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Technical Note

Mechanism of Absorption of the Dipeptide α-Methyldopa-phe in Intestinal Brush-Border Membrane Vesicles

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INTRODUCTION

In a previous report it was shown that dipeptides containing α-methyldopa as one of the amino acids were much more efficiently absorbed (higher membrane permeability) than α-methyldopa itself, suggesting that peptide transport is structurally less specific than the amino acid transporters (1). The experimental system used in the previous report was as an *in situ* perfused intestinal segment. Since brush-border and cytosolic enzymes are present in that system, the observed nonlinear absorption could be due to nonlinear metabolism. However, since no free α-methyldopa was observed during the intestinal perfusions, this suggests very limited brush-border metabolism. The intestinal brush-border membrane system (2,3) has the advantage of no cytosolic enzymes and that the uptake into the cytosol can be measured directly for both prodrug and drug concentration. The results of an investigation into the mechanism of transport of the dipeptide α-methyldopa-phe using this vesicle system are reported below.

Membrane Vesicle Preparation and Uptake Experiment. Male Wistar rats, 200–250 g (Sankyo Lab. Co., Toyama, Japan), were used. They had access to a standard food and water prior to the experiments. Intestinal brush-border membrane vesicles were prepared by the method of Kessler *et al.* (2) with some modification as described previously (3). The uptake of the α-methyldopa-phe dipeptide by membrane vesicles was performed at 37°C using a rapid filtration technique described previously (3). The influx of the dipeptide was initiated by adding an aliquot of 90 μl of a solution (270 m*M* mannitol and 20 m*M* Mes/Tris buffer of pH 5.5) containing the dipeptide to 10 μl of the membrane vesicle suspension which was incubated for 5 min at 37°C. Unless otherwise noted, the incubation medium was composed of 270 m*M* mannitol and 20 m*M* Hepes/Tris of pH 7.5. The uptake was terminated at 30 sec by adding 1 ml of ice-cold stop solution containing 300 m*M* mannitol and 20 m*M* Mes/Tris, pH 5.5. The diluted samples were applied immediately on a Millipore filter (HAWP, 0.45-μm pore size) and washed rapidly twice with 4 ml of ice-cold stop solution. L-α-Methyldopa-L-phenylalanine trapped on the Millipore filter was extracted with 500 μl of 0.01 *N* HCl by shaking for 20 min in the glass tube with a Teflon-sealed cap and the resultant extract was assayed by HPLC.

Assay Procedure. The HPLC system was equipped with a constant flow pump, BIP-1 (Japan Spectroscopic Co., Tokyo, Japan), and a UV detector, UVIDEC-100-V (Japan Spectroscopic Co.), an automatic sample injector, ASL350 (Japan Spectroscopic Co.), and an integrator, Chromatopac CR3A (Shimadzu Corp., Kyoto, Japan). The analytical conditions used were as follows: the analytical column was a strong cation-exchange column (Partisil 10-SCX, 4.6 × 250 mm, Whatman). The mobile phase, 15% acetonitrile, 85% 0.05 *M* ammonium phosphate buffer, pH 2.5, was used at a flow rate of 1 ml/min. The eluent was monitored at 280 nm (0.0025 full-scale). Protein was measured by the method of Bradford (4), using the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA) with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The concentration dependency of the initial uptake (at 30 sec) of L-α-methyldopa-L-phenylalanine in the presence of an inward proton gradient (pH in = 7.5; pH out = 5.5) is shown in Fig. 1, indicating one saturable and one nonsaturable uptake. The apparent kinetic parameters for the uptake at 37°C were evaluated by a nonlinear least-squares analysis of the data using NONLIN computer program (5) according to the following equation:

$$J = J_{max}C/(K_t + C) + k_dC$$

and estimated to be

$$K_t = 0.341 \pm 0.018 \text{ m}M,$$
$$J_{max} = 0.113 \pm 0.022 \text{ nmol/30 sec/mg protein},$$
$$K_d = 0.0586 \pm 0.0124 \text{ nmol/30 sec/mg protein/m}M.$$

The value of K_t and k_d were very similar to those (K_t = 0.83 m*M* and k_d = 0.076 nmol/30 sec/mg protein/m*M*) reported (3) for cefixime uptake by the intestinal membrane vesicles, whereas the maximum uptake rate of L-α-methyldopa-L

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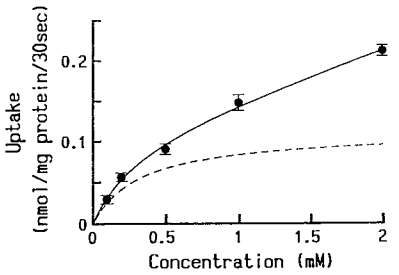

Fig. 1. Concentration dependence of L-α-methyldopa-L-phenylalanine uptake by intestinal brush-border membrane vesicles. Membrane vesicles were preloaded with 20 m*M* Hepes/Tris buffer (pH 7.5) containing 270 m*M* mannitol. Uptake of L-α-methyldopa-L-phenylalanine was measured at 37°C for 30 sec by incubating the membrane vesicles in 20 m*M* Mes/Tris buffer (pH 5.5) containing 270 m*M* mannitol. Each point represents the mean ± SE of 3 to 10 experiments. The solid and dashed lines indicate total and saturable uptakes, respectively.

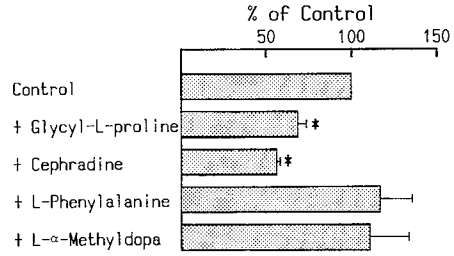

Fig. 2. Inhibitory effect of dipeptide, β-lactam antibiotic, and amino acids on the uptake of L-α-methyldopa-L-phenylalanine by intestinal brush-border membrane vesicles. The preloaded and uptake conditions were the same as described in the legend to Fig. 1. Concentrations of L-α-methyldopa-L-phenylalanine and inhibitors were 2 and 20 m*M*, respectively. Osmolarities were adjusted to the control study by changing concentration of mannitol. Each bar represents the mean ± SE of four to six experiments. The bars marked with an asterisk are significantly different from the control study ($P < 0.05$).

phenylalanine was about 20-fold smaller than that (3) of cefixime uptake in the presence of the similar proton gradient.

To determine the extent to which the observed uptake was due to surface binding a study was done in 990 mOsm buffer using 20 m*M* Mes/Tris buffer (pH 5.5) containing 970 mOsm mannitol to rupture the vesicles. The observed uptake was reduced to 44 ± 8% ($n = 3$) compared to normal membranes. Thus the observed uptake is mainly intravesicular, although surface binding is significant.

In order to confirm that this newly synthesized peptide is transported via the peptide carrier system, the inhibitory effect of the dipeptide, glycyl-L-proline, was examined. As shown in Fig. 2, 20 m*M* glycyl-L-proline inhibited significantly the α-methyldopa peptide uptake in the presence of the proton-gradient, whereas the individual amino acids L-phenylalanine and L-α-methyldopa showed no inhibitory effects at the same concentration of 20 m*M*. A cephalosporin antibiotic, cephradine, which has been reported to be transported via intestinal dipeptide carrier systems, also inhibited significantly the α-methyldopa-phe peptide uptake (6,7). The present results more directly support the hypothesis that the L-phenylalanine peptide of L-α-methyldopa is recognized and taken up by the dipeptide transport system, in agreement with the previous findings obtained from the *in situ* perfusion experiment (1).

The direct evidence of peptide carrier mediated transport for this peptide type prodrug, when combined with previous results for β-lactam antibiotics (8,9), ACE inhibitors, and peptide-type prodrugs (10–12), indicates that the mucosal cell peptide carrier is an important mechanism for effective oral delivery of peptides and peptide analogues and prodrugs. Determination of the structural requirements of this pathway will considerably aid the development of effective oral drugs in this structural class.

REFERENCES

1. M. Hu *et al. Pharm. Res.* 6:66–70 (1989).
2. M. Kessler *et al. Biochim. Biophys. Acta* 506:136–154 (1978).
3. A. Tsuji *et al. J. Pharmacol. Exp. Ther.* 241:594–601 (1987).
4. M. M. Bradford. *Anal. Biochem.* 72:248–254 (1976).
5. C. M. Metzler, G. K. Elfring, and A. J. McEwen. *A Users Manual for NONLIN and Associated Programs,* The Upjohn Company, Kalamazoo, Mich., 1974.
6. E. Nakashima, A. Tsuji, S. Kagatani, and T. Yamana. *J. Pharm. Dyn.* 7:452–464 (1984).
7. P. J. Sinko and G. L. Amidon. *Pharm. Res.* 5:645–650 (1988).
8. A. Tsuji. In *Frontiers of Antibiotic Research,* Academic Press, New York, 1987, pp. 255–268.
9. A. Tsuji, H. Hirooka, I. Tamai, and T. Terasaki. *J. Antibiot.* 39:1592–1597 (1986).
10. M. Hu and G. L. Amidon. *J. Pharm. Sci.* 77:1007–1011 (1988).
11. D. I. Friedman and G. L. Amidon. *Pharm. Res.* 6:1043–1047 (1989).
12. D. I. Friedman and G. L. Amidon. *J. Pharm. Sci.* 78:995–998 (1989).