Biochimica et Biophysica Acta, 373 (1974) 523—526

BBA Report

BBA 71215

INTESTINAL ABSORPTION OF PEPTIDES
PEPTIDE UPTAKE BY SMALL INTESTINE OF *RANA PIPIENS*

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(Received October 7th, 1974)

Summary

The absorption of glycyl-L-leucine is studied using the small intestine of *Rana pipiens* perfused through the vascular bed. It is found that the transfer into the vascular bed of glycine and leucine from the peptide in the lumen occurs in part by a process independent from those for the transfer of the free amino acids. This process is particularly effective for the transfer of glycine.

In various mammalian species, amino N can be taken up from the lumen of the small intestine not only in the form of free amino acids, but also as small peptides. Peptides taken up in this way are hydrolysed to the constituent amino acids which are then transferred into the extracellular fluids including the circulation of the intestinal wall. Some peptides are hydrolysed by enzymes located in the luminal facing brush border membrane of the absorbing cells; others appear to be transported intact across this membrane to be hydrolysed by enzymes located within the cells [1—2]. Because of the fact that, particularly under in vitro conditions (e.g. everted sac experiments), there is considerable appearance of free amino acids in mucosal fluids during peptide absorption [3], it is often difficult to discover whether any particular peptide is hydrolysed at the surface of the brush border membranes or hydrolysed within the cell.

We report here experiments that investigate the uptake of the dipeptide glycyl-L-leucine in the small intestine of *Rana pipiens* using a vascularly perfused preparation [4] which reduces the complications that characterize classical in vitro techniques. With this technique the mesenteric bed of the small intestine is perfused in situ with frog Ringer bicarbonate solution (Ca^{2+}, 0.5 mM) containing bovine serum albumin, (fraction V, 1 g·100 ml^{-1}),

via a nylon cannula in the coeliacomesenteric artery. The venous effluent is collected through a nylon cannula in the portal vein and the recovery of the arterial inflow remained above 90% (often 95%) throughout the experiments. The lumen in the intestine is cannulated with 3 mm diameter nylon tubes and circulated on a single pass system at a flow rate of 2 ml/min with frog Ringer bicarbonate solution. Oxygen is supplied to the tissue from both sides (25° C; gas mixture 95% O_2 /5% CO_2 (v/v)). Glycine was estimated chemically [4] and L-leucine by an L-amino acid oxidase method [5]. This preparation permits the rapid clearance from the mucosal epithelium of the products of absorption, thereby reducing their accumulation within the tissue, and allows easy sampling of both luminal and vascular effluents [6].

With increasing concentrations of an equimolar mixture of L-leucine and glycine in the luminal solution, the rate of transfer of the two amino acids into the vascular effluent from the lumen showed saturation over the range 0.5—10 mM. For L-leucine the half-saturation constant (K_t) of the transfer function was 3.1 ± 0.6 mM (5) and for glycine the K_t was 0.9 ±0.1 mM (5). Thus at a luminal concentration of 10 mM the transfer systems for L-leucine and glycine were completely saturated and this was confirmed in each experiment by increasing the luminal concentration of both amino acids to 15 mM with no further increase in their transport rates. Rates of transfer of amino N are shown in column (a), Table I.

TABLE I

RATES OF TRANSFER OF AMINO N INTO PORTAL VEIN FROM LUMEN OF SMALL INTESTINE CONTAINING FREE AMINO ACIDS AND DIPEPTIDE

(a) concentration of L-leucine and glycine, each 10 mM/l. (b) concentration of L-leucine and glycine, each 10 mM/l, of glycyl-L-leucine, 5 mM/l. (c) concentration of glycyl-L-leucine, 5 mM/l. Lumen fluid contains Na-Ringer. Each value, mean ± S.E. of mean of 4 observations rounded to nearest integer.

Amino N transferred as: (μmol·g dry wt^{-1}·h^{-1})	Lumen			
	(a) Gly+L-Leu	(b) Gly+L-Leu +Gly-L-Leu	(c) Gly-L-Leu	(b—a)
L-Leucine	123 ± 17	227 ± 21	182 ± 17	104 ± 24
Glycine	58 ± 8	180 ± 5	174 ± 16	122 ± 9
Total	181 ± 17	408 ± 26	356 ± 32	227 ± 27

However, the addition of 5 mM glycyl-L-leucine to a mixture of 10 mM L-leucine and glycine (column (b), Table I) increased the rate of appearance of L-leucine in the vascular effluent by 85% and of glycine by 210%. The addition of 5 mM dipeptide to the amino acid mixture thus more than doubled the rate of appearance of amino nitrogen in the vascular effluent. No peptide was detected in the vascular effluent.

When dipeptide alone (5 mM) was present in the lumen (column (c) Table I) the transfer of glycine was increased threefold compared with that from the 10 mM amino acid mixture. However, L-leucine transfer was increased to a much smaller extent. Evidently the efficiency of capture of glycine, poorly handled by free amino-acid transport systems, when presented in the form of dipeptide is very high.

Transfer of amino nitrogen from this dipeptide need not occur exclusively by a "peptide transport" system. The rate of transfer by such a system for peptide transport can be estimated by subtracting the rates of amino N transfer, observed when the amino acid transfer systems are saturated, from the transfer rates observed in the additional presence of the peptide. These data are given in column (b—a), Table I. Comparison of the total transfer of amino N from the peptide alone (column (c)), with this estimate of the activity of the peptide transport system shows that the latter accounts for 60% of all the amino nitrogen appearing in the vascular effluent when the dipeptide is present in the lumen. The remaining amino N must be handled by systems accessible to free amino acids in the lumen.

Amino acid uptake by the small intestine is known to depend [7] on sodium and it has been suggested that peptide uptake may also require the presence of sodium [8,9], Experiments were therefore undertaken in which the sodium in the luminal fluid was entirely replaced by potassium, the vascular perfusate being unaltered. Under these conditions uptake of glycine from the amino acid mixture was inhibited to a greater extent than that of L-leucine and the overall transfer of total amino N into the vascular effluent was reduced by half in the absence of sodium in lumen fluids (column (a), Table II.)

TABLE II

RATES OF TRANSFER OF AMINO N INTO PORTAL VEIN FROM LUMEN OF SMALL INTESTINE CONTAINING FREE AMINO ACIDS AND DIPEPTIDE

(a), (b) and (c) as Table I. Lumen fluid contains Na-free Ringer. Each value, mean ± S.E. of mean of 4 observations rounded to nearest integer.

Amino N transferred as: (μmol·g dry wt^{-1}·h^{-1})	Lumen			
	(a) Gly+L-Leu	(b) Gly+L-Leu +Gly-L-Leu	(c) Gly-L-Leu	(b—a)
L-Leucine	73 ± 7	166 ± 15	110 ± 20	93 ± 10
Glycine	15 ± 2	139 ± 16	107 ± 17	124 ± 16
Total	87 ± 7	305 ± 29	218 ± 36	217 ± 26

Addition of 5 mM peptide to the amino acid mixture (column (b), Table II) greatly increased the total uptake of both amino acids but the rates of transfer were lower than in the presence of sodium (column (b), Table I). Transfer of both amino acids from the dipeptide alone (column (c), Table II) was again much greater than from the mixture (column (a), Table II) but still lower than in the presence of sodium (column (c), Table I). This is not due to an influence on hydrolysis, for in separate experiments it was found that the rate of hydrolysis of the dipeptide in situ was not affected by the substitution of the sodium: 421 ± 60 μmol/g/h (5) with sodium, 432 ± 69 μmol/g/h (6) with no sodium. The uptake of amino N by the peptide transport system as estimated by subtracting the saturated amino acid component

(a) from the total uptake in the presence of dipeptide and amino acid mixture (b) is given in column (b—a), Table II. By comparing these estimates with those obtained in the presence of sodium (Table I) it appears that the activity of the peptide transport system was not affected by the removal of sodium.

TABLE III

RATES OF TRANSFER OF Gly AND L-Leu INTO PORTAL VEIN FROM LUMEN OF SMALL INTESTINE CONTAINING FREE AMINO ACIDS AND DIPEPTIDE

(a), (b) and (c) as in Tables I and II. Values are means ± S.E. of means of 4 observations of ratio: (rate of transfer of Gly)/(Rate of transfer of L-Leu).

	(a) Gly+L-Leu	(b) Gly+L-Leu +Gly-L-Leu	(c) Gly—L—Leu	(b—a)
Na^+ in lumen	0.52 ± 0.14	0.81 ± 0.06	0.96 ± 0.05	1.39 ± 0.31
No Na^+ in lumen	0.21 ± 0.03	0.84 ± 0.05	1.01 ± 0.09	1.33 ± 0.06

In Table III are shown the rates of transfer of glycine relative to those of leucine; the data emphasise the poor uptake of glycine from the amino acid mixture. From the peptide alone glycine is transferred as fast as leucine, and for the peptide transfer system, estimated as (b—a), Tables I and II, the glycine taken up in this way may even be preferentially transferred across the epithelium.

These experiments demonstrate that in *R. pipiens* there exists an uptake mechanism for glycyl-L-leucine which increases the efficiency of transfer of amino nitrogen from the lumen of the small intestine to the blood stream. Nevertheless, uptake of amino nitrogen from the dipeptide glycyl-L-leucine is not exclusively via such a peptide route, a proportion is also transferred by systems accessible to amino acids present free in the lumen. The activity of the system for peptide uptake does not appear to depend upon the presence of Na^+ in the lumen.

This work is supported by a grant from the Medical Research Council.

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