ABSORPTION OF TWO TYROSINE CONTAINING TRI-PEPTIDES FROM THE SMALL INTESTINE AND RECTUM OF THE RAT

C.E.Heading, C.S. Rogers and S. Wilkinson, Dept. of Paramedical Sciences, North East London Polytechnic, Romford Rd., London E15 4LZ and Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS.

There is increasing interest in small peptides, such as for example, phosphono-peptides and enkephalin derivatives, that may be of therapeutic use. Ideally such peptides would be active after oral administration. Although it has been known for several years that many small peptides are capable of passing from the gut lumen into intestinal epithelial cells prior to their hydrolysis, much less is known of the factors which enable some peptides to reach mesenteric blood intact. In view of this, we have studied two tripeptides Tyrosyl-Glycyl-Glycine (T.G.G.) and Tyrosyl-DAlanyl-Glycine (T.A.G.) to establish whether they can cross the gut wall intact, and if so by what means.

The ability of the peptides to be hydrolysed by intestinal mucosa was measured by the method of Boullin, et al, (1973) while the everted sac technique of Wilson & Wiseman (1954) was used to study absorption from the small intestine and from the rectum (Heading, et al 1978). Sample analysis was effected using T.L.C. with additional processes pre and post development when required. Separation difficulties required samples containing T.A.G. to be chromatographed before and after hydrolysis, but subsequent use of radiolabelled TAG (Tyrosyl-DAlanyl 2-^{14}C glycine) permitted assay by scintillation counting of developed chromatograms. Radio labelled TAG of initial specific activity 0.176 mCi mMol^{-1}was used at mucosal concentrations of 3 x 10^5 c.p.m. ml^{-1}.

As expected hydrolysis, as judged by cleavage of one or more peptide bond, was slower for T.A.G. than for T.G.G. At 10 mM substrate concentration the rates were 6.49 $\pm$ 2 μmol g^{-1} hr^{-1} and 190 $\pm$ 35 μ mol g^{-1}hr^{-1} respectively (n = 3). Using intestinal sacs, absorption of each peptide was studied at 20 mM mucosal concentration for periods of up to 2 hr. and at 10, 20 and 40 mM for 1 hr. T.G.G. failed to appear in serosal fluid at detectable levels ($<$ 0.5 mM), while in all cases T.A.G. was detected in serosal fluid and its appearance was both concentration and time dependant. Subsequent use of radio-labelled peptide enabled intact peptide to be detected in serosal fluid within 15 min. and with mucosal concentrations as low as 1.25 mM. When in a variety of experiments Na^+ in the bathing fluid was replaced by K^+ absorption of T.A.G. was reduced by upwards of 25%, but never totally eliminated. The involvement of some passive transport was confirmed when absorption was only reduced by 12% in experiments conducted at 4°C instead of 37 C. However, when peptide was added to mucosal and serosal fluids at equal concentrations, accumulation of the peptide in the serosal fluid was observed, indicating that absorption did not require to be down a concentration gradient. These results seem similar in character to those of Addison et al (1975) who found evidence of a Na^+ dependant system capable of transporting tri-peptides into intestinal cells, against a concentration gradient.

Using sacs of rectum T.G.G. again failed to appear in serosal fluid at detectable levels, but T.A.G. crossed to the serosal surface at a rate similar to that seen with the intestinal sacs, to reach a serosal concentration approximately one tenth of the mucosal concentration by 1 hr. This absorption was only slightly reduced by replacement of Na^+ by K^+ in the bathing fluid, indicating that transport was occurring by a Na^+ independent system.

Addison, J.M., Burston, D., et al Clin.Sci.Mol.Med.(1975) 49: 305-312.
Boullin, D.J. Crampton, R.F., et al (1973) Ibid. 45: 849-858.
Heading, C.E., Rogers, C.S. & Wilkinson, S. (1978) J.Physiol. 278: 21P
Wilson, T.H. & Wiseman, G. (1954). J.Physiol. 123: 116-125.