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Kinetics of the Mediated Transport and the Passive Net Flux of Phenylalanine Across the Rat Small Intestine *in vivo*

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The kinetics of L-phenylalanine absorption by rat jejunum, *in vivo*, has been studied with luminal perfusion (0.68 ml/min) during successive periods at different substrate concentrations.

The non-saturable passive component, measured by inhibiting the active transport with 60 mM methionine, was a linear function of the substrate concentration with an apparent mass-transfer coefficient of 1.42 nmoles/cm/min/mmoles/l.

The transport component, estimated from the difference between total absorption and the passive component, displays saturation kinetics with an apparent transport constant (Km) of 7.5 mM and maximal transport rate (Vmax) of 107 nmoles/cm/min.

Active transport seems to be the main component in absorbing phenylalanine proceeding from the digestion of food proteins.

The small intestine ability to actively transport amino acids from the mucosal to the serosal side has been shown in numerous *in vitro* studies (14). This transport across the brush border epithelial membrane follows saturation kinetics. There is evidence, however, that amino acids, under a favourable concentration gradient, also cross the digestive epithelium by a non-saturable passive diffusion process (2, 3, 13, 15). Intestinal absorption under physiological conditions may thus be explained by a combination of saturable and diffusion-like processes acting in parallel, whose relative importance will depend on the effective concentration of the food derived amino acids in jejunum and ileum, and their corresponding kinetic parameters.

The active and passive components for phenylalanine absorption in rat intestine *in vivo* have been estimated according to equations based on a model that takes both kinetics into consideration (2). On a wide range of concentrations (0-50 mM), the disappearance of the amino acid from the intestinal lumen was easily accounted for by a major non-saturable component and a minor saturable one of about 6 mM Km.

In this paper the kinetics of phenylalanine absorption *in vivo* by rat jejunum, is studied under a different approach. As well as the passive component for sugar absorption can be efficiently measured by blocking the active transport with phlorizin (4, 10, 11), the passive component of the phenylalanine absorption has been measured as the absorption remaining when the active transport becomes competitively inhibited by a high concentration of methionine.

Materials and Methods

Wistar rats male and female weighing between 100 and 250 g were used. Absorption was studied according to the *in vivo* technique of PONZ *et al.* (10). After a 20-24 h fast, the animals were anesthetized with urethane (125 mg/100 g). A segment 10-20 cm in length, from the proximal jejunum was single-pass perfused at a flow rate of 0.68 ml/min. The absorption periods were of 2 min. The perfusate (0.9% NaCl) contained L-phenylalanine (Merck) and L-U- ^{14}C-Phe (R.C. Amersham) at the desired concentrations.

Luminal disappearance of amino acid was taken to be equivalent to absorption. Radioactivity was measured by conventional liquid scintillation technique (LKB Model Counter Wallas 1215 RackBeta II).

The active transport of phenylalanine was blocked by adding its competitive inhibitor L-methionine (Merck), at 60 mM concentration (12).

Phenylalanine absorption was measured in the same animal both in the absence and presence of methionine, the latter value was taken as that of the passive diffusion component, and the difference between both conditions as the value for the active transport. From the mean values of the passive and non-passive components at different concentrations of substrate in a group of animals, the corresponding kinetic parameters were determined: the apparent Michaelis constant, Km, and the maximum rate, Vmax, for active transport, by the double reciprocal plot according to LINEWEAVER-BURK (7); and the apparent mass-transfer coefficient of the passive component, K_D, from the relation between passive absorption and substrate concentration. Linearity was checked by regression analysis and the respective constants were derived from the least squares fit.

Results

Steadiness of phenylalanine absorption along successive periods. Under continuous perfusion, the amino acid absorbed during periods of two minutes, at intervals of ten minutes, was measured. Phenylalanine concentration was 1 mM and 20 mM for the first and second hour respectively. The amino acid absorption along the successive periods was constant for each concentration (Figure 1).

Absorption as a function of substrate concentration. Phenylalanine was assayed at concentrations between 0.5 mM and 50 mM.

Figure 2 shows the absorption rate as a function of the initial phenylalanine con-

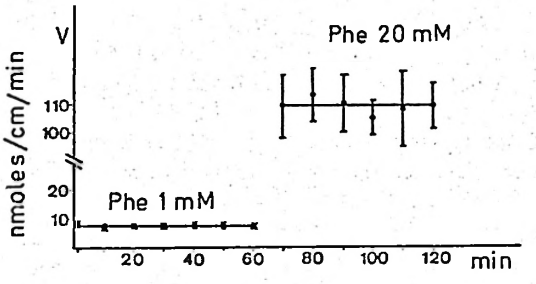

Fig. 1. *Steadiness of the phenylalanine intestinal absorption throughout a two hour period.* Absorption periods of two minutes. Each point is the mean ± S.E. of 2 determinations on each of 6 animals.

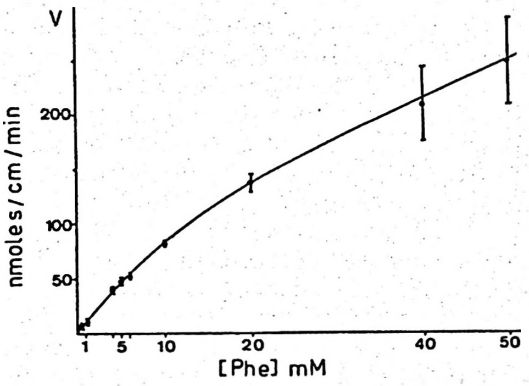

Fig. 2. *Rate of phenylalanine absorption at various concentrations in the intestinal lumen.*
Each point is the mean ± S. E. of 2 determinations on each of 4-6 animals.

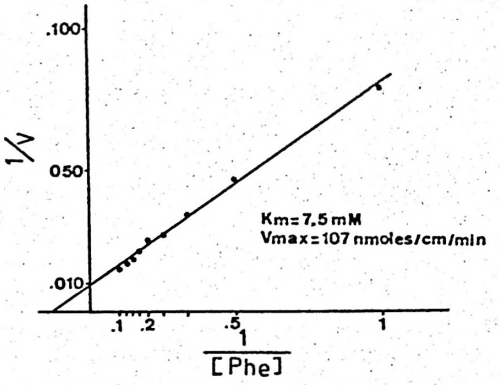

Fig. 4. *Double reciprocal plot for the active transport of phenylalanine.*

centration. The plot, which at low concentrations seems to correspond to a saturable process, becomes later almost a straight line, suggesting the existence of a saturable component and a non-saturable one.

Diffusion and active transport components. In one group of animals, phenylalanine absorption at 1, 2, 3, 4 and 5 mM concentrations both in absence and presence of 60 mM methionine was measured in successive periods (duplicated for each concentration) in the same jejunum segment. In another group the same experiment was carried out with 5, 6, 8 and 10 mM concentrations. Absorption in the presence of methionine was very small, so that even with a phenylalanine concentration of 10 mM, the change in concentration at efflux from the intestine was merely in the order of 4%.

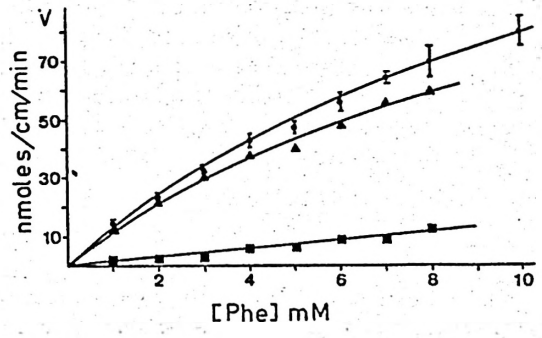

Fig. 3. *Kinetics for the* in vivo *intestinal absorption of phenylalanine.*
Each point is the mean of 2 determinations on each of 5 animals. (●) Total absorption, (▲) Active transport, (■) Diffusion (+ Met 60 mM).

Figure 3 shows the rate of phenylalanine absorption in the absence (total) and presence of methionine (diffusion), as well as that for the active transport (difference between total and diffusion), for concentrations from 1 to 10 mM. It may be seen that the saturable active component represents from 95 to 80% of the total absorption, while the passive one only accounts for 5 to 20% of the total. As could be expected, the relative importance of the saturable component decreases on increasing the substrate concentration.

The values for the diffusion component (absorption in the presence of 60 mM methionine), fit quite well those of a straight line ($r = 0.982$), whose slope, the mass transfer coefficient (K_D), has a value of 1.42 nmoles/cm/min/mmoles/l.

The double reciprocal plot (Figure 4) was obtained from the active transport values. The points fit quite well those of a straight line (r = 0.9977), allowing to calculate an apparent Km of 7.5 mM and a Vmax of 107 nmoles/cm/min.

Discussion

Phenylalanine absorption along successive periods under identical experimental conditions seems to be constant for at least two hours. Therefore, the adopted methodology permits highly comparative studies of the factors that may affect amino acid absorption as it happens with sugar absorption (10).

In the single-pass perfusion experiments with absorption periods of two minutes, the changes in fluid volume from net water movements were negligible and have not been taken into account.

The use of 60 mM methionine as inhibitor of phenylalanine transport has proven to be enough effective since the rate of the remaining phenylalanine absorption was a linear function of the substrate concentration (1-10 mM). With this range of concentrations, absorption depends mainly (95-80%) on a transport system that becomes competitively inhibited by methionine. On the other hand, the passive diffusion component (K_D = 1.42 nmoles/cm /min/nmoles/l) is not negligible (5-20%) and should be taken into account to determine the kinetic parameters of the active transport.

When the passive component is substracted, the apparent Km obtained (7.5 mM) is similar to that determined by ANTONIOLI *et al.* (2). These authors, however, attribute phenylalanine absorption basically to passive diffusion, due perhaps to the concentrations of up to 50 mM used in their work.

Other references on *in vivo* Km had yielded higher values. For PÉNZES *et al.* (9) in rat, values ranged between 10 and 42 mM, however, they had disregarded the passive diffusion component. WINNE *et al.* (15), also in rat, after correction of the diffusion component, obtained a Km of 17 mM, as their lowest value, but they measured absorption as the appearance of the substrate in the venous blood.

In vitro, the reported Km values for phenylalanine in rat intestine are smaller: 3.3 mM (5) and 1.4 mM (6). The difference with the values from the present work *in vivo* may be attributed to the greater thickness of unstirred layers (15), especially on account of the low perfusion rate used (8).

These results suggest that the intestinal absorption of the phenylalanine from digested proteins takes place through active transport as well as through passive diffusion. The importance of either component depends mostly on the free amino acid concentration reached in the small intestine lumen. Results from ADIBI *et al.* (1), in man, indicated that the phenylalanine concentration at the intestinal lumen after a protein rich meal was 1.76 mM, quite higher than that inside the enterocyte and much higher than that in plasma, i.e. with a favourable gradient for passive absorption. Nevertheless, even under conditions of favourable gradient, the active transport, in agreement with the kinetic parameters obtained, is likely to be the main component for the absorption of phenylalanine from digestion of food proteins.

Resumen

Se estudia en yeyuno de rata, *in vivo*, con perfusión luminal (0,68 ml/min) durante períodos sucesivos, la cinética de la absorción intestinal de la L-fenilalanina.

El componente pasivo, no saturable, se mide por inhibición del transporte activo con metionina 60 mM, y resulta ser función lineal de la concentración del sustrato con una constante de transferencia de masa, K_D = 1,42 nmoles/cm/min/mmoles/l.

El componente de transporte, calculado por dife-

rencia entre la absorción total y el componente pasivo, exhibe una cinética de saturación con una constante de transporte aparente, Km = 7,5 mM y una velocidad máxima, Vmax = 107 nmoles/cm /min.

De acuerdo con los parámetros cinéticos obtenidos es probable que el transporte activo sea el principal componente para la absorción de fenilalanina derivada de la digestión de proteínas de los alimentos.

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