

AMINO ACIDS AND CARBOHYDRATES ABSORPTION BY Na⁺-DEPENDENT TRANSPORTERS IN THE PYLORIC CECA OF *Hoplias malabaricus* (ERYTHRINIDAE)

ABSORÇÃO DE AMINOÁCIDOS E CARBOIDRATOS POR TRANSPORTADORES Na⁺-DEPENDENTES NOS CECOS PILÓRICOS DA TRAÍRA *Hoplias malabaricus* (ERYTHRINIDAE)

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SUMMARY

Information about amino acids and carbohydrate absorption in fish is important to formulate an adequate diet to obtain optimal growth. Therefore, the objective of this study was to investigate if Na⁺-dependent transporters are involved on the absorption of glycine, L-glutamine, L-leucine, L-lysine, L-proline, L-alanine, and the carbohydrates fructose and glucose in the pyloric ceca of *Hoplias malabaricus*. The pyloric ceca were mounted in a system of continuous perfusion "in vitro". Amino acids and carbohydrates were placed on the mucosal side at concentrations of 10, 20, and 40mM. The serosal side of the pyloric ceca was positive in relation to the mucosal side. The addition of glycine, L-glutamine, L-leucine, L-lysine, L-proline (all tested concentrations), and glucose (at concentrations of 20 and 40mM) increased the positivity of the serosal side, indicating the presence of Na⁺-dependent transporters in the absorption of these substances. L-alanine and fructose did not change the positivity of the serosal side. The pyloric ceca seem to be the main site of nutrient absorption in the digestive tract of *H. malabaricus*.

Key words: *Hoplias malabaricus*, amino acids, carbohydrates, intestinal absorption, Na⁺-dependent transporters, pyloric ceca.

RESUMO

O conhecimento dos mecanismos de absorção de aminoácidos e carboidratos em peixes é importante para se formular uma ração adequada à obtenção de um crescimento ótimo. Portanto, o presente estudo teve por objetivo determinar se transportadores Na⁺-dependentes estão envolvidos na absorção de glicina, L-glutamina, L-leucina, L-lisina, L-prolina, L-alanina e dos carboidratos frutose e glicose nos cecos pilóricos de *Hoplias malabaricus*. Os cecos pilóricos foram montados em

um sistema de perfusão contínua "in vitro". Aminoácidos e carboidratos foram adicionados ao lado mucoso dos cecos nas concentrações de 10, 20 e 40mM. O lado seroso dos cecos pilóricos apresentou-se positivo em relação à mucosa. A adição de glicina, L-glutamina, L-leucina, L-lisina, L-prolina (em todas as concentrações utilizadas), e glicose (nas concentrações de 10 e 20mM) aumentou a positividade do lado seroso, indicando a existência de transportadores Na⁺-dependentes para a absorção dessas substâncias. L-alanina e frutose não modificaram a positividade da serosa. Com base nos resultados obtidos, os cecos pilóricos parecem ser o principal sítio de absorção de nutrientes no trato digestivo de *H. malabaricus*.

Palavras-chave: *Hoplias malabaricus*, aminoácidos, carboidratos, absorção intestinal, transportadores Na⁺-dependentes, cecos pilóricos.

INTRODUCTION

The pyloric ceca are digitiforms expansions situated in the proximal intestine, caudal to the pyloric sphincter. Anatomical evidence suggests that the pyloric ceca are an adaptation formed in order to increase the area of the proximal intestine, being more developed in carnivorous fishes and reduced, or even absent, in herbivorous fishes (BUDDINGTON & DIAMOND, 1987). The histological composition of the ceca is nearly identical to that of the anterior intestine, suggesting that digestive function and peristaltic capabilities are very similar in both sections (WILLIAMS &

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NICKOL, 1984). There are variations in number and in anatomic microscopic characteristics of the pyloric ceca of different species (GENTILE *et al.*, 1989). Variations in length and number of pyloric ceca could also be observed in fishes of the same species and with similar size (BERGOT *et al.*, 1975). The pyloric ceca, which are responsible for a greater part of the digestion of lipids and proteins, receive pancreatic and hepatic secretions (SIRE *et al.*, 1981; DIMES *et al.*, 1994), and participate of the absorption of amino acids, carbohydrates (BUDDINGTON & DIAMOND, 1987), Na^+ , and water (BOGÉ *et al.*, 1988; KERSTETTER & WHITE, 1994).

The amino acids are transported across the brush border membrane of the intestine by Na^+ -dependent transporters, Na^+ -independent transporters and a carrier-independent, non saturable pathway (apparently diffusion) (VILELLA *et al.*, 1990). In the European eel, *Anguilla anguilla*, transporters for neutral, imino and basic amino acids have been described (VILELLA *et al.*, 1988, 1989, 1990). The absorption of carbohydrates in the intestine of fishes is also Na^+ -dependent. Consequently, the partial transfer of amino acids and carbohydrates towards the intracellular medium is energized by the movement of Na^+ down its electrochemical potential gradient (FERRARIS & AHEARN, 1984). This flow of Na^+ alters the transepithelial potential difference (TPD) of the intestine, and the measurement of TPD changes can give an idea of the transport of amino acids and carbohydrates by Na^+ -dependent transporters.

Since the transport of amino acids and carbohydrates in the pyloric ceca of fishes was studied in a few teleost species (BUDDINGTON & DIAMOND, 1987), the aim of this study was to determine if Na^+ -dependent transporters are involved on the absorption of amino acids and carbohydrates in the pyloric ceca of a carnivorous teleost, *Hoplias malabaricus*.

MATERIAL AND METHODS

Specimens of *H. malabaricus* (Erithrynidae) (250-600g) of both sexes were captured by nets placed in ponds situated near the town of Silveira Martins (Southern Brazil). Fishes were maintained in a continuously aerated 500L freshwater tank using a water reuse system (22°C), with aeration and without food for at least three days before experimentation. The fishes were killed by section of the spinal cord and the abdominal cavity was opened to expose the pyloric ceca. This organ was separated and cleaned with control

physiological solution (mM): 120.0 NaCl, 3.0 CaCl_2 , 5.5 KCl, 1.45 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10.0 NaHCO_3 and 2.5 glucose, adjusted at pH 7.8 with HCl. The pyloric ceca were then mounted in a system of continuous perfusion similar to that described by ANDO & KOBAYASHI (1978), with a flow of 0.2ml/min through the organ, and gassed with air. TPD (expressed in mV with the polarity of the serosal relative to mucosal side) was measured in an open circuit for periods of 10min with a digital multimeter (impedance input of 10M Ω) plugged to a pair of calomel electrodes connected to the external and the internal solutions of the pyloric ceca by 3% agar-saline bridges. This system was first mounted without the pyloric ceca and dismantled when the potential difference between the calomel electrodes was more than 0.1mV.

Control experiments: these experiments were made with the pyloric ceca mounted in the system of continuous perfusion only with control physiological solution. The TPD was measured during intervals of 20min during 7h to verify if the preparation presented some variation of the TPD as a function of time.

Experiments with amino acids and carbohydrates: Measurements of the TPD were made with the control physiological solution until stabilization of the TPD of the pyloric ceca. After stabilization the amino acids L-alanine (Merck), glycine (Synth), L-lisine, L-glutamine, L-leucine, and L-proline (Nuclear) and the carbohydrates fructose and glucose (Merck) were added to mucosal side. Mannitol (Nuclear) was added to serosal side to maintain the osmolarity of the solutions. TPD was recorded for at least 40min, with intervals of 10min until stabilization. TPD values were used for statistical analysis by one-way analysis of variance and Duncan test using the SPSS program (version 1986) and to design the curve-fitting graph (Slide Write Plus, Advanced Graphics Software, Inc.). Data were expressed as mean \pm SE, and minimum significant level was $P < 0.05$.

RESULTS

When the pyloric ceca were mounted in the perfusion system and bathed on both sides with physiological solution the serosa was positive relative to the mucosa. The TPD attained a steady state in 30-40min, with a mean $0.9 \pm 0.05\text{mV}$ ($n=30$). After stabilization there was no significant changes in the TPD through the 7h of control experiments.

Addition of L-lysine, glycine, L-leucine, L-proline, and L-glutamine increased the positivity

of the serosal side in all concentrations examined (Figure 1 and 2). Addition of glucose (concentrations of 20 and 40mM) also increased the positivity of the serosal side (Figure 3). There was a significant correlation between the TPD and the concentration of these amino acids and glucose, which could be described by the equations in the figure legends. L-alanine (Figure 2) and D-fructose (Figure 3) did not change the positivity of the serosa throughout 40min of experiment.

DISCUSSION

The species studied (*H. malabaricus*) presented the TPD of the pyloric ceca similar to that of the intestine of typical freshwater fishes, in which the serosal side is positive in relation to the mucosal side (FERRARIS & AHEARN, 1984). The medium intestine and rectum of this species also have the same pattern (serosa positive) (PAVANATO *et al.*, 1996). Probably the increase of positivity of the serosal side of the pyloric ceca of *H. malabaricus* by the addition of L-proline, L-lysine, L-leucine, L-glutamine, and glycine is due to a flow of positive charges from the mucosal to the serosal side because

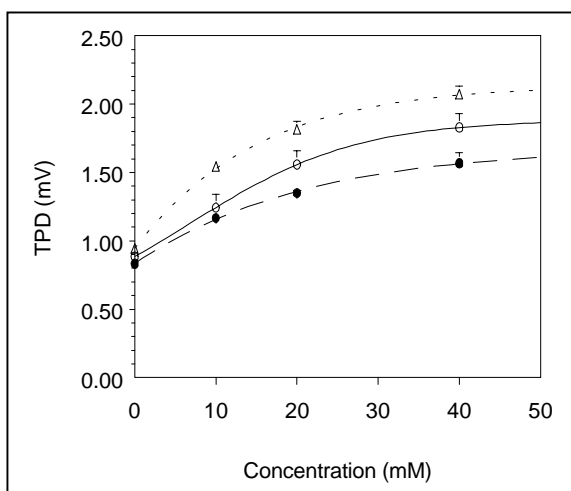


Figure 1 - Effect of L-lysine (○), glycine (●), and L-leucine (Δ) addition on transepithelial potential difference (TPD) of the pyloric ceca of *Hoplias malabaricus*. Values obtained after stabilization. The following equations were fitted to the data:

$$(\circ) y = \frac{0.39 + 1.49}{1 + e^{-\left(\frac{x-7.14}{10.13}\right)}} \quad r^2 = 0.99$$

$$(\bullet) y = \frac{-5.08 + 6.76}{1 + e^{-\left(\frac{x-36.6}{18.7}\right)}} \quad r^2 = 0.99$$

$$(\Delta) y = \frac{-10.1 + 12.2}{1 + e^{-\left(\frac{x-31}{13.9}\right)}} \quad r^2 = 0.99$$

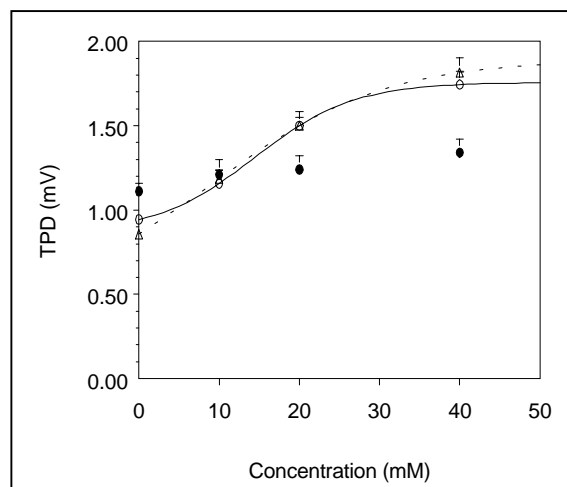


Figure 2 - Effect of L-proline (○), L-alanine (●), and L-glutamine (Δ) addition on transepithelial potential difference (TPD) of the pyloric ceca of *Hoplias malabaricus*. Values obtained after stabilization. The following equations were fitted to the data:

$$(\circ) y = \frac{0.86 + 0.89}{1 + e^{-\left(\frac{x-14.4}{6.15}\right)}} \quad r^2 = 0.99$$

$$(\bullet) y = \frac{0.4 + 1.49}{1 + e^{-\left(\frac{x-8.8}{10.8}\right)}} \quad r^2 = 1.0$$

of the cotransport of Na^+ with the amino acids. Addition of arginine and L-alanine (but not L-lysine, L-glutamine, glycine, and L-proline) at the mucosal side also altered significantly TPD of the medium intestine of *H. malabaricus* (VIEIRA, 1996). Flow of Na^+ toward serosal side in response to the addition of amino acids was also observed in several teleosts species, increasing the positivity of the serosal side (FERRARIS & AHEARN, 1984; VILELLA *et al.*, 1988; BOGÉ & PÉRES, 1990).

The fact that L-alanine did not change the TPD of pyloric ceca of *H. malabaricus* does not indicate that there is no absorption of this substance in this organ. This amino acid may be absorbed in the pyloric ceca by a non-electrogenic mechanism (diffusion, for example), and consequently do not alter the TPD.

The uptake of glucose into intestinal epithelial cells of teleosts is also a Na^+ -dependent symport (FERRARIS & AHEARN, 1984; BOGÉ & PÉRES, 1990) that uses the electrochemical gradient mucosal side - intracellular medium for Na^+ created by the Na^+ - K^+ ATPase to induce the transport of this carbohydrate against a concentration gradient (AHEARN *et al.*, 1992). Therefore, the increase of the positivity of the serosal side of the pyloric ceca of *H. malabaricus* after addition of glucose may

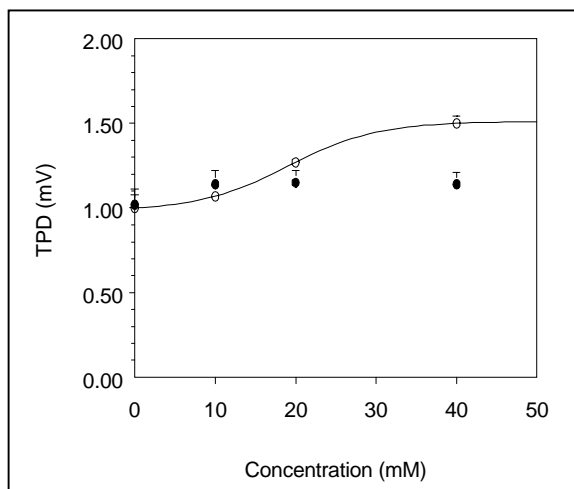


Figure 3 - Effect of glucose (○) and D-fructose (●) addition on transepithelial potential difference (TPD) of the pyloric caeca of *Hoplias malabaricus*. Values obtained after stabilization.

$$y = \frac{0.98 + 0.53}{1 + e^{-\left(\frac{x-19.05}{5.56}\right)}} \quad r^2 = 1.0$$

also be due to the increase of Na^+ absorption. In some carnivorous teleosts like *Pseudopleuronectes americanus* intestinal glucose transport was very low and did not change the TPD (THOMPSON & KLEINZELLER, 1985). However, in spite of the fact that *H. malabaricus* is a carnivorous species, the addition of glucose (but not fructose) changed the TPD of the pyloric caeca, indicating that there is glucose absorption in this part of the digestive tract. Fructose probably is absorbed by a Na^+ - independent mechanism or occurs in other part of the gut.

Experiments with the pyloric caeca of some teleosts species (*Salmo gairdneri*, *Micropterus salmoides*, *Morone saxatilis*, and *Gadus morhua*) indicated that nutrient uptakes in this organ are similar to those in proximal intestine. The pyloric caeca have been considered by some authors as an adaptation to increase the effective surface area of the proximal intestine (BUDDINGTON & DIAMOND, 1987). Since the addition of several amino acids on the mucosal side changed significantly the TPD of the pyloric caeca of *H. malabaricus* but not that of the medium intestine (VIEIRA, 1996), it is possible to speculate that the pyloric caeca are the main site of nutrient absorption in this species. Additional experiments with radioactive amino acids must be done to confirm this hypothesis. Moreover, this study also confirmed that

the pyloric caeca of *H. malabaricus* absorb L-proline, L-lysine, L-leucine, L-glutamine, glycine, and glucose. Therefore, the development of commercial food for this species can include these substances.

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