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Interactions Among Amino Acid Transport Systems in Snail *Helix aspersa* Intestine

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Interactions among the transport of diverse amino acids in everted intestine of snail *Helix aspersa* have been studied. The uptake of 0.5 mM methionine is clearly inhibited by high concentrations (40 mM) of leucine, and not by proline or lysine, whereas the last two amino acids inhibit cycloleucine uptake. Methionine strongly inhibits proline and lysine uptake, which is significantly inhibited by their analogs hydroxiproline and arginine, respectively. Results suggest that in *Helix* intestine the transport systems for basic amino acids and iminoacids are shared with high affinity by methionine whereas the neutral amino acids transport systems do not seem to be shared, or are so very weakly, by the basic ones or by the imino acids.

Key words: Intestinal transport, Amino acids, Snail, *Helix aspersa*.

Various studies have tried to characterize the interactions among diverse intestinal amino acid transport systems, at both the mucosal (12, 16) and basolateral (6, 7, 14) enterocyte membranes. Explaining such interactions between neutral and basic amino acids was the purpose of many of those papers. Mutual inhibitions, especially noticeable with neutral amino acids as inhibitors, have been described. The neutral amino acid capacity to stimulate basic amino acid uptake by various mammal intestinal preparations (17, 20, 23-25), has likewise been repeatedly observed.

The present work continues the study of the amino acid transport systems in snail *Helix aspersa* intestine (8, 9, 11) analyzing the interactions between the transport of neutral amino acids, lysine and proline.

Materials and Methods

Amino acid uptake by the intestinal tissue has been measured in 2 min or 30 min incubation periods. The technique used was described in a previous paper (3). Preparations of everted intestine from

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1 to 4 animals were suspended in 10 ml of physiological solution (4), pH 7.2, with the amino acid (^{14}C) at the required concentration, with stirring (100 cycles/min) and O_2 bubbling. Once the incubation period was finished, the preparations were separated from the medium through vacuum filtration, washed with ice cold physiological solution, dried on wet filter paper and weighed. Each intestine was then introduced in 0.5 ml 0.1 M NO_3H and after 24 h in cold storage, aliquot samples were taken to determine the radioactivity, allowing to estimate the amount of L-amino acid transferred to the tissue water by comparing it with the radioactivity in the sample from the incubation medium. The amino acid uptake was measured in μmoles per gram of wet weight in the incubation time mean (mean ± SE) and the results of the interaction experiments are expressed as percent values of the control uptake.

In experiments without Na^+ in the incubation medium this cation was replaced by Tris, the pH being adjusted to 7.2 and the medium osmolarity being kept constant.

The labelled amino acids L-[methyl ^{14}C] methionine (56.7 mCi/mmol), 1-aminocyclopentan-1-[^{14}C]carboxilic acid (59 mCi/mmol), L-[U^{14} C] proline (250 mCi/mmol) and L-[U^{14} C] lysine ClH (348 mCi/mmol) were from Amersham International, inert L-lysine ClH and cycloleucine from Sigma and salts and other L-amino acids from Merck.

Results

Inhibition of L-methionine uptake. — The effect of various amino acids at a high concentration (40 mM) on 0.5 mM methionine uptake by the tissue, has been studied (fig. 1).

Leucine exerts a strong inhibitory effect on methionine transport. In agreement with previous observations (8) the measured residual methionine uptake could correspond to a lineal process of simple diffusion.

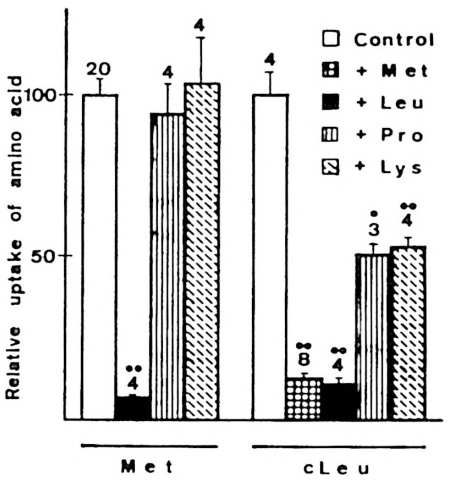

Fig. 1. *Relative values of the effect of several amino acids (40 mM) on the total uptake of 0.5 mM methionine and 0.5 mM cycloleucine.*
Methionine and cycloleucine absolute control values were 0.708 μmoles g^{-1} w.w. 2 min^{-1} and 0.250 μmoles g^{-1} w.w. 2 min^{-1}, respectively. No symbols means nonsignificant difference, • $p < 0.01$ and •• $p < 0.001$. Vertical bars denote S.E. of the mean of the number of separate determinations given on the top of the histograms.

L-proline does not produce a significant inhibition on the uptake of 0.5 mM and 0.2 mM methionine (results not shown). Similarly, the presence of lysine does not show any inhibitory effect on methionine transport at concentrations of 0.05, 0.2, 0.5 (fig. 1) and 2 mM.

Inhibition of cycloleucine uptake. — Both methionine and leucine at a 40 mM concentration exert a strongly inhibitory effect on 0.5 mM cycloleucine transport, both inhibition being of similar magnitude.

Similarly the imino acid proline and the basic amino acid lysine at a 40 mM concentration significantly inhibit cycloleucine transport, although to a lesser extent than that observed for the neutral amino acids (fig. 1).

Inhibition of L-proline uptake. — Figure 2 shows the effects of high concentrations (40 mM) of hydroxyproline (OH Pro), methionine, cycloleucine and lysine on 0.5 mM L-proline uptake. Hydroxyproline clearly inhibits proline uptake. Similar effects were observed for other proline concentrations in a previous work (9).

Methionine, likewise, exerts a strongly inhibitory effect, quite greater than that observed with hydroxyproline, which suggets that methionine shares proline carriers.

The inhibition produced by cycloleucine on proline uptake is slightly inferior to that observed with hydroxyproline. Lysine inhibition, on the contrary, is lower and less significant ($p < 0.1$).

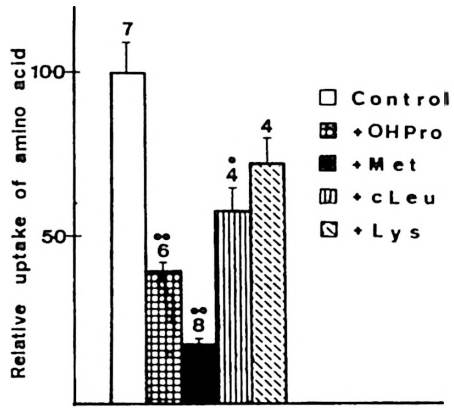

Fig. 2. *Relative values of the effect of several amino acids (40 mM) on the total uptake of 0.5 mM proline.*
Absolute value of proline control being 0.202 μmoles g^{-1} w.w. 2 min^{-1}. Statistical significance and symbols as stated in figure 1.

Inhibition of L-lysine uptake. — With the basic amino acid lysine, two incubation periods (2 and 10 min) as well as two different concentrations, 0.5 and 10 mM, have been used.

In two minutes incubation periods (fig. 3) and with the lower lysine concentration no significant inhibition by 0.5 mM methionine, 40 mM proline or the absence of Na^+, has been observed, where-

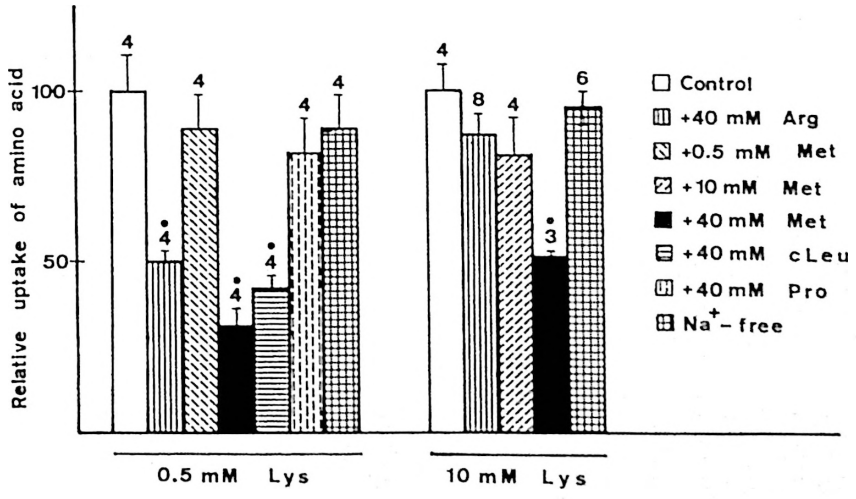

Fig. 3. *Relative values of the effect of different experimental conditions on 0.5 and 10 mM lysine uptake in two minute incubation periods.*
Absolute values of uptake were 0.092 μmoles g^{-1} w.w. 2 min^{-1} and 0.868 μmoles g^{-1} w.w. 2 min^{-1} for 0.5 and 10 mM lysine, respectively. Statistical significance and symbols as stated in figure 1.

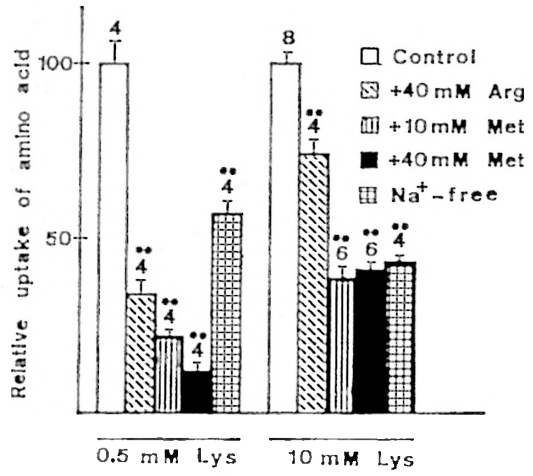

Fig. 4. *Relative values of the effect of different experimental conditions on 0.5 mM lysine uptake in ten minute incubation periods.*
Absolute values of uptake were 0.449 μmoles g^{-1} w.w. 10 min^{-1} and 3.651 μmoles g^{-1} w.w. 10 min^{-1} when lysine concentration in the medium was 0.5 and 10 mM, respectively. Statistical significance and symbols as stated in figure 1.

as arginine, methionine and cycloleucine at 40 mM concentration, inhibit the uptake significantly. With 10 mM lysine, instead, neither 40 mM arginine, 10 mM methionine nor Na^+ absence produces appreciable inhibitions, whereas 40 mM methionine clearly inhibits it. In longer incubation periods (10 min) (fig. 4) the inhibitions by arginine and methionine become much more apparent and Na^+ absence is shown to also reduce lysine uptake. Arginine at a 40 mM concentration produces a marked lysine uptake inhibition, stronger with a 0.5 mM lysine concentration than with that of 10 mM, as could be expected from a competition process. Methionine at 10 mM and 40 mM concentrations produces an inhibitory effect much higher than that by arginine. Such an inhibition is also appreciable when the lysine : methionine concentration ratio is 1:1, in contrast to what was observed for 2 min incubation periods.

Finally, Na^+ absence, with 10 min incubations periods, clearly has an inhibitory effect, more evident with 10 mM lysine than with 0.5 mM lysine.

Discussion

Interactions between neutral and basic amino acids. — The presence of 40 mM lysine does not produce a significant decrease of methionine uptake at the various assayed concentrations in snail intestine, although it does inhibit cycloleucine transport markedly. On the other hand, both methionine and cycloleucine inhibit lysine transport, the former very strongly. The fact that neutral amino acids inhibit the transport of the basic ones has repeatedly been observed when the neutral amino acid concentration is high in relation to that of the basic one in chicken (10) as well as in rat (5, 15), rabbit (26) and guinea pig (17).

The results in *Helix* do not allow to confirm the stimulations of the basic amino acid transport in the presence of similar concentrations of neutral amino acids reported by some authors (15, 17, 23).

Inhibitions of the basic amino acid over the neutral ones have likewise been described (10, 13, 18, 22), although the inhibition in this sense seems to be less pronounced than in the opposite sense (1, 19). Most authors propose models in which all the transport systems for basic amino acids are shared by the neutral ones, while the basic amino acids do not share all of the neutral ones (10, 19, 27). This seems to be also the case for some invertebrates (2) and it proves to be acceptable for the interpretation of the present results in *Helix*, which seem to suggest the presence of some type of transport system for neutral amino acids not shared by the basic amino acids.

Interactions between neutral amino acids and imino acids. — The results show a behaviour similar to the one observed for neutral — basic amino acid interaction: strong inhibition on the imino acid transport by the neutral amino acids, and weak in the opposite sense. STEVENS and WRIGHT (28) admit in rabbit intestine an imino acid transport system which seems to transport phenylalanine but not alanine or methionine, and transport systems for neutral amino acids competitively inhibible by alanine and phenylalanine. The latter transport systems are also shared by proline although it inhibits only 30% of the phenylalanine transport. MUNCK (19) proposes the existence of five transport systems able to transport neutral amino acids, two of which would be shared with the imino acids.

Interactions between basic amino acids and imino acids. — Studies on the reciprocal inhibition between basic amino acids and imino acids are not frequent. They, nevertheless, seem to be mutually weak inhibitors (10, 18, 21). The results in *Helix aspersa* hardly reveal reciprocal weak inhibitions between them, both groups probably sharing, therefore, some type of transport system.

Resumen

Se estudian las interacciones entre el transporte de diversos aminoácidos en intestino evertido del caracol, *Helix aspersa*. La entrada de metionina 0,5 mM se inhibe claramente por elevadas concentraciones (40 mM) de leucina, y no de prolina o lisina, los cuales inhiben la penetración de cicloleucina. La metionina disminuye fuertemente la entrada de prolina y de lisina, que también se inhibe por sus análogos estructurales hidroxiprolina y arginina respectivamente. Los resultados sugieren que en el intestino de *Helix* los sistemas de transporte para aminoácidos básicos e iminoácidos son compartidos con elevada afinidad por la metionina, mientras que los sistemas de transporte para aminoácidos neutros no parecen ser compartidos, o lo son muy débilmente, ni por los básicos ni por los iminoácidos.

Palabras clave: Transporte intestinal, Aminoácidos, Caracol, *Helix aspersa*.

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