 8). Recent studies with purified brush-border membrane vesicles from mammalian small intestine have shown that dipeptide transport occurs via a Na^+-independent, H^+-coupled, non-concentrative mechanism (3-5). We have recently reported that enterocytes isolated from chicken small intestine accumulate glycyl-sarcosine (Gly-Sar) and that this accumulation appears to be indirectly driven by an inward H^+ gradient and dependent upon external Na^+ (1).

Regarding the kinetic characteristics of this transport system, different results have also been obtained depending on the biological preparation, the experimental condition or the animal species employed (1, 4). The aim of the present work was to further investigate the kinetics of this transport system in isolated cells at different extracellular pH.

Material and Methods

Cell isolation and measurement of Gly-Sar uptake. — Intestinal epithelial cells have been isolated from 4-6 wk-old Hubbard chickens using the hyaluronidase method developed by KIMMICH (6) with minor modifications as previously described (1). The media composition was in mM: 80 NaCl, 100 mannitol, 3 K_2HPO_4, 1 $MgCl_2$, 1 $CaCl_2$, 20 Tris-HCl (pH, 7.4) and 1 mg bovine serum albumin. In some

* To whom all correspondence should be addressed.

experiments NaCl was substituted by the appropriate concentration of mannitol. The pH, 6.0 was achieved by changing Tris-HCl to Tris-Citric Acid isotonic buffer.

Materials. — Gly-Sar and carnosine were obtained from Sigma. The ^{14}C-Gly-Sar was supplied by the Radiochemical Centre, Amersham. All other chemicals were of the highest purity available.

Results and Discussion

The Gly-Sar uptake as a function of its concentration was investigated by varying the concentration of the peptide from 0.1 to 50 mM at pH 6.0 and 7.4. The relationship between total uptake and Gly-Sar concentration in the incubation medium was non-linear at low concentrations and became linear at high concentrations (fig. 1). The shape of these curves suggest the involvement of at least two components in

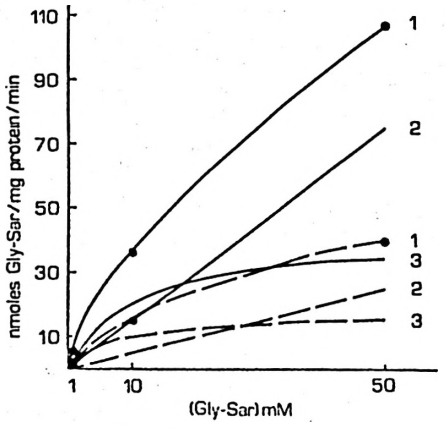

Fig. 1. *Gly-Sar uptake by isolated chicken enterocytes.*
Cells were incubated for 1 minute in Tris-HCl (20 mM) buffer, pH 7.4 (- - -) or in Tris-isocitric acid (20 mM), pH 6.0 (——). Curve 1, total uptake; curve 2, passive component obtained graphically (see text); curve 3, saturable component (difference curve).

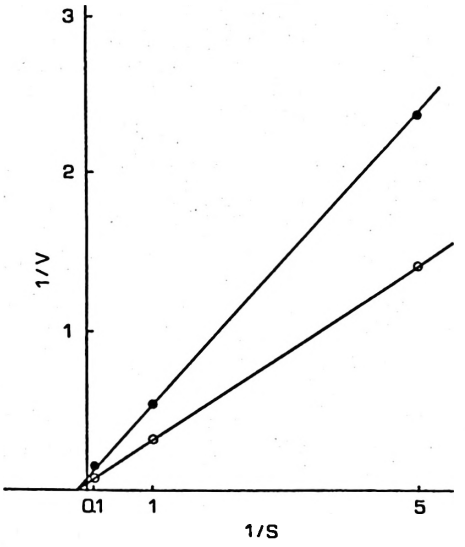

Fig. 2 *Lineweaver-Burk plot of Gly-Sar saturable component uptake by isolated enterocytes incubated at pH 7.4 (●) or 6.0 (○).*
Values of V are expressed in nmoles/µl cell water/minute.

the uptake process, a saturable component upon which a diffusive component is superimposed. This latter component was assessed by the graphic method of NEAME and RICHARDS (9). The active component was estimated by subtracting the passive transfer from the total uptake. The values of the apparent kinetic constants (Km' and Jmax') can be obtained by fitting the data with an unweighed single rectangular hyperbola and were: Km' 8.5 ± 1.5 mM (pH, 6.0) and 8.0 ± 1.2 mM (pH, 7.4) and that of Jmax' were 39 ± 2 nmol / mg protein / min (pH, 6.0) and 17.5 ± 0.7 nmol / mg protein / min (pH, 7.4). The results, when plotted by the Lineweaver-Burk method (fig. 2), yielded a straight line, indicating the presence of a single active transport system at the two pH used. The kinetic constants calculated by this method are: Km', 6.5 ± 1.2 mM (pH, 6.0); 6.2 ± 1.5 mM (pH, 7.4) and Jmax', 31.5 ± 2.5 nmol Gly-Sar / mg protein / min (pH, 6.0); 14.3 ± 2 nmol / mg protein / min

Table I. *Effect of carnosine upon Gly-Sar uptake by isolated enterocytes.*
Results are given in nmol Gly-Sar / µl cell water and are means ± S.E. of 6 independent determinations.

Gly-Sar [mM]:	0.1			1		
Time (min):	5	15	30	5	15	30
Control	0.12 ± 0.02	0.22 ± 0.01	0.30 ± 0.03	1.20 ± 0.04	2.26 ± 0.04	3.11 ± 0.13
Carnosine (mM)						
1	0.10 ± 0.007	0.16 ± 0.01[c]	0.24 ± 0.01	1.10 ± 0.07	2.02 ± 0.08[b]	2.46 ± 0.08[b]
5	0.08 ± 0.008	0.12 ± 0.005[a]	0.16 ± 0.006[a]	0.86 ± 0.007[b]	1.62 ± 0.08[a]	1.52 ± 0.08[a]
10	0.06 ± 0.004[c]	0.09 ± 0.005[a]	0.12 ± 0.01[a]	0.65 ± 0.07[a]	1.25 ± 0.09[a]	1.37 ± 0.12[a]

[a] $p < 0.001$; [b] $0.05 > p > 0.001$; [c] $0.025 > p > 0.01$

(pH, 7.4), very close to those obtained by non-linear regression analysis, and similar to those determined in rabbit brush-border vesicles by other authors (3).

Studies with intestinal rings (8) and intestinal brush-border membrane vesicles (3) have shown that peptide transport is optimal at pH 5.5-6.0. This pH value might stimulate Gly-Sar transport by at least two mechanisms: i) The value of Gly-Sar isoelectric pH is 5.68 (2). Therefore, the concentration of the transportable zwitterionic species is maximal in this pH range, and ii) Since the external pH is 1.5-2.0 acidic units compared with intracellular pH, an inward proton gradient that stimulates peptide-H^+ coupled transport would exist. Our results showed that the diffusive component at pH 6.0 was greater than at pH 7.4 (1.5 vs 0.5 nmol / mg protein / min /mM), which agree with what could be expected in the light of the first mechanism. Regarding the effect of pH on Km' and Jmax', when a cation is cotransported with the organic solute, the presence of the cation-gradient may stimulate solute transport by increasing Jmax' or by decreasing Km' or by both. Our results indicate that at pH 6.0 the Jmax' increased while the Km' remained in the same order of magnitude.

In order to know if Gly-Sar transport system should be used by other dipeptides we studied the effect of carnosine (β-alanyl-hystidine), a non-hydrolizable dipeptide (10), upon Gly-Sar uptake. Table I shows the results obtained when the carnosine concentrations were 1, 5 and 10 mM. The concentrations of Gly-Sar employed in these experiments were 0.1 and 1 mM because, as shown in fig. 1, at these concentrations the passive component was less important. As can be seen, the presence of carnosine inhibits Gly-Sar uptake and when the concentration of carnosine increases the uptake of Gly-Sar further decreases. Taking the values at 1 min incubation and after corrections for the diffusive component the inhibition constant, K_I, was 12 mM (1). These results suggest that Gly-Sar and carnosine share a common transport system. Similar results had been obtained with rabbit brush-border membrane vesicles (3).

In conclusion, the results obtained support the general view that the uptake of Gly-Sar at lower concentrations is mainly realized by a saturable transport system, whose Jmax' changed with the pH of the medium.

Acknowledgement

This work was supported by a grant from the Spanish «Comisión Interministerial de Ciencia y Tecnología» PB86-0513.

Resumen

Se estudian las características cinéticas del transporte de glicil-sarcosina (Gly-Sar) por enterocitos aislados de pollo, midiendo su concentración intracelular, y discriminando entre los componentes saturable y no saturable del transporte total. El componente no saturable es mayor a pH 6,0 que a pH 7,4, y la Jmax' también aumenta al disminuir el pH externo, mientras que la Km' permanece en el mismo orden de magnitud. La carnosina inhibe competitivamente la captación de Gly-Sar, indicando que ambos comparten un mismo sistema de transporte activo.

Palabras clave: Transporte de dipéptidos, Células aisladas, Intestino de pollo, Cinética.

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