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ELECTRICAL PROPERTIES OF A Na^+-DEPENDENT PHENYLALANINE TRANSPORT IN LIZARD (*LACERTA GALLOTI*) DUODENUM

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Abstract—1. The unidirectional transepithelial fluxes of L-phenylalanine across lizard duodenum were determined in flux chambers.

2. Phenylalanine was preferentially transferred from the mucosal to the serosal fluid.

3. This transport was accompanied by an accumulation of substrate from the mucosal medium into the tissue to a similar level and against a concentration gradient.

4. There was no net movement of phenylalanine when the sodium was substituted by choline.

5. The influx of L-phenylalanine into the epithalial cells of lizard duodenum was examined by incubating slices of intestine in radioactively-labelled solutions of the substrate for 2 min.

6. The steady-state uptake was assessed after similar incubations lasting 45 min.

7. Phenylalanine influx obeys the Michaelis–Menten equation with a K_m of 5.1 and is dependent on the presence of sodium ions in the incubation medium.

8. Phenylalanine has been used to induce changes in short-circuit current (ΔI_{sc}) across intestine. ΔI_{sc} was a hyperbolic function of amino acid concentration characterized by the parameters J_m (maximum change in ΔI_{sc}) and K_m (concentration needed to attain an ΔI_{sc} equal to half the J_m).

9. ΔI_{sc} determined K_m constants showed good agreement with values obtained from direct measurements of phenylalanine uptake into tissue.

INTRODUCTION

Amino acid transport across intestinal mucosa has been amply studied in several species of mammals and several kinetic models have been proposed to explain the transport mechanisms (Wiseman, 1968; Curran *et al.*, 1967; Preston *et al.*, 1974; Sepúlveda and Smith, 1978; Paterson *et al.*, 1979, 1980a; Alvarado and Lerminier, 1982). Nevertheless, studies on amino acid intestinal absorption in reptiles are scarce. Everted intestinal sacks have been used to investigate the active and passive transport of single amino acid in the lizard *Uromastyx hardwickii* (Quadri *et al.*, 1970). The isolated intestinal epithelium of the tortoise (*Testudo hermanni*) actively transports glycine, alanine and histidine (Baillien, 1969) whilst glutamic acid is passively transported.

Several studies performed in mammalian intestine have reported that changes induced in bioelectric parameters by amino acid addition to incubation medium can be used to determine the transport of neutral amino acids across intestinal tissue (Schultz and Zalusky, 1965; Kohn *et al.*, 1968; Syme and Levin, 1977). The influence of amino acids on transmural potential difference and short-circuit current have also been studied in some reptilian intestines. The addition of actively-transported amino acids to the mucosal bath greatly increases potential difference, whereas the addition of passively transported amino acids does not have this effect (Baillien and Schoffeniels, 1962). Similar changes are also observed after adding D-glucose (Gilles-Baillien and Schoffeniels, 1965).

For the present survey, the intestine of the lizard *Lacerta galloti* was chosen, being a species with which we already have experience in studies on glucose transport (Lorenzo *et al.*, 1982). Our aim was to examine certain salient features of phenylalanine transport *in vitro* and to compare them with the known properties of those systems in mammals and other reptiles.

MATERIALS AND METHODS

Lizards (*Lacerta galloti*) were captured at Hoya Fría (Tenerife) and kept in a terrarium until they were required. Under ether anesthesia, the duodenum was removed and placed in ice-cold bathing solution gassed with 5% CO_2 in O_2. The Ringer solution with which intestines were bathed had the following composition: NaCl 107 mM; $NaHCO_3$ 25 mM; Na_2HPO_4 0.2 mM; Na_2HPO_4 1.8 mM; KCl 4.5 mM; $CaCl_2$ 1.25 mM; $MgSO_4$ 1 mM (pH 7.2).

Unidirectional transmural fluxes of phenylalanine under open circuit conditions were measured in a flux chamber with an aperture of 0.21 cm^2, as described in detail elsewhere (Bolaños *et al.*, 1984). The tissues were bathed on each side with Ringer at 30°C circulating through the chamber. ^{14}C-L-phenylalanine isotopes were added to one face of the tissue and their rate of appearance in the opposing solution was assessed by taking samples of 20 μl at regular 20 min intervals for 1 hr. Samples were taken in duplicate to avoid errors, the same volume being replaced with unmarked Ringer solution. At the end of the experiment, the tissue within the aperture was excised, rinsed, blotted and cut into sections which were weighed, dissolved in 30% KOH and counted to determine the level of the substrate in the intestine. The unidirectional fluxes were determined from the standard equations (Schultz and Zalusky, 1964).

The uptake by intestinal slices was determined by incubating them at 30°C in a solution of the required concentratioin of ^{14}C-L-phenylalanine in the appropriate buffer using standard techniques (Cartier *et al.*, 1979). Following the incubation, the tissues were rinsed with ice-cold isotonic

mannitol solution, blotted, weighed and dissolved separately in 0.1 ml 30% KOH solution for counting in a liquid scintillation spectrometer. The uptake was calculated by comparison of the radioactivity in the slice with the specific activity of the incubation medium counted under identical conditions. The appropriate sodium concentration was obtained by replacing the required amount with equimolar quantities of choline. For the assessment of the initial rate of entry, the incubations were performed for 2 min, while for the determination of the equilibrium uptake they lasted for 45 min. For the kinetic study of the initial rate of influx, an estimate of the extracellular space of the preparation was required; this was obtained by determining the entry of ^{14}C-polyethylene glycol (PEG, mol. wt 4000) into parallel samples incubated under identical conditions. The apparent Michaelis constant, K_m, and the maximum transport rate, V_m, were deduced from the Lineweaver–Burk plot. In order to evaluate the passive transport component, 60 mM of methionine was added to the incubation medium.

For determining concentration-dependent, amino acid-induced current, lizard duodenum was mounted in a Ussing chamber. All tissues were bathed in Ringer solution gassed with 95% O_2 + 5% CO_2 at 30°C for continuous recording of short-circuit current. Small volumes of amino acid-containing solutions were added to solutions bathing the mucosal surface of each preparation; increases in amino acid-induced current being recorded as described previously (Bolaños *et al.*, 1984). The final concentration of amino acid in the musocal medium varied from 0.2 to 30 mM. Choline was used to substitute Na^+ partially, in both mucosal and serosal solutions of the intestine.

RESULTS

Transmural fluxes

The unidirectional fluxes of L-phenylalanine (1 mM) are reported in Table 1. In standard conditions, a relevant active transfer of amino acid takes place across the duodenum. The absence of sodium modified the amino acid transport in both directions and the net flux was drastically reduced.

When the level of labelled phenylalanine in tissue was examined at the end of the experiment, it could be seen that a concentration gradient between the tissue and the bathing medium was only established when the amino acid was present in the mucosal medium (Table 2). When sodium ions were absent no accumulation occurred.

Accumulation and influx

The accumulation of phenylalanine in slices of lizard intestine during incubation of 45 min is strongly inhibited by the lack of sodium in the incubation medium (Table 3). The initial rate of influx (2 min) is greatly depressed in the absence of sodium.

Table 1. Unidirectional transepithelial fluxes

	J^{Phe}_{m-s} (1 mM)	J^{Phe}_{s-m}	$J^{Phe}_{net.}$ (nmol/cm^2 hr)
Control (10)	246.7 ± 12.8	4.46 ± 0.52	243.3 ± 12.7
Na^+ free (8)	30.61 ± 3.9*	14.61 ± 1.7*	16.0 ± 4.2*

Lizard intestine was mounted in a flux chamber and bathed with Ringer solution. ^{14}C-L-phenylalanine (1 mM) was added to one face of the tissue and the rate of the appearance of the isotope in the opposite solution was monitored. The results are the means ± SEM of the number of the different animals given in parentheses. *Significant difference with the control series according to the *t*-test. $P < 0.005$.

Table 2. Phenylalanine in tissue (μmol/ml tissue water)

	Substrate in mucosal medium	Substrate in serosal medium
Control (5)	1.3541 ± 0.07	0.3092 ± 0.02
Na^+ free (5)	0.3620 ± 0.03*	0.2234 ± 0.01*

After determining unidirectional transepithelial fluxes, at the end of the experiments the level of ^{14}C-L-phenylalanine in tissue was assessed and expressed in μmol/ml on the assumption of 80% tissue water. The results are the means ± SEM of the number of the different animals given in parentheses. *Significant difference with the control values according to the *t*-test.

Table 3. Phenylalanine uptake (μmol/g fresh tissue)

	2 min	45 min
Control (27)	0.5487 ± 0.04	0.9320 ± 0.04
Na^+ free (10)	0.2106 ± 0.005*	0.4322 ± 0.03*

Uptake of phenylalanine by slices of lizard duodenum. Tissue slices were incubated in Ringer solutions (or Na^+ free) containing 1 mM ^{14}C-L-phenylalanine for 2 min. The results are the means ± SEM of the number of the experiments given in parentheses. *Significant difference with the control values according to the *t*-test.

The kinetics of phenylalanine influx were explored by incubating tissue samples for 2 min in Ringer solution (or Na^+ free). Phenylalanine was assayed at concentrations between 1 and 30 mM. Figure 1 shows the absorption rate as a function of the initial phenylalanine concentration. The plot 1a, which at low concentrations seems to correspond to a saturable process, later becomes almost a straight line, suggesting the existence of a saturable component and a non-saturable one. In a group of animals, phenylalanine uptakes were evaluated in the presence of 60 mM of methionine. Figure 1b shows the rate of the absorption total and in the presence of methionine. Non-diffusive components were evaluated as the difference between total absorption and diffusion (+60 mM met.). Passive component was 27% of the total.

The K_m value of the phenylalanine transport calculated from uptake measurement was 5.5 m and $V_m = 0.07$ μeq/g 2 min.

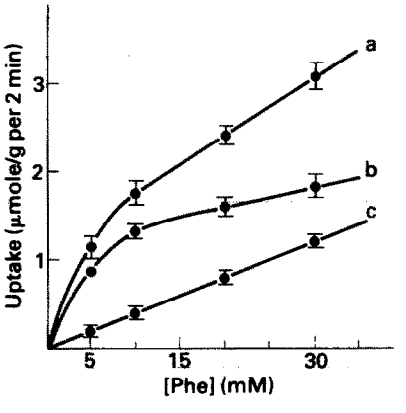

Fig. 1. Kinetics for the intestinal absorption of L-phenylalanine. (a) Total absorption. (b) Non-diffusive component. (c) Diffusion (+60 mM methionine).

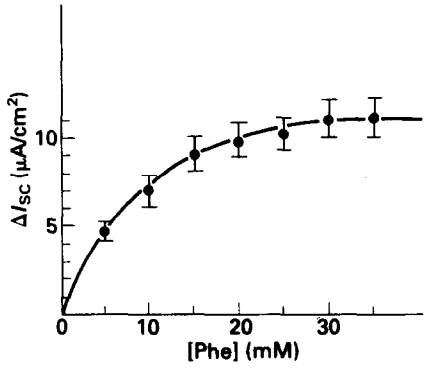

Fig. 2. Concentration dependence of L-phenylalanine-induced current in lizard duodenum in presence of 136 mM Na^+.

Use of ΔI_{sc} *as a measure of* L-*phenylalanine entry into tissue*

Small increases in the amounts of phenylalanine added to the mucosal side of the preparation of duodenum mounted in an Ussing chamber caused a pronounced rise in the induced current. Increasing the concentration of amino acid to above 30 mM led to a progressive saturation of the response (Fig. 2). This shows saturation kinetics similar to those obtained from uptakes of phenylalanine into tissue (Fig. 1a).

The Lineweaver–Burk plot of the transport data from amino acid-induced current allows us to calculate the kinetic parameters of the transport system for phenylalanine. The influence of Na^+ on these parameters is shown in Fig. 3. Increasing Na^+ concentration has no consistent effect on V_m, whilst K_m decreases as sodium concentration increases: 5.5, 14.5, 23.1 and 48.2 mM for 100, 75, 50 and 25%, respectively.

DISCUSSION

The present work provides an analysis of the kinetics of amino acid transport in the lizard intestine and the first direct comparison between chemically and electrically determined K_m and V_m constants.

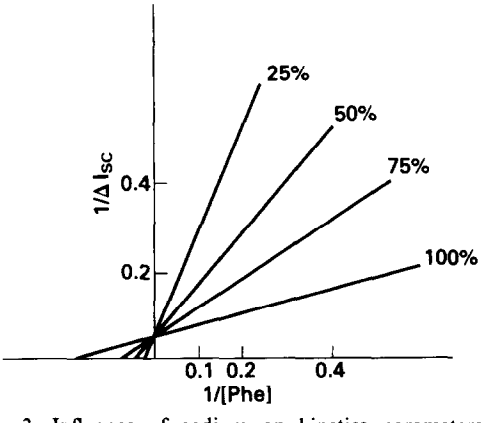

Fig. 3. Influence of sodium on kinetics parameters of L-phenylalanine transport obtained from the I_{sc} and expressed in μeq/g 2 min.

Results shown in Table 1 reveal that lizard duodenum possesses the capacity to transport L-phenylalanine against a concentration gradient and this transport is fully dependent on sodium in the incubation medium. The transport observed in the absence of sodium may be caused by the presence of the sodium ions in the tissue itself and not because phenylalanine transport may take place independently of sodium. This hypothesis has been suggested by several authors for the mammalian intestine (Alvarado *et al.*, 1982).

Another salient assumption is that phenylalanine influx into the tissue (Fig. 1) constitutes the sum of a diffusive component and a single saturable system, as was also found in mammalian intestines (Robinson *et al.*, 1980; Schultz and Curran, 1970; Torre and Ponz, 1983). Influx and accumulation are markedly dependent on the presence of external sodium ions. The kinetic parameters of the transport system obtained after 2 min incubation at 30°C are 1.59 μmol of phenylalanine/g wet wt × 2 min for the V_m and 5 mM for the apparent K_m. In other experiments carried out at other times with 5 min incubation periods the values obtained were similar. These values for K_m are very similar to those described for guinea pigs and dogs (Cartier *et al.*, 1978). Values reported for K_m in the rat intestine *in vitro* (Finch and Hird, 1960; Larsen *et al.*, 1964) are smaller than those obtained by us, suggesting that in the lizard intestine the affinity for the phenylalanine carrier is smaller than in the rat intestine.

A model used to describe amino acid entry on the Na^+-dependent high-affinity system supposes the amino acid to bind first followed by Na^+, subsequent entry of amino acid and Na^+ taking place with a stoichiometry of 1 (Paterson *et al.*, 1980b). The apparent K_m of amino acid for this system, its dependence on the Na^+ ion and the dissociation constant of the amino acid from its carrier site (KD) can be determined from measurements of I_{sc}.

The K_m value of phenylalanine for the Na^+-dependent high-affinity carrier calculated from ΔI_{sc} measurements using 136 mM Na^+ was 5.5 mM. This corresponds to values of 5.1 mM determined from direct measurements. The agreements found between these two different sets of measurements was generally good, providing further independent evidence for the presence of a high-affinity low-capacity component to neutral amino acid uptake by lizard duodenum. These observations agree with those described for rabbit ileum.

It is concluded from these experiments that the ΔI_{sc} method can be used to study the kinetic characteristics of amino acid uptake into lizard duodenum.

Using this method we have verified that changing the sodium concentration affects the K_m for phenylalanine influx, leaving the V_m unchanged. This is equivalent to saying that the permeability coefficients of all forms of the carrier are the same; this has been confirmed in the mammalian intestine using uptakes (Alvarado and Lerminier, 1982; Torre and Ponz, 1983).

From these results we deduce that the properties of phenylalanine transport in the lizard intestine are similar to those described for the other amino acids in mammalian intestines (Schultz and Curran, 1970;

Alvarado and Mahmood, 1974). On the other hand, these results agree with those from the tortoise intestine (*Testudo graeca*) where it is shown that the intestine of the turtle, like those of the rabbit and other mammals (Hajjar *et al.*, 1972) has a Na^+-dependent amino acid transport system in the membrane of the brush border; consequently, the active step of transport must be located at the mucosal membrane.

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