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Metabolism and Transport of the Pentapeptide Metkephamid by Brush-border Membrane Vesicles of Rat Intestine

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Abstract—Intestinal metabolism and transport of the pentapeptide metkephamid (Tyr-D-Ala-Gly-Phe-*N*-Me-Met-NH_2) were studied using isolated brush-border membranes from the rat. Analysis of the metabolic fragments of enzymatic hydrolysis revealed that cleavage of the N-terminal peptide bond leads to the formation of tyrosine and a tetrapeptide D-Ala-Gly-Phe-*N*-Me-Met-NH_2. The inactivation was due to aminopeptidase N activity and could be inhibited by peptidase inhibitors puromycin, bacitracin and certain dipeptides. Transport studies demonstrated uptake of the intact pentapeptide into the intravesicular space of the vesicles. The transport was a first-order process; no participation of known intestinal peptide carrier systems in the transport of metkephamid could be shown. Modelling of simultaneous metabolism and transport kinetics suggests strategies to improve the fraction absorbed of a peptide by either decreasing its affinity to the metabolizing enzymes (increase K_m) or decreasing the concentration of the metabolizing enzymes e.g. by delivering the peptide to an absorption site with reduced enzymatic activity (decrease V_{max}) or increasing its absorption velocity.

There are two barriers that restrict the systemic availability of peptide-drugs by oral administration: low permeability of intestinal epithelia (diffusional or transport barrier) which may be related to their poor lipophilic character and large molecular weight, and enzymatic degradation in the intestinal lumen, enterocytes or liver (enzymatic barrier). Intestinal permeability refers to the intestinal wall's property to modify the flow of substances across the membrane by putting a certain degree of resistance to the free diffusional flow and depends on the physicochemical nature of the permeant. For di- or tripeptides effective intestinal transport of the intact molecule has been demonstrated occurring via an intestinal peptide carrier system located in the brush-border membrane of the enterocytes. Examples include the hydrolysis-resistant peptides glycyl-sarcosine, glycyl-sarcosyl-sarcosine, and carnosine (β-Ala-His) (Mathews 1983), further angiotensin-converting enzyme inhibitors (Friedman & Amidon 1989) and β-lactam antibiotics (Tsuji et al 1981, 1987; Sugawara et al 1991). There is some controversy about whether larger peptides (tetra- and pentapeptides) might be taken up via carrier-mediated mechanisms which has been shown for a tetrapeptide (Kramer et al 1990). Also, for a cyclic somatostatin analogue, an octapeptide, intestinal carrier-mediated transport has been confirmed (Fricker et al 1992). However, the structural requirements for active transport of peptides with larger molecular weight is unknown.

Successful oral delivery of peptide drugs not only requires a sufficient permeability of the active species through the intestinal wall, but also requires a high stability of the peptide towards intestinal metabolism (Wiedhaup 1981). The metabolic barrier is seen in the rapid removal of the peptide from the absorption site due to metabolism by peptidases and proteases associated with the membrane and lumen. Therefore, to improve the stability of peptides in the intestinal tract, it is necessary to understand the metabolic pathways and inactivation kinetics of such compounds in the intestine. In particular, in the case of oligopeptides, metabolism by enzymes located in the brush-border membrane of the enterocyte may represent a rate limiting step in the overall availability of the compound, since there are several peptidases integral to the brush-border membrane whose active sites are positioned external to the enterocyte's surface membrane. Amino-oligopeptidase (aminopeptidase N), acidic aminopeptidase (aminopeptidase A), Gly-Leu peptidase, Asp-Lys peptidase, dipeptidylaminopeptidase, carboxypeptidase P and dipeptidylcarboxypeptidase are well characterized peptidases of the intestinal brush-border membrane (Gray 1989). Of these enterocyte's surface peptidases, amino-oligopeptidase plays a vital role in the metabolism of peptides because of its broad specificity for amino acid side chains at the NH_2-terminus of the peptide substrate and its wide tissue distribution (Stratford & Lee 1986). Considerable peptidase activity is also localized in the cytosol of the enterocyte. However, different studies using peptides with various chain-lengths have shown that up to 90% of the capacity of the absorptive cell to hydrolyse tetra- and polypeptides is associated with the brush-border, whereas, with respect to the hydrolysis of dipeptides, only 10% of the activity is associated with the brush-border, compared with 90% with the cytosol (Matthews 1976).

In the present study, the role of the intestinal brush-border membrane as a barrier to absorption of a pentapeptide and its transport mechanism is assessed. Metkephamid (Tyr-D-Ala-Gly-Phe-*N*-Me-Met-NH_2), which has been synthetically modified from the naturally occurring metenkephalin (Tyr-Gly-Gly-Phe-Met) to increase its enzymatic resistance and thus biological stability, has been selected. The potential for

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oral absorption of metkephamid has been previously assessed in rats (Su et al 1985).

Measurable plasma concentrations of the peptide could not be detected following oral administration indicating either a nearly complete presystemic elimination of the compound or a pronounced inability towards intestinal transport. The present study helps to distinguish between feasible factors that may be responsible for that low bioavailability.

Materials and Methods

Chemicals

Metkephamid was kindly provided by Ely Lilly and Company (Indianapolis, USA). Puromycin HCl, amino acid standards, L-leucine-4-nitroanilide and bacitracin were purchased from Fluka (Buchs, Switzerland). Phe-Phe-Phe, D-Ala-Gly, Ala-Phe, Asp-Phe, Gly-Pro and Ala-Tyr were purchased from Bachem Biochemica (Bubendorf, Switzerland). [^{3}H]Glucose, was obtained from NEN. All other chemicals were of analytical purity and obtained from Sigma Chemicals (Buchs, Switzerland).

Preparation of brush-border membrane vesicles (BBMVs)

BBMVs were prepared from combined duodenum, jejunum and upper ileum by a magnesium-precipitation method (Hauser et al 1980; Stieger & Murer 1983). The intestines from two adult male Wistar rats, 180–240 g, were rinsed with 0·9% NaCl (4°C) and freed of mucus, the mucosa was scraped off the luminal surface with glass slides and put immediately into buffer containing (mM): 12 Tris-HCl, 5 EGTA, and 300 D-mannitol (pH 7·1, 4°C). Ice-cold distilled water (120 mL) was added, and the mixture was homogenized in a blender (Omnimix, Sorvall) for 3 min. Magnesium chloride solution was added to achieve a final concentration of 10 mM. The homogenate was left at 4°C for 15 min and afterwards centrifuged at 3000 *g* for 15 min (Sorvall centrifuge, SS-34 rotor, Dupont). The supernatant was centrifuged at 27 000 *g* for 30 min. An additional purification step was undertaken by suspending the pellet in 30 mL 5 mM HEPES/KOH buffer, pH 7·5 and 270 mM D-mannitol and centrifuging at 27 000 *g* for 30 min; thereafter the pellet was suspended in 500 μL buffer containing 5 mM HEPES/KOH pH 7·5 and 290 mM D-mannitol (transport buffer) and homogenized by syringing through a 22-gauge needle. The BBMVs were characterized by determination of their protein content by the Lowry method (Bio Rad assay kit) with bovine serum globulin as standard, enrichment of brush-border marker enzyme leucine-aminopeptidase by measuring the formation of *p*-nitroaniline from L-leucine-4-nitroanilide at 410 nm (UV) and 37°C in the presence of BBMVs as compared with enzyme activity in the homogenate, scanning electron microscopy (freeze-fracture technique) and their ability to transport D-glucose in the presence of a sodium gradient (see below). [^{3}H]D-Glucose was quantified by liquid scintillation counting after dissolution of the filters in scintillation fluid.

Metabolism studies

Metkephamid solutions (45 μL) at different concentrations were incubated with 5 μL BBMV suspension or cytosol at 37°C in 50 mM MES/KOH buffer solutions at pH 6·5 (37°C). The protein concentration in the incubate was 2–4 mg mL^{-1}. The enzymatic reaction was stopped by addition of 0·2% Triton-X-100 in 0·2 M perchloric acid. Initial rates were calculated from the degradation product concentration after 45 s of incubation. This incubation time was adjusted so that not more than 5% of the substrate was hydrolysed. Metabolism studies in the presence of enzyme inhibitors (see figures) were conducted by incubating the vesicle suspension with a solution of peptide and inhibitor.

Transport studies

Metkephamid. Peptide transport was measured at 37°C by a rapid filtration technique described previously (Hopfer et al 1973). BBMVs (20 μL, approx. 30 mg protein mL^{-1}) were incubated with 180 μL peptide solution (1–16 mM) for defined periods of time (1–60 min) in pH 6·5 to 7·5 buffers at 37°C. The peptide solution contained 1 mM puromycin HCl to inhibit the activity of the proteolytic enzymes during the transport experiments. The osmolarity of the peptide solutions was adjusted to 350 mOsm with D-mannitol using a semi-micro osmometer (Roebling, Dietikon, Switzerland). Hyperosmolar buffers (568–1064 mOsm) were prepared by addition of D-mannitol to the buffer solutions. Transport was terminated by adding an aliquot of the vesicle incubate to 2 mL ice-cold stop solution containing (mM) 100 D-mannitol, 150 NaCl and 5 Tris-HCl, at pH 7·4. Immediately after stopping the reaction, the vesicles were sucked through a prewashed (with stop solution) nitrocellulose filter with a pore size of 0·45 μm (Sartorius, Instrumenten-Gesellschaft, Zürich, Switzerland). The filter was washed twice again with 4 mL stop solution. The blank value, which includes nonspecific adsorption of the peptide to the vesicle surface and filter material, was determined by mixing the peptide with the stop solution and then adding the vesicle suspension followed by immediate filtration. This value was subtracted from the uptake data. All the experiments done under a given condition were repeated 2–5 times.

D-Glucose. D-Glucose transport was quantified in the presence of an initial gradient of NaCl (200 mM outside, 0 inside). The buffer in all cases consisted of (mM) 5 HEPES-KOH, 290 mannitol, 0·2 D-glucose and 200 NaCl, pH 7·7 (at room temperature, 21°C).

Preparation of cytosol

Mucosa scrapings were suspended in 50 mM MES/KOH buffer pH 6·5 containing 250 mM D-mannitol and homogenized with a Potter-Elvehjem homogenizer at 2000 rev min^{-1} for 2 min. Destruction of the enterocytes was confirmed by microscopy. The homogenate was centrifuged at 100 000 *g* for 1 h and the supernatant separated as the cytosol fraction.

Analytical methodologies

Analyses were performed on a Merck high pressure liquid chromatograph equipped with an L-6200 pump, an F-1050 fluorescence spectrophotometer, an L-4250 UV-vis detector, an L-3000 photo diode array detector, an AS 4000 autosampler and a D-2500 Chromato-integrator (Merck-Hitachi, ABS, Dietikon, Switzerland). The degradation product following enzymatic cleavage of metkephamid, tyrosine, was identified by HPLC using amino acid analysis following pre-

column fluorescence derivatization with *o*-phthaldialdehyde and by its UV spectrum using diode array detection.

Chromatographic conditions for the assay of tyrosine and metkephamid were as follows: stationary phases -LiChrosorb RP8, 5 μm (Merck 250 × 4 mm) for metkephamid, LiChrosorb RP18.5 μm (Merck 250 × 4 mm) for tyrosine; mobile phase −0·01 M sodium heptanesulphonic acid in 50 mM phosphate buffer pH 4·0 and acetonitrile (73:27; v/v) for metkephamid, 0·01 M sodium heptanesulphonic acid in water, phosphoric acid, acetonitrile, water (730:1·5:260:1000; v/v) for tyrosine; flow 1·5 mL min^{-1}; ambient temperature. Detection was by UV absorbance at 205 nm. Intraday and interday coefficients of variation of the analytical method were <3 and <5%, respectively, with detection limits of 5–10 ng (injected amounts). Pre-column fluorescence derivatization of tyrosine was performed as follows. To 100 μL incubate, 250 μL methanol was added, the mixture was vortexed and centrifuged. Supernatant (300 μL) was mixed with 100 μL borate buffer pH 9·5 and 100 μL of *o*-phthaldialdehyde reagent solution (200 mg *o*-phthaldialdehyde + 243·2 mg *N*-acetylcysteine dissolved in a mixture of methanol (4·5 mL) and borate buffer (0·5 mL)). The mobile phase consisted of monobasic sodium phosphate (66·7 mM) + dibasic sodium phosphate (66·7 mM) (65·3:34·7, v/v) and acetonitrile (90:10, v/v) and separation was on a LiChrospher 100 RP8, 5 μm reversed phase column (Merck 250 × 4 mm) at a flow of 1·5 mL min^{-1}. Excitation wavelength was 344 nm, emission 443 nm. Filters were extracted with 250 μL 0·2 M perchloric acid containing 0·2% Triton-X-100. Phe-Phe-Phe was used as an internal standard. Calibration curves were prepared over the range 0·104–2·08 μg metkephamid per filter.

Computations

Numerical computations for the transport/metabolism model were performed employing a Runge-Kutta algorithm (NONLIN).

Results and Discussion

Vesicle characterization

Closed and right-side out oriented vesicles of approximately 200 nm in diameter were obtained by the differential precipitation technique as demonstrated by scanning electron microscopy (Fig. 1). The vesicles were enriched 14 ± 0·7-fold (s.d.) over the starting homogenate (n = 5) which can be considered as normal for this preparation (Kessler et al 1978; Biber & Murer 1991). Control experiments with each batch

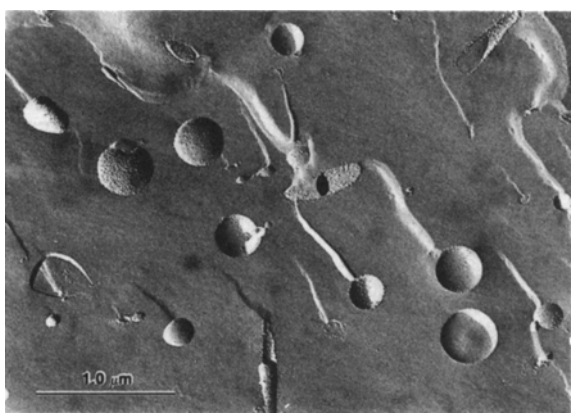

FIG. 1. Freeze-fracture scanning electron microscopic photograph of brush-border membrane vesicles.

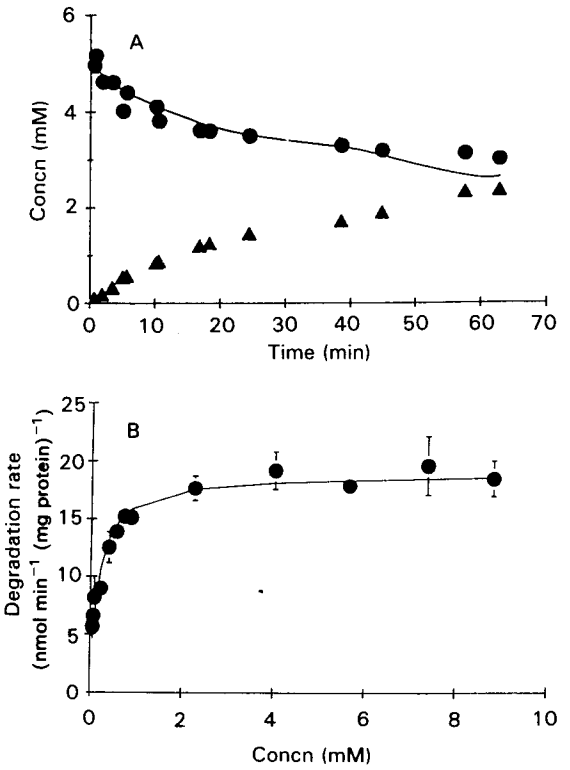

FIG. 2. Degradation of metkephamid by pooled brush-border enzymes of duodenum, jejunum and ileum of the rat in 50 mM MES-KOH buffer, pH 6·5. A. Simultaneous metkephamid (●) degradation and formation of tyrosine (▲), one of the principal metabolites (protein concn 3·6 μg mL^{-1}, 37°C). B. Effect of metkephamid concentration on the rate of its degradation (incubation time 45 s, 37°C). The solid line through the symbols was drawn in accordance with $V = V_{max}/(K_m + C)$, (means ± s.d., n = 3).

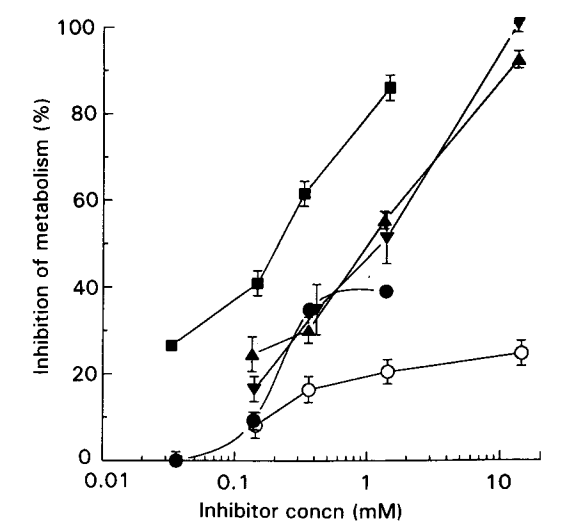

FIG. 3. Inhibition of brush-border metabolism of metkephamid by aminopeptidase inhibitors and dipeptides (means ± s.d., n = 3). ○ D-Ala-Gly, ● bacitracin, ▼ L-Ala-L-Phe, ■ puromycin, ▲ L-Ala-L-Tyr.

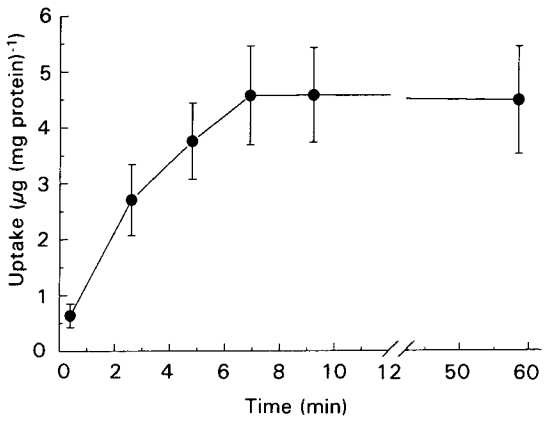

FIG. 4. Time course of metkephamid transport by intestinal BBMVs (means ± s.d. n = 15). The inner solution in the vesicles contained 5 mM HEPES/KOH buffer + 290 mM mannitol, pH 7·7. Incubations were performed in a solution containing (mM): 4 metkephamid, 1 puromycin, 250 mannitol and 50 MES/KOH buffer pH 6·7. The pH of the buffer solution is given for room temperature. At 37°C the pH values decreased to 7·5 and 6·5, respectively.

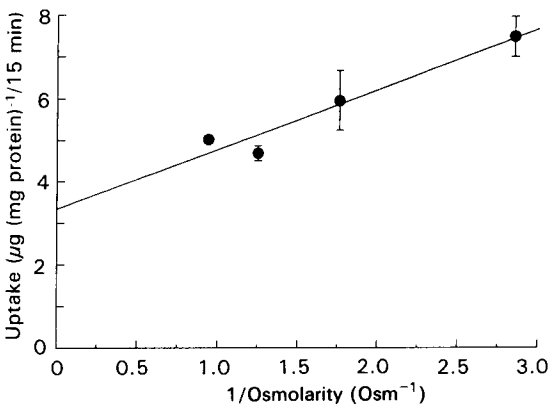

FIG. 5. Dependence of metkephamid transport on extravesicular medium osmolarity (means ± s.d. n = 5). The vesicles were prepared in 5 mM HEPES/KOH buffer + 290 mM mannitol, pH 7·7. Incubation solutions contained (mM) 4 metkephamid and 1 puromycin in 50 MES/KOH buffer pH 6·7 and were adjusted to 351, 568, 800 and 1064 mOsm by addition of mannitol.

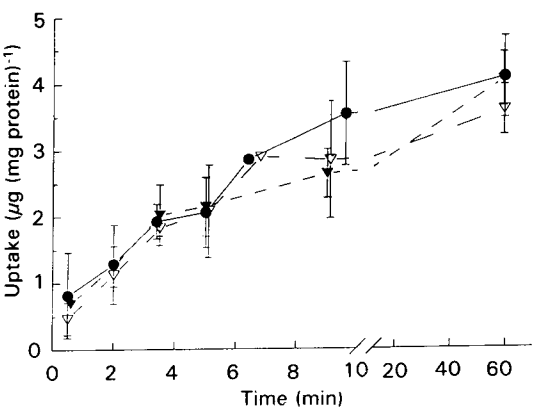

FIG. 6. Independence of metkephamid transport on the presence of sodium (means ± s.d. n = 5). Outer solution (mM): 50 MES/KOH, 50 mannitol, 100 NaCl (▽) or 50 MES/KOH, 50 mannitol, 100 KCl (▼) or 50 MES/KOH, 250 mannitol (●), all pH 6·5 (37°C). All outer solutions contained (mM) 4 metkephamid and 1 puromycin. Inner solution (mM): 5 HEPES/KOH and 290 mannitol, pH 7·5 (37°C).

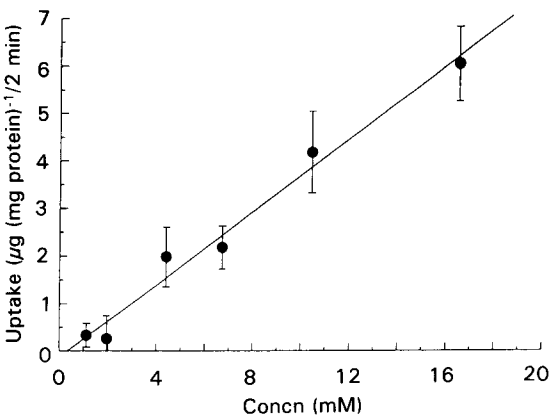

FIG. 7. Dependence of transport rate of metkephamid on the peptide concentration in the incubation medium. Outer solutions were composed of (mM): 50 MES/KOH buffer pH 6·5 (37°C), 250 mannitol, 1 puromycin. Metkephamid concentrations were: 1·12, 1·95, 4·39, 6·72, 10·44 and 16·53 mM. (Means ± s.d. n = 5).

of vesicles showed a sodium dependent overshoot in the uptake of D-glucose and thus revealed the functional integrity of the vesicles (Murer & Kinne 1980).

Enzymatic hydrolysis of metkephamid

In the presence of isolated BBMVs, metkephamid was hydrolysed with a pseudo-first-order half-life of 14 min yielding tyrosine and a tetrapeptide. The kinetics of degradation of the parent compound and the formation of tyrosine is shown in Fig. 2A. The rates of metkephamid degradation and tyrosine formation were similar. This is demonstrated by the continuous line in Fig. 2A which is the predicted metkephamid concentration calculated on the basis of tyrosine formation. This implies that the inactivation of the peptide by cleavage of the N-terminal amino acid peptide bond is the overall dominating degradation pathway. The saturability of the enzymatic reaction was challenged by increasing substrate concentrations from 0·06 to 9 mM. The formation rate of the degradation product increased with increasing substrate concentrations and approached a maximum velocity V_{max} asymptotically (Fig. 2B). The behaviour of the system could be well described by the Michaelis–Menten relationship. V_{max} and K_m were determined by nonlinear regression analysis to be 18·9 nmol min^{-1} (mg protein)$^{-1}$ and 172 μM, respectively. The observed metabolism of metkephamid is most likely due to the activity of aminopeptidases in the brush-border membrane, since the biotransformation of metkephamid could be inhibited in the presence of aminopeptidase inhibitors (puromycin, bacitracin) or certain dipeptides such as Ala-Tyr or Ala-Phe (Schwarz et al 1981) (Fig. 3). The inhibition was concentration-dependent; the strongest inhibition of metabolism was observed in the presence of puromycin. The inhibitory effect of dipeptides on metkephamid metabolism may be attributed to a competitive inhibition of aminopeptidase in the presence of dipeptides containing aromatic amino acids (Kenny et al 1987). Similar inhibitory effects of dipeptides containing aromatic amino acids have been observed in metabolism studies with endopeptidase 24.11 which has been attributed to the presence of a hydrophobic binding pocket near the catalytic centre of the enzyme (Kenny et al 1987).

k_{abs} (min^{-1})	0.000287	0.00574	0.000287	0.000287
V_{max} ($\mu g\ min^{-1}$ (mg protein)$^{-1}$)	12.474	12.474	12.474	1.2474
K_m ($\mu g\ mL^{-1}$)	113.5	113.5	1135	113.5
F_{abs} (%)	3.44	38.60	5.58	24.91

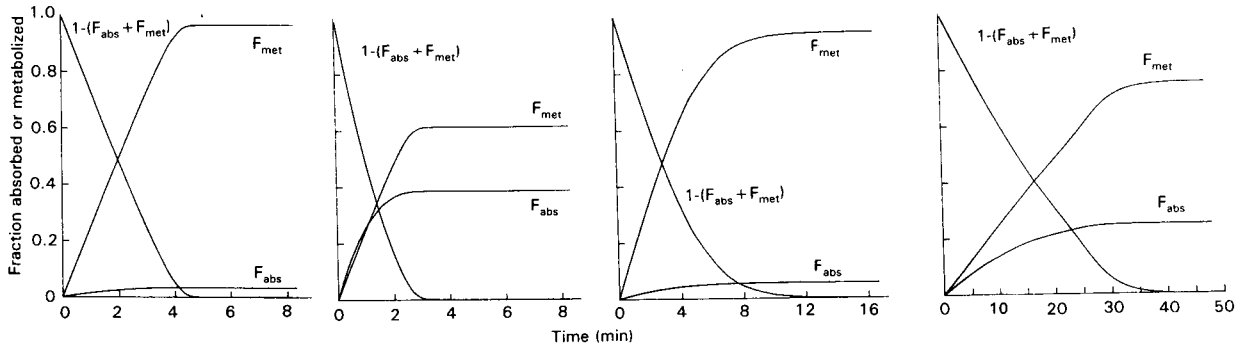

FIG. 8. Simulation of the time course of transport and concurrent metabolism of metkephamid in brush-border membrane vesicles.

Compared with the brush-border metabolism, the degradation of metkephamid due to the metabolic enzyme activity in the cytosol of the enterocytes was found to be negligible. This activity was less than 0·5% of the activity of the brush-border membrane as determined by the initial rate method. These findings are not surprising, since the aminopeptidases in the cytoplasm, cytoplasmic di- and tripeptidase and proline dipeptidase have their main substrate specificity towards dipeptides, tripeptides and dipeptides containing proline (Adibi & Kim 1981). From a structure stability point of view metkephamid might have been cleaved at three cleavage sites due to the similarity in the structure of metkephamid and metenkephalin (Thorsett & Wyvratti 1987). Endopeptidase 24.11 is responsible for the hydrolysis of the Gly^3-Phe^4 bond which may be inhibited by thiorphan. The same peptide bond may also be hydrolysed by angiotensin-converting enzyme (ACE) which may be inhibited by captopril. Dipeptidylaminopeptidase cleaves the Gly^2-Gly^3 peptide bond. Tyrosine and the tetrapeptide Gly-Gly-Phe-Met are released following the hydrolysis of Tyr^1-Gly^2 by the aminopeptidase activity (Turner et al 1987). There is evidence indicating that the aminopeptidase, the endopeptidase 24.11 and ACE could each contribute to metenkephalin metabolism in the gastrointestinal tract with the greatest contribution from aminopeptidases (Geary et al 1982; Kashi & Lee 1986). The majority of the structure-stability and structure-analgesia studies in the area of enkephalins performed so far indicate that the replacement of the glycyl residue in position 2 of metenkephalin by D-alanine increases the stability of the peptide by reducing its affinity towards the aminopeptidase (Schwarz et al 1981). Also a significant decrease in affinity towards endopeptidase 24.11 expressed as a shift in K_m from 86 to 680 μM is observed when the terminal carboxy is amidated (Turner et al 1987).

Transport studies

Metkephamid transport was studied in the presence of puromycin, to inhibit the enzymes responsible for the metabolism of the compound. The time course of metkephamid uptake in the presence of puromycin shows that an equilibrium between the peptide in the extravesicular and intravesicular space was reached after approximately 10 min (Fig. 4). The plateau remained remarkably constant over time which demonstrated the effectiveness of the enzyme inhibition. The calculated internal volume of the vesicles, corrected for binding, based on the equilibrium peptide concentration and the protein concentration per time point, ranged from 0·94 to 1·54 μL (mg protein)$^{-1}$, which is a value that can be confirmed by literature data (Biber & Murer 1991). Since the uptake values represent the sum of transport into the intravesicular space plus the binding of the peptide to the external and internal membrane surface as well as to structures present in the intravesicular space (e.g. the cytoskeletal elements), the amount of binding was analysed by determining the uptake of the peptide as a function of the osmotic-sensitive intravesicular space. This space was manipulated by addition of the impermeant solute D-mannitol to the extravesicular space. Extrapolation of the resulting straight line to infinite osmolarity revealed a binding of 45% (Fig. 5). Thus it can be shown that the observed uptake is mainly intravesicular, although surface binding is significant. Significant binding of solutes to the BBMVs has also been reported by other investigators, e.g. for octreotide 75% (Fricker et al 1992), for α-methyldopa-Phe 44% (Tsuji et al 1990) and for choline 50% (Kessler et al 1978).

To elucidate further the mechanism of transport of the peptide into the vesicle space, various tests for the existence of active transport systems were performed. The dependence of uptake on an inwardly directed proton gradient was examined by maintaining a pH gradient in the uptake experiment ($pH_{intravesicular} = 7{\cdot}5$, $pH_{extravesicular} = 6{\cdot}5$) and comparison with data from experiments with equal pH values on both sides of the membrane ($pH_{intravesicular} = 7{\cdot}5$, $pH_{extravesicular} = 7{\cdot}5$). There was no significant difference between transport rates under both conditions. These results could also be confirmed using a different experimental approach. Uptake was quantified in vesicles in the presence of carbonylcyanid-4-trifluormethoxyphenylhydrazone (FCCP), a protonophore. At an extravesicular pH of 6·5 the mean uptake of metkephamid averaged $0{\cdot}50 \pm 0{\cdot}032$ (n=4) and $0{\cdot}51 \pm 0{\cdot}0091$ μg (mg protein)$^{-1}$ min^{-1} (n=4) (means $\pm$ s.d.) in the presence and absence of FCCP, respectively. Transport rates also were not significantly different at extravesicular pH values of 6·5 and 7·5. The independence of transport on the pH thus far

demonstrates that uptake is not mediated by the intestinal proton-coupled transport system for small peptides. To further confirm the independence of metkephamid transport and peptide carrier-mediated uptake, the permeation of metkephamid through the brush-border membrane in the presence of a high concentration of actively transported Gly-Pro (50 mM) and Asp-Phe (6·5 mM) was investigated. There was no significant difference in metkephamid uptake in the presence of either of these peptides. Similarly, no dependence of transport of the pentapeptide on the presence of sodium ions could be detected as was observed in the uptake studies with the octapeptide octreotide (Fricker et al 1992) (Fig. 6). The concentration dependency of metkephamid uptake in the presence of an H^+ gradient was examined. There was no indication of saturability of the relationship between uptake and the medium concentration of the peptide (Fig. 7). This leads to the conclusion that the peptide is absorbed passively through the brush-border membrane and according to a first-order process.

Modelling of simultaneous transport and metabolism

Normally, transport and metabolism are concurrent during the absorption phase. Therefore, to obtain a quantitative description of the two parallel processes, a model was established. The model is based on the assumption that transport and metabolism of the peptide depend on the surface area and enzyme concentration, respectively, which are assumed to be directly proportional to the protein concentration in the incubation medium. The transport of the peptide may be described by

$$\frac{dM_{abs}}{dt} = A\, P_{eff}\, C = k_{abs}\, C \qquad (1)$$

where M_{abs} is the amount of peptide absorbed per mg protein, t is the time, A is the area of the absorbing surface, P_{eff} is the effective membrane permeability and C is the concentration of the peptide in the incubation fluid. A and P_{eff} can be combined to give k_{abs}, the apparent first-order absorption rate constant. The constant k_{abs} was determined from the slope of the uptake-rate vs peptide concentration plot (Fig. 8). The formation rate of the metabolite M_{met} is given by:

$$V\frac{dC_{met}}{dt} = d\frac{M_{met}}{dt} = \frac{V_{max}C}{K_m + C} \qquad (2)$$

where V is the volume and the incubation medium, C_{met} is the metabolite concentration in the incubate and V_{max} and K_m are the parameters of the Michaelis–Menten equation. V_{max} and K_m were 12·47 μg min^{-1} (mg protein)$^{-1}$ and 113·5 μg mL^{-1}, respectively.

The time profiles of the absorbed and metabolized fractions of the dose are shown in Fig. 8. Simulations utilizing the intrinsic parameters of membrane transport and metabolism show that metabolism is clearly the dominating process which eventually leads to 96·5% of the dose being metabolized and 3·5% being absorbed. There are various strategies to increase the fraction of the peptide absorbed intact which can be shown in the following simulations: the fraction absorbed may be increased by an increase in the absorption velocity, e.g. by a change in the physicochemical parameters of the peptide or by other techniques such as a change in the membrane permeability of the peptide or by the addition of permeation enhancers. Thus, an increase in the absorption rate constant k_{abs} will lead to a corresponding increase in the fraction absorbed. Similarly, a change in the parameters of the metabolic process will also affect the fraction of the peptide dose absorbed intact. An increase in the Michaelis–Menten constant, e.g. in the case of concurrently added competitive enzyme inhibitors, will increase F_{abs}. Finally F_{met} will be decreased and F_{abs} conversely increased when the maximum rate of metabolism, V_{max} is decreased. Since V_{max} is proportional to the catalytic rate constant k_{cat} and the available enzyme concentration E_o, a decrease in V_{max} in the gastrointestinal tract may be given by changes in the distribution of metabolizing enzymes e.g. in the longitudinal direction of the intestinal tract.

Conclusions

Using the brush-border membrane vesicle model, transport as well as metabolism of a pentapeptide in the intestine has been demonstrated. Modelling of the simultaneous diffusion and saturable metabolism processes clearly indicate that strategies to deliver the peptide to an absorption site with little enzymatic degradation, and measures to increase the absorption velocity of the compound, will be successful in increasing the fraction of the peptide absorbed intact.

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References

Adibi, S. A., Kim, Y. S. (1981) Peptide absorption and hydrolysis. In: Johnson, L. R. (ed.) Physiology of the Gastrointestinal Tract. Raven Press, New York, pp 1073–1095

Biber, J., Murer, H. (1991) Methodological guide for studying epithelial transport with isolated membrane vesicles. In: Yudilevich, D. L. (ed.) Cell Membrane Transport. Plenum Press, New York, pp 163–186

Fricker, G., Drewe, J., Vonderscher, J., Kissel, T., Beglinger, C. (1992) Enteral absorption of octreotide. Br. J. Pharmacol. 105: 783–786

Friedman, D. I., Amidon, G. L. (1989) Intestinal absorption mechanism of dipeptide angiotensin converting enzyme inhibitors of the lysyl-proline type: lisinopril and SQ 29, 852. J. Pharm. Sci. 78: 995–998

Geary, L. E., Wiley, K. S., Scott, W. L., Cohen, M. L. (1982) Degradation of exogenous enkephalin in the guinea-pig ileum: relative importance of aminopeptidase, enkephalinase and angiotensin converting enzyme activity. J. Pharmacol. Exp. Ther. 221: 104–111

Gray, G. M. (1989) Dietary protein processing: intraluminal and enterozyte surface events. In: Forte, G. (ed.) Handbook of Physiology: The Gastrointestinal System. Vol III, Oxford University Press, pp 411–420

Hauser, H., Howell, K., Dawson, R. M. C., Bowyer, D. E. (1980) Rabbit small intestinal brush border membrane preparation and lipid composition. Biochim. Biophys. Acta 602: 567–577

Hopfer, U., Nelson, K., Perotto, L., Isselbacher, K. J. (1973) Glucose transport in isolated brush border membranes from rat small intestine. J. Biol. Chem. 248: 25–32

Kashi, S. D., Lee, V. H. L. (1986) Enkephalin hydrolysis in homogenates of various absorptive mucosae of the albino rabbit: similarities in rates and involvement of aminopeptidases. Life Sci. 38: 2019–2028

Kenny, A. J., Stephenson, S. L., Turner, A. J. (1987) Cell surface peptidases. In: Kenny, A. J., Turner, A. J. (eds) Mammalian Ectoenzymes. Elsevier, pp 169–210

Kessler, M., Acusto, O., Storelli, C., Murer, H., Müller, M., Semenza, G. (1978) A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestine brush border membranes. Biochim. Biophys. Acta 506: 136–154

Kramer, W., Girbig, F., Gutjahr, U., Kleemann, H. W., Leipe, I., Urbach, H., Wagner, A. (1990) Interaction of renin inhibitors with the intestinal uptake system for oligopeptides and β-lactam antibiotics. Biochim. Biophys. Acta 1027: 25–30

Matthews, D. M. (1976) Peptide absorption. Gastroenterology 71: 151–161

Mathews, D. M. (1983) Intestinal absorption of peptides. Biochem. Soc. Trans. 11: 808–810

Murer, H., Kinne, R. (1980) The use of isolated membrane vesicles to study epithelial transport processes. J. Membr. Biol. 55: 81–95

Schwarz, J.-C., Malfroy, B., De La Baune, S. (1981) Biological inactivation of enkephalins and the role of enkephalin-dipeptidyl-carboxypeptidase ("Enkephalinase") as neuropeptidase. Life Sci. 29: 1715–1740

Stieger, B., Murer, H. (1983) Heterogeneiety of brush border membrane vesicles from rat small intestine prepared by a precipitation method using Mg/EDTA. Eur. J. Biochem. 135: 95–101

Stratford, R. E., Lee, V. H. (1986) Aminopeptidase activity in homogenates of various absorptive mucosae in the albino rabbit: implications in peptide delivery. Int. J. Pharm. 30: 73–82

Su, K. S. E., Campanale, K. M., Mendelsohn, L. G., Kerchner, G. A., Gries, C. L. (1985) Nasal delivery of polypeptides I: nasal absorption of enkephalins in rats. J. Pharm. Sci. 74: 394–398

Sugawara, M., Iseki, K., Miyazaki, K. (1991) H^+ coupled transport of orally active cephalosporins lacking an α-amino group across brush-border membrane vesicles from rat small intestine. J. Pharm. Pharmacol. 43: 433–435

Thorsett, E.D., Wyvratti, M. J. (1987) Inhibition of zinc peptidases that hydrolyze neuropeptides. In: Turner, A. J. (ed.) Neuropeptides and Their Peptidases. Verlag Chemie, Weinheim, pp 229–292

Tsuji, A., Nakashima, E., Kagami, I., Yamana, T. (1981) Intestinal absorption mechanism of amphoteric β-lactam antibiotics I: comparative absorption and evidence for saturable transport of amino-β-lactam antibiotics by in situ rat small intestine. J. Pharm. Sci. 70: 768–772

Tsuji, A., Tamai, I., Hirooka, H., Terasaki, T. (1987) β-Lactam antibiotics and transport via the dipeptide carrier system across the intestinal brush-border membrane. Biochem. Pharmacol. 36: 565–567

Tsuji, A., Tamai, I., Nakanishi, M., Amidon, G. L. (1990) Mechanism of absorption of the dipeptide α-methyldopa-Phe in intestinal brush-border membrane vesicles. Pharm. Res. 7: 308–309

Turner, A. J., Hooper, N. M., Kenny, A. J. (1987) Metabolism of neuropeptides. In: Turner, A. J., Kenny, A. J. (eds) Mammalian Ectoenzymes. Elsevier, pp 211–248

Wiedhaup, K. (1981) The stability of small peptides in the gastrointestinal tract. In: Breimer, D. D., Speiser, P. (eds) Topics in Pharmaceutical Sciences. Elsevier, Amsterdam, pp 307–324