Biochimica et Biophysica Acta, 553 (1979) 175—178

BBA Report

BBA 71385

INTESTINAL HANDLING OF TWO TETRAPEPTIDES BY RODENT SMALL INTESTINE IN VITRO

DAVID BURSTON, ELIZABETH TAYLOR and DAVID M. MATTHEWS

Department of Experimental Chemical Pathology, Vincent Square Laboratories of Westminster Hospital, London SW1 (U.K.)

(Received December 13th, 1978)

Key words: Tetrapeptide absorption; Intestinal transport; (Jejunum)

Summary

Uptake of free Leu and Ala and uptake of these amino acids from the tetrapeptides Leu-Gly-Gly-Gly and Ala-Gly-Gly-Gly has been studied in rings of everted rodent jejunum in vitro. When mediated uptake of free Leu was virtually saturated addition of Leu-Gly-Gly-Gly gave no significant increase in uptake of Leu. Uptake of Leu and of Ala from the tetrapeptides was strongly inhibited by Met, as was uptake of these amino acids from free solution. The results did not suggest that either tetrapeptide was taken up intact by the jejunum.

For some years it has been generally believed that dipeptides and tripeptides, but not higher peptides, enter the absorptive cells of the small intestine [1—4] and two recent investigations in man [5] and the rat [6] have supported this view. There are however a very great number of possible tetrapeptides alone, and few of these have been studied from the point of view of intestinal absorption. In 1976, Chung et al. suggested that Leu-Gly-Gly-Gly might be taken up intact by rat small intestine in vivo [7]. Because of this, and because of the ability of microorganisms to transport peptides of more than 3 residues [8], we have studied intestinal handling of the tetrapeptides Leu-Gly-Gly-Gly and Ala-Gly-Gly-Gly by rodent small intestine in vitro. Free and peptide-bound amino acids other than Gly were in the L-form.

Leu-Gly-Gly-Gly was purchased from Vega-Fox Biochemicals, Tucson, AZ, and Ala-Gly-Gly-Gly from Bachem Fine Chemicals, Torrance, CA. Amino acids were obtained from British Drug Houses Ltd., Poole, Dorset, Koch-Light Laboratories, Slough, Buckinghamshire and the Sigma Chemical Company, St. Louis, MO. All other reagents were analytical grade.

In experiments using 3 ml of incubation medium experimental procedure and measurements of uptake into rings of everted hamster jejunum were as previously described [9—11]. When 0.5 ml of incubation medium was used each ring of rat or hamster jejunum was incubated in a round-bottomed glass test tube instead of a conical flask; otherwise the procedure was the same. Briefly, incubations were carried out in a Tris-phosphate saline medium, pH 7.2, under oxygen for 2 min at 37°C in a shaking incubator. A 2 min uptake was taken to approximate conditions of influx [10]. After removal from the incubation medium the ring was rinsed in NaCl (154 mM) at 4°C and after blotting on hard filter paper (Whatman No. 50) was eluted for 5 min in 1 ml of sulphosalicylic acid (60 g/l) at 100°C. After centrifugation the supernatant was analysed by ion exchange chromatography on a Locarte automatic loading amino acid analyser. Uptake was expressed as $\mu mol \cdot g^{-1}$ initial wet wt. $\cdot min^{-1}$, after correction for substrate in the inulin space [12].

The significance of differences between means was assessed by the *t*-test.

Most experiments were carried out in the hamster. In the first type of experiment (Table I), uptake of Leu was measured at a concentration (40 mM) at which it was expected that mediated uptake of the free amino acid would be virtually saturated [11]. Repetition of this procedure in the presence of Leu-Gly-Gly-Gly (10 mM) did not cause a significant increase in uptake of Leu, although uptake of Leu from the peptide (10 mM) alone was substantial. This suggested that uptake of Leu from the peptide was the result of uptake of free Leu released by hydrolysis at the brush border, rather than uptake of intact tetrapeptide followed by intracellular hydrolysis, since uptake of peptides appears to be independent of that of free amino acids [3]. If the tetrapeptide had been taken up by a system independent of that for free Leu, and hydrolysed in the intestinal wall, a large increment in Leu would have been expected. A small increment might be the result of uptake of the peptide by simple diffusion [13].

In the second type of experiment (Table II) uptakes of Leu and of Ala from free solution and from the tetrapeptides Leu-Gly-Gly-Gly and Ala-Gly-Gly-Gly were measured in the absence of and in the presence of a high concentration of Met, a powerful competitive inhibitor of uptake of free Leu and free Ala; in one experiment with Leu-Gly-Gly-Gly, Ile was used as inhibitor. Uptake of the free amino acids was strongly inhibited in the presence of Met, and their uptake from the tetrapeptides did not escape the inhibitory

TABLE I

UPTAKE OF Leu FROM FREE SOLUTION AND FROM Leu-Gly-Gly-Gly BY RINGS OF EVERTED HAMSTER JEJUNUM

The volume of incubation medium was 3 ml. Values for uptake are the means of observations in 6 rings, two from each of 3 animals. Standard errors are given.

Substrate(s)	Concn. (mM)	Uptake of Leu ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
Leu	10	2.0 ± 0.16
Leu-Gly-Gly-Gly	10	1.4 ± 0.11
Leu	40	2.3 ± 0.36
Leu	40	2.8 ± 0.41
+ Leu-Gly-Gly-Gly	10	

TABLE II

UPTAKE OF Leu and Ala FROM FREE SOLUTION AND FROM TETRAPEPTIDES BY RINGS OF EVERTED HAMSTER JEJUNUM WITH AND WITHOUT Met OR Ile AS INHIBITOR OF UPTAKE OF FREE AMINO ACIDS

Inhibitor concentration was 100 mM in all experiments. The volume of incubation medium was 0.5 ml. Values for uptake are the means of observation in 6 rings, two from each of 3 animals. Standard errors are given.

Substrate	Concn. (mM)	Inhibitor	
			Uptake of Leu (μmol·g^{-1}·min^{-1})
Leu	1	—	0.42 ± 0.04
Leu	1	Met	0.07 ± 0.03
Leu-Gly-Gly-Gly	1	—	0.21 ± 0.04
Leu-Gly-Gly-Gly	1	Met	0.03 ± 0.02
Leu-Gly-Gly-Gly	1	Ile	0.01 ± 0.02
			Uptake of Ala (μmol·g^{-1}·min^{-1})
Ala	10	—	2.1 ± 0.31
Ala	10	Met	0.37 ± 0.08
Ala-Gly-Gly-Gly	10	—	1.8 ± 0.15
Ala-Gly-Gly-Gly	10	Met	0

TABLE III

UPTAKE OF Leu FROM FREE SOLUTION AND FROM Leu-Gly-Gly-Gly BY RINGS OF EVERTED RAT JEJUNUM WITH AND WITHOUT Met AS INHIBITOR

The concentration of Met was 100 mM. The volume of incubation medium was 0.5 ml. Values for uptake are the means of observations in 5 or 6 rings, one or two from each of 3 animals. Standard errors are given.

Substrate	Concn. (mM)	Inhibitor	Uptake of Leu (μmol·g^{-1}·min^{-1})
Leu	5	—	0.44 ± 0.07
Leu	5	Met	0.15 ± 0.03
Leu-Gly-Gly-Gly	5	—	0.23 ± 0.04
Leu-Gly-Gly-Gly	5	Met	0

effect. This type of experiment was also carried out in the rat (Table III), with similar results to those in the hamster. The results of the second type of experiment also suggested that uptake of Leu and of Ala from the peptides was the result of hydrolysis followed by uptake of free amino acids, rather than uptake of intact peptides followed by hydrolysis.

The results provide no evidence suggesting uptake of intact Leu-Gly-Gly-Gly or Ala-Gly-Gly-Gly, and in no experiment was intact tetrapeptide found in the intestinal tissue. Though these are only two of a great number of possible tetrapeptides, the results obtained with them do not alter the earlier conclusion that as far as we know only di- and tripeptides are taken up intact by the absorptive cells of the small intestine.

A point of interest is that in the experiment on uptake of Leu-Gly-Gly-Gly (10 mM) by hamster jejunum (Table I), the mean final concentration of Leu appearing in the medium was only 0.1 mM. A concentration of this magnitude could not possibly account for the observed extent of uptake of Leu

from Leu-Gly-Gly-Gly (10 mM), which is more than 3 times that of uptake of Leu from free Leu (1 mM). This suggests that the effective concentration of Leu released in the brush border region must be very much higher than that appearing in the bulk phase of the medium. The question of whether this can be accounted for by the diffusion barrier of the unstirred layer [14], or whether Ugolev's hypothesis of 'membrane hydrolysis' [15] must be invoked to explain it requires further investigation.

This work was supported by a grant from the Medical Research Council of Great Britain.

References

1 Matthews, D.M., Craft, I.L., Geddes, D.M., Wise, I.J. and Hyde, C.W. (1968) Clin. Sci. 35, 415—424
2 Peters, T.J., Donlon, J. and Fottrell, P.F. (1972) in Transport Across the Intestine (Burland, K.L. and Samuel, P.D., eds.), pp. 153—167, Livingstone, Edinburgh
3 Matthews, D.M. (1975) Physiol. Rev. 55, 537—608
4 Matthews, D.M. (1977) in Peptide Transport and Hydrolysis, Ciba Foundation Symposium 50, New Series (Elliott, K. and O'Connor, M., eds.), pp. 5—14, Elsevier, Amsterdam
5 Adibi, S.A. and Morse, E.L. (1977) J. Clin. Invest. 60, 1008—1016
6 Smithson, K.W. and Gray, G.M. (1977) J. Clin. Invest. 60, 665—674
7 Chung, Y.C., Silk, D.B.A. and Kim, Y.S. (1976) Clin. Res. 24, 282A
8 Payne, J.W. (1975) in Peptide Transport in Protein Nutrition (Matthews, D.M. and Payne, J.W., eds.), pp. 283—364, North-Holland Publishing Company, Amsterdam
9 Matthews, D.M., Addison, J.M. and Burston, D. (1974) Clin. Sci. Mol. Med. 46, 693—705
10 Sleisenger, M.H., Burston, D., Dalrymple, J.A., Wilkinson, S. and Matthews, D.M. (1976) Gastroenterology 71, 76—81
11 Schedl, H.P., Burston, D., Taylor, E. and Matthews, D.M. (1979) Clin. 56, 25—31
12 Cheng, B., Navab, F., Lis, M.T., Miller, T.N. and Matthews, D.M. (1971) Clin. Sci. 40, 247—259
13 Matthews, D.M., Gandy, R.H., Taylor, E. and Burston, D. (1978) Clin. Sci. 56, 15—23
14 Wilson, F.A. and Dietschy, T.M. (1974) Biochim. Biophys. Acta 363, 112—126
15 Ugolev, A.M., Timofeeva, N.M., Smirnova, L.F., De Laey, P., Gruzdkov, A.A., Iezuitova, N.N., Mityushova, N.M., Roshchina, G.M., Gurman, E.G., Gusev, V.M., Tsvetkova, V.A. and Shcherbakov, G.G. (1977) in Peptide Transport and Hydrolysis, Ciba Foundation Symposium 50, New Series (Elliott, K. and O'Connor, M., eds.), pp. 221—238, Elsevier, Amsterdam