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Active Transport of L-Phenylalanine by Snail Intestine, *Helix (Cryptomphalus) aspersa* *

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Snail intestine was found to actively accumulate L-phenylalanine. The uptake of L-phenylalanine was a saturable function of the amino acid concentration and sodium-dependent. The K_T for L-phenylalanine uptake increased as the Na^+ concentration was reduced, being 2.2 mM at 71.4 mM Na^+, 5.9 mM at 10 mM Na^+ and 9.9 mM at 0 mM Na^+. Variations in Na^+ concentration were without effect on the V_{max}. At low concentrations of L-phenylalanine (0.5 and 1 mM) the tissue was able to concentrate the amino acid even in the absence of Na^+.

L-phenylalanine uptake was competitively inhibited by L-methionine (K_I=0.58 mM). This finding suggests that both amino acids might share a common transport system, being the affinity for L-methionine higher than for L-phenylalanine.

Studies on amino acids intestinal absorption in mollusks are very scarce. Active transport has been reported to occur in the intestine of polyplacophore *Cryptochiton stelleri* (7) and cephalopod *Eledone moschata* (17). There seems to be also an active transport of L-proline in *Helix pomatia*, although such a transport is lacking for L-lysine and L-glutamic acid (11). Furthermore, GERENCSER (5) found an amino acid-dependent increase in short circuit current and transmural potential difference in the intestine of the marine gasteropod *Aplysia californica*, when certain amino acids were added to the mucosal bathing solution. From the results, he estimated the apparent K_m for stimulation of short circuit current by mucosally added glycine, valine and alanine. Tryptophan, phenylalanine, proline and histidine also affected the intestinal electric parameters. Neither aspartic acid nor cysteine seemed to use a transport system.

Previous results from our laboratory (2, 3) have shown that *Helix (Cryptomphalus) aspersa* intestine actively transported monosaccharides. To further investigate the transport characteristics of snail intestine, the present work was designed to investigate the transport of L-phenylalanine.

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Materials and Methods

The technique used to study phenylalanine uptake has been described elsewhere (3). Briefly, four pieces of everted intestine of *Helix (Cryptomphalus) aspersa*, weighing about 20 mg altogether, were incubated at 30° C in 5 ml of physiological saline solution (2, 10) containing L-phenylalanine labelled with ^{14}C for 10 or 15 min. The incubation medium was continuously bubbled with 95 % O_2, 5 % CO_2 and shaked at 65 oscillations/min and 1.5 cm amplitude. At the end of the experiment, the pieces were washed by gentle shaking in ice-cold physiological solution, dried on wet filter paper and weighed together to determine their wet weight. The tissue was extracted for 24 h in 0.5 ml of 0.1 N HNO_3 at 4° C. Samples of 100 and 150 μl were taken respectively from the bathing solutions and from the extracts of the tissue for radioactivity counting in a liquid scintillation counter (Nuclear Chicago).

Results are expressed as net influx rates of L-prenylalanine in micromoles transferred to the tissue per gram of wet weight at the indicated time. In order to estimate the amino acid concentration reached in the total tissue water, the dry weight of the preparations was also determined.

L-(U-^{14}C)phenylalanine (513 mCi/mmol) was supplied by Radiochemical Centre Amersham.

Results

Kinetics of L-phenylalanine uptake. The concentration-dependent L-phenylalanine uptake into snail intestine, in control conditions (71.4 mM Na^+) and after 15 min incubation period, shows saturation kinetics. For all the assayed concentrations of L-phenylalanine, the concentration of the amino acid in the tissue water was higher than that in the bathing solution. These results reveal that snail intestine is able to actively transport L-phenylalanine.

Effect of varying concentrations of Na^+ on L-phenylalanine uptake into snail intestine. To find out if L-phenylalanine uptake was sodium-dependent, experiments at various concentrations of Na^+ in the incubation medium were carried out. For osmotic purpose the above cation was substituted by Tris in all cases.

The reduction in Na^+ concentration in the bathing solution from 71.4 mM to 15 mM does not significantly affect the net influx of L-phenylalanine into the tissue. However, the uptake of L-phenylalanine is significantly inhibited when the Na^+ concentration drops to 10 mM, and further impairment is observed at 0 mM Na^+, regardless of the amino acid concentrations used. Nevertheless, under these two experimental conditions, the transference of the amino acid from medium to tissue must include a process of mediated transport, since a saturation kinetics (fig. 1) is observed.

Furthermore, the absence of Na^+, does not prevent the amino acid from reaching an intra-tissue concentration superior to that of the incubation medium, at least at the lowest concentrations of L-phenylalanine (fig. 2).

Since the L-phenylalanine influx to the tissue may include a diffusion component besides the mediated transport, a study of its uptake in the presence of high concentrations of L-methionine, which in other tissues behaves as a competitive inhibitor of transport, was undertaken as well. With 50 mM L-methionine, the entry of L-phenylalanine into the tissue water is a lineal function of its concentration in the medium, which indicates that under these experimental conditions only the passive diffusion process is available for L-phenylalanine (fig. 1). This result must be attributed specifically to the presence of L-methionine and not to an increase in osmotic pressure in the

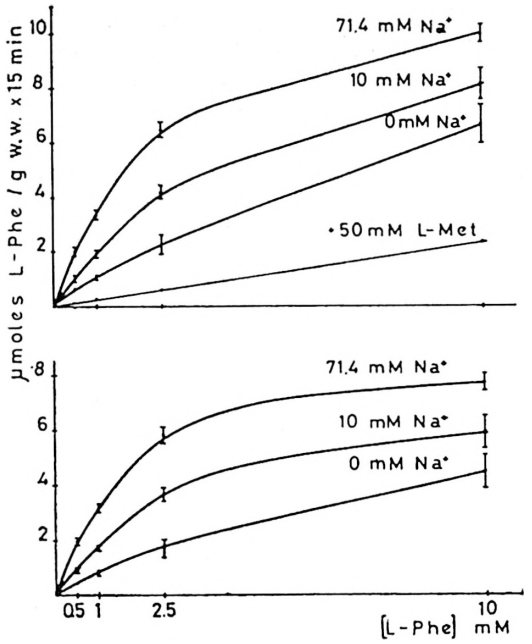

Fig. 1. *Effect of varying Na^+ concentrations on the kinetics of L-phenylalanine accumulation in snail intestine.*
Upper graph: total values of entry into the tissue. Lower graph: influx to the tissue by specific transport mechanisms.

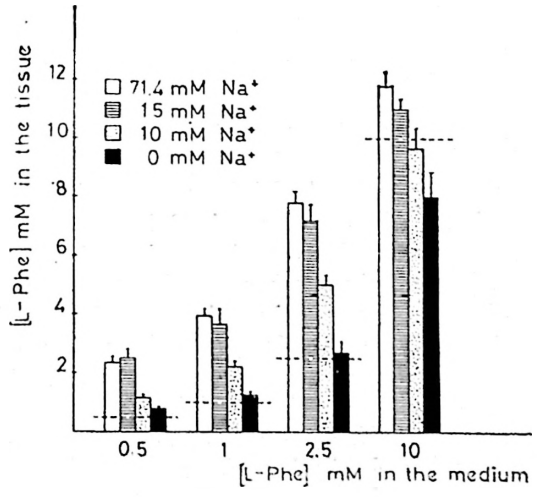

Fig. 2. *Effect of Na^+ on the capacity of intra tissue accumulation of L-phenylalanine.*

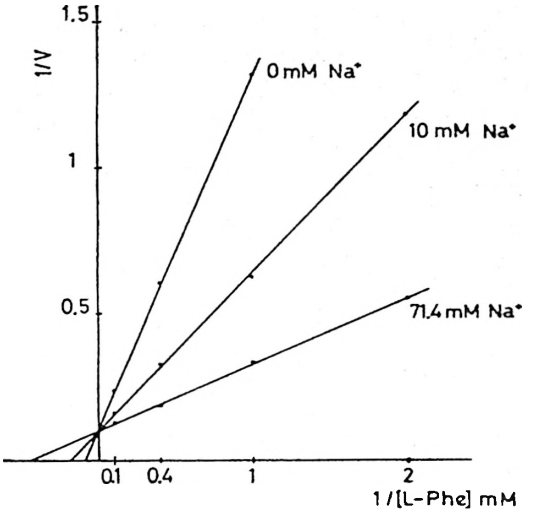

Fig. 3. *Influence of Na^+ on the kinetic parameters of L-phenylalanine transport.*

medium, since it is not observed with 50 mM D-mannitol.

By substracting the values for L-phenylalanine uptake found in the presence of 50 mM L-methionine (diffusion component) from those obtained in the absence of the inhibitor, the entry rates of the amino acid by a non-passive process of mediated transport will be obtained, at any Na^+ concentration (fig. 1).

The Lineweaver-Burk plot of the transport data, thus estimated (fig. 3), allows us to calculate the kinetic parameters of the transport system for L-phenylalanine under the specified conditions. It may be observed that a sodium concentration decrease in the medium does not affect the saturation rate of the process (V_{max} = 10 µmoles of L-Phe/g w.w./15 min), whereas below 15 mM Na^+, it causes increasing rises in the K_T, from 2.2 mM with 71.4 mM Na^+ to 9.9 mM in the absence of Na^+.

Competitive inhibition of L-phenylalanine influx by L-methionine. The effect of varying concentrations of L-methionine,

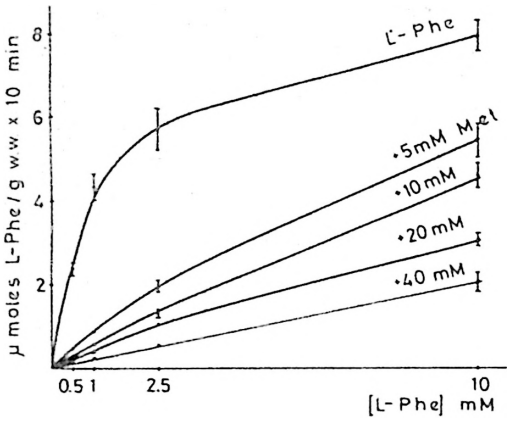

Fig. 4. *Effect of various L-methionine concentrations on the penetration rate of L-phenylalanine.*

on the uptake of L-phenylalanine into snail intestine, measured after 10 min incubation, is displayed in figure 4. L-phenylalanine uptake becomes all the more inhibited the greater the L-methionine concentration assayed is. With 40 mM methionine, the entry of L-phenylalanine into the tissue seems not to be mediated by a transport system but by a diffusion process (fig. 4).

The Lineweaver-Burk plot (fig. 5) of the rate of active uptake of L-phenylalanine (taking as transport rates the difference between the uptakes measured in the absence, i.e. total uptake, and in the presence of 40 mM L-methionine, i.e. diffusion component), shows that the saturation rate of L-phenylalanine transport (V_{max} = 7.14 µmoles L-Phe/g w.w./10 min) is unaffected by the presence of 5 mM methionine, while its apparent constant increases from K_T = 1.07 mM (absence of methionine) to K_T = 10.28 mM (with 5 mM methionine).

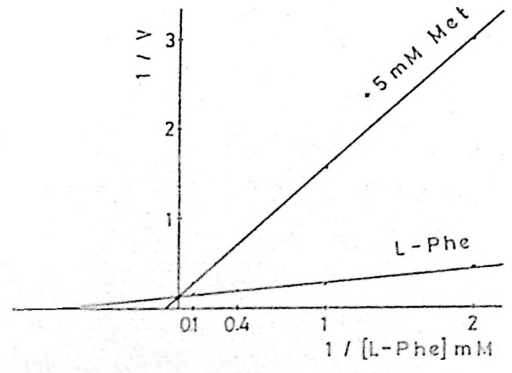

Fig. 5. *Competitive inhibition by L-methionine on intestinal transport of L-phenylalanine.*

The expresion $K_T' = K_T(1 + [I]/K_I)$ allows us to estimate the value of the inhibition constant for L-methionine, K_I, when its concentration, [I], is 5 mM. This value comes to about 0.58 mM.

Discussion

The present results show that snail intestine possesses the capacity to transport L-phenylalanine against a concentration gradient. The kinetic parameters of the transport system obtained after 15 min incubation at 30° C are 10 µmoles of L-Phe/g w.w/15 min for the V_{max} and 2.2 mM for the apparent K_T. In other experiments carried out at other times with 10 min incubation periods the obtained values were, V_{max} = 7.14 µmoles of L-Phe/g w.w/10 min and K_T = 1.07 mM. These values for K_T are very similar to those described by other autors in various species of mammals and under *in vivo techniques* (4, 8, 15, 16).

The reduction in Na^+ concentration in the incubation medium inhibits the rate of the L-phenylalanine influx into the tissue. Nevertheless, when the Na^+ concentration in the medium is 15 mM, the amino acid uptake does not differ significantly from the one corresponding to a normal medium (71.4 mM Na^+). Further decreases in Na^+ concentration are necessary for the inhibition to appear.

From a kinetic point of view, the inhibition brought about by suppression of Na^+ is accompanied by increases in the

transport constant, without altering the saturation rate of the process. The K_T rises from 2.2 mM to 5.9 and 9.9 mM when the Na^+ concentration in the medium is reduced from 71.4 to 10 and 0 mM respectively. Similar results have been found by other authors in various species of mammals (8, 14).

In the absence of Na^+, the tissue/incubation medium concentration ratio reaches values above 1 at concentrations of 0.5 mM (1.56) and 1 mM (1.24) L-phenylalanine. These values would be certainly increase if the data were corrected by the extracellular space. These findings indicate that some capacity for intra tissue accumulation against a concentration gradient still remains at 0 mM Na^+. If the intracellular accumulation of L-phenylalanine were only dependent upon the Na^+ gradient across the luminal membrane, those results would be hardly explainable even if a certain residual amount of the cation on the external side of the membrane is assumed to be present (9). Other explanations are possible, such as the coupling of the active transport of the amino acid with the unequal distribution of other ions or with an electrochemical gradient. GERENCSER (5) has measured in *Aplysia californica* intestine a transmural potential difference serosa negative, which becomes more pronunced on adding actively transported substrates to the mucosal side. He concluded that an active transport of Cl^- from mucosal to serosal could account for the transmural potential and that this Cl^- transport might be in some way related to those for sugars and amino acids (6).

The non-linearity between the inverses of the K_T for L-phenylalanine at the distinct Na^+ concentrations and the latter ones favors in each case the non-compulsory model for the formation of the amino acid-carrier-Na^+ ternary complex (1).

The experiments on transport inhibition of L-phenylalanine by various L-methionine concentrations show that the inhibitory effect is competitive. The addition of 5 mM L-methionine increases the K_T from 1.07 mM to 10.28 mM, without modifying the V_{max}. A common transport system for both neutral amino acids must, therefore, exist in snail intestine. The K_I for L-methionine is 0.58 mM (which provides an indirect measurement of its transport constant), lower than the K_T obtained for L-phenylalanine in the same experiments. This finding suggests that the postulated common carrier possesses greater affinity for L-methionine than for L-phenylalanine. The same phenomenon is reported for other species (4, 12, 13).

Resumen

El intestino de caracol presenta capacidad para transportar activamente L-fenilalanina. La disminución de Na^+ en el medio de incubación inhibe la penetración del aminoácido al tejido por mecanismos de transporte. Dicha inhibición se acompaña de aumentos en la constante de transporte, desde 2,2 mM (71,4 mM Na^+, medio normal) a 5,9 (10 mM Na^+) y 9,9 mM (0 mM Na^+), sin que se altere la velocidad de saturación del proceso. Persiste la capacidad de acumulación intratisular de L-fenilalanina en ausencia de Na^+, al menos con las concentraciones 0,5 y 1 mM del aminoácido.

La L-metionina inhibe competitivamente el transporte de L-fenilalanina (K_I = 0,58 mM). Puede deducirse que ambos aminoácidos utilizan el mismo sistema de transporte y que el transportador común posee mayor afinidad por la L-metionina que por la L-fenilalanina.

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