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Prodrugs of peptides. 15. 4-Imidazolidinone prodrug derivatives of enkephalins to prevent aminopeptidase-catalyzed metabolism in plasma and absorptive mucosae

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Summary

Aminopeptidase-catalyzed hydrolysis of Leu-enkephalin and Met-enkephalin is responsible for the rapid metabolism of these pentapeptides in human plasma and at various mucosal sites. The objective of this study was to circumvent this metabolic inactivation by the prodrug approach. A series of 4-imidazolidinone derivatives were prepared by condensing the enkephalins with various aldehydes and ketones and their hydrolysis kinetics were studied in aqueous solution and in the presence of enzymes. Whilst the enkephalins were rapidly hydrolyzed by a purified aminopeptidase and in both human plasma solutions and rabbit intestinal homogenates, the 4-imidazolidinone derivatives were almost totally resistant to enzymatic cleavage in these media. On the other hand, these derivatives are readily converted to the parent peptides by spontaneous hydrolysis. The rate of hydrolysis depended on the structure of the carbonyl component, being apparently increased with increasing steric effects within this component. Thus, the derivative prepared from cyclopentanone showed a half-life of hydrolysis of 3.1 h at pH 7.4 and 37°C whereas the compound made from propionaldehyde had a half-life of 149 h. It is concluded that 4-imidazolidinone formation may be a useful prodrug approach to protect the N-terminal amino acid residue of enkephalins against cleavage by aminopeptidases and to obtain transport forms with improved lipophilicity.

Introduction

Peptides are becoming an important new class of drugs, however, their application as clinically useful compounds is seriously hampered due to substantial delivery problems. Peptides in general show poor biomembrane penetration characteristics and rapid enzymatic degradation at the absorption sites as well as in the systemic circulation (Humphrey and Ringrose, 1986; Lee and Yamamoto, 1990). In recent years, we have been studying the prodrug approach as a means to solve or diminish these delivery problems of peptide drugs (for a review, see Bundgaard, 1991). The strategy in this approach is conversion of the peptides into prodrug or transport forms which are more lipophilic than the parent peptides and capable of protecting these against proteolytic degradation at the mucosal absorption barrier or in the blood but at the same time also being

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capable of reverting to the parent peptides following absorption by a spontaneous or unspecific plasma enzyme-catalyzed reaction.

In a previous work (Rasmussen and Bundgaard, 1991), we have shown that 4-imidazolidinone formation may be a useful approach to protect the N-terminal amino acid residue of di- and tripeptides against cleavage by aminopeptidases. 4-Imidazolidinones are readily formed by condensing compounds containing an α-aminoamide moiety such as peptides with a free N-terminal amino group with aldehydes or ketones (Scheme 1) (for references, see Klixbüll and Bundgaard, 1984). The usefulness of these derivatives as prodrug forms is due to their ready hydrolysis in aqueous solution with release of the parent peptide, the rate of hydrolysis being dependent on the pH and the structure of the R_3 and R_4 substituents (Scheme 1), i.e. the structure of the parent peptide. Whilst this structural influence has previously been delineated (Klixbüll and Bundgaard, 1984; Rasmussen and Bundgaard, 1991), information is only sparsely available on the effect of the R_1 and R_2 substituents on the rate of hydrolysis (Klixbüll and Bundgaard, 1985).

This study was aimed at obtaining such knowledge using the pentapeptides leucine and methionine enkephalin (**I** and **II**) as model peptides. To this end, we have studied the kinetics of hydrolysis of various 4-imidazolidinones formed with different aldehydes and ketones (**III–XI**) in aqueous buffer solutions. To obtain information on the possible capability of the derivatives to protect the enkephalins against enzymatic degradation, stability studies were also performed in human plasma, rabbit intestinal homogenates and in buffer solutions containing leucine aminopeptidase and other proteolytic enzymes. Finally, the lipophilicity characteristics of the derivatives were determined.

Materials and Methods

Apparatus

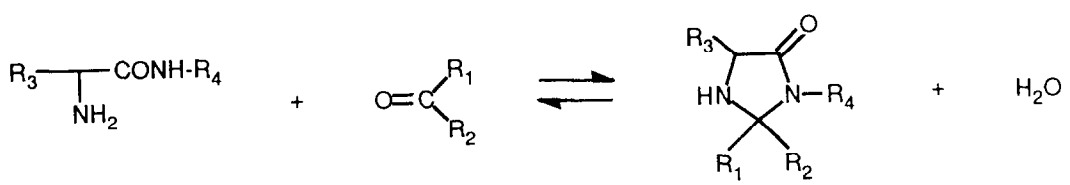
High-performance liquid chromatography (HPLC) was carried out with a system consisting of a Shimadzu pump model LC-6A, a Shimadzu SPD-6A variable-wavelength UV detector and a Rheodyne 7125 injection valve with a 20 μl loop. Two different reversed-phase columns were used: a deactivated Supelcosil LC-8-DB column (33 × 4.6 mm; 3 μm particles) with a Supelguard precolumn (both from Supelco Inc., U.S.A.) and a Chrompack column (100 × 3 mm) packed with Chromsphere C-18 (5 μm particles). Readings of pH were carried out on a Radiometer PHM Autocal instrument.

Chemicals

Leucine enkephalin, methionine enkephalin, L-tyrosine, Gly-Gly-Phe-Leu and other peptides (all having the L configuration), were purchased from Bachem AG, Budendorf, Switzerland or Sigma Chemical Co., St. Louis, MO, U.S.A. Leucine aminopeptidase (cytosol, type III-CP; from porcine kidney), carboxypeptidase A (type I; from bovine pancreas) and α-chymotrypsin (type II; from bovine pancreas, 56 units/mg) were obtained from Sigma. Buffer substances and all other chemicals and solvents used were of reagent grade.

Preparation of 4-imidazolidinones

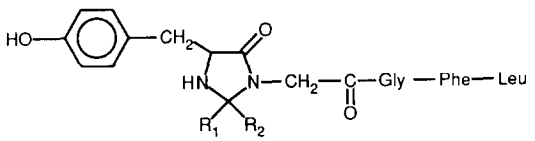
The 4-imidazolidinone derivatives of Leu-enkephalin and Met-enkephalin (**III–XI**) were prepared in situ by adding an excess of the appropriate aldehyde or ketone to a solution of 3 mg of the enkephalins in 5 ml of 0.01 M phosphate buffer of pH 7.4. This procedure has previously been described for the preparation of the 4-imidazolidinones derived from the enkephalins and

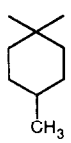


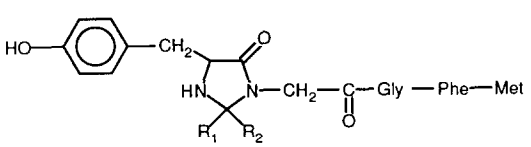
Scheme 1.

I Tyr —Gly —Gly —Phe— Leu (Leu-enkephalin)

II Tyr —Gly —Gly —Phe—Met (Met-enkephalin)



	R_1	R_2
III	$-CH_3$	—H
IV	$-C_2H_5$	—H
V	$-CH_3$	$-CH_3$
VI	$-C_2H_5$	$-CH_3$
VII	$-CH(CH_3)_2$	$-CH_3$
VIII		
IX		
X		



	R_1	R_2
XI	$-CH_3$	$-CH_3$

acetaldehyde (Summers et al., 1980; Summers and Lightman, 1981). The amounts used of the carbonyl compounds were 0.5 ml of acetaldehyde, propionaldehyde, 2-butanone, 3-methyl-2-butanone, cyclohexanone and 1 ml of acetone, cyclopentanone and 4-methylcyclohexanone.

The mixtures were stirred at room temperature and the reaction progress followed by HPLC. The peak due to the enkephalins disappeared gradually with the simultaneous appearance of a new peak with a greater retention time. No other peaks were seen. The conversion of the enkephalins to their 4-imidazolidinone derivatives was complete after about 24 h. In all cases, the reaction mixture contained less than 10% of unreacted enkephalin. This reaction mixture containing a large molar excess of aldehyde or ketone remained stable for several days at room temperature.

Kinetic measurements

Hydrolysis in buffer solutions. The decomposition of the 4-imidazolidinone derivatives was studied in aqueous buffer solution at 37°C. The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate solutions. The total buffer concentration generally used was 0.02 M. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The rates of decomposition were determined by using HPLC procedures capable of separating the derivatives from their parent peptides. Mobile phase systems of 0.1% phosphoric acid containing acetonitrile (15–30% v/v) and methanol (0–5% v/v) were used, the acetonitrile and methanol concentration being adjusted for each compound to give a retention time of 2–7 min. Triethylamine was added to the mobile phases at a concentration of 10^{-3} M in order to improve the peak shape. In all cases, the 4-imidazolidinone derivative was eluted later than the parent enkephalin. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 215 nm. Quantitation of the compounds was carried out by measuring the peak heights.

The reactions were initiated by adding 50–100 μl of the above-mentioned reaction solution to 10

ml of buffer solution, pre-equilibrated at 37°C, in screw-capped test tubes. The solutions were kept in a water-bath at 37°C and at appropriate times, samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual derivative against time.

Hydrolysis in plasma, gut homogenate and enzyme solution. The decomposition of some of the 4-imidazolidinone derivatives as well as of Met-enkephalin and Leu-enkephalin was studied at 37°C in 80% human plasma, 10% rabbit intestinal homogenate and in 0.05 M phosphate buffer solutions (pH 7.4) containing leucine aminopeptidase, α-chymotrypsin or carboxypeptidase A at a concentration of 20, 70 and 1.0 U ml^{-1}, respectively. The intestinal homogenate was prepared as described by Møss et al. (1990). The decomposition of Met-enkephalin and Leu-enkephalin was initiated by adding 50 μl of a stock solution of the peptides in methanol to 5 ml of the test solutions, the initial concentration being 10^{-4} M. The reactions of the 4-imidazolidinones were started by adding 100 μl of the reaction solutions described above to 5 ml of the test solutions. The reaction mixtures were kept in a water-bath at 37°C and at appropriate intervals samples of 100 μl were withdrawn and added to 100 μl of a 2% (w/v) solution of zinc sulfate in methanol-water (1:1 v/v) to deproteinize the samples and stop the reactions. After immediate mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed by HPLC for remaining 4-imidazolidinone or peptide as described above. Pseudo-first-order rate constants for the degradation were determined as described above.

Determination of partition coefficients

The apparent partition coefficients (P) of the peptides and their derivatives were determined in octanol-water systems at 20–22°C. The aqueous phase was a 0.05 M phosphate buffer solution of pH 7.40. The buffer solution and octanol were mutually saturated before use. The compounds were dissolved in the aqueous buffer phase and the octanol-buffer mixtures were shaken for 15 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after extraction, could readily be measured by HPLC. Centrifugation was used to separate the two phases. The partition coefficients were calculated from

$$P = \left(\frac{C_i - C_w}{C_w}\right)\left(\frac{V_w}{V_o}\right) \quad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous phase before and after distribution, respectively, V_w is the volume of the aqueous phase, and V_o the volume of the octanol phase.

Results and Discussion

Kinetics of hydrolysis

The kinetics of hydrolysis of various 4-imidazolidinone derivatives of Leu-enkephalin and Met-enkephalin (**III–XI**) was studied in aqueous solution at 37 or 60°C over the pH range 1–10. However, some derivatives were studied only at pH 7.40. Under the experimental conditions used, all derivatives were hydrolyzed completely to the parent enkephalin as revealed by HPLC analysis. At constant pH and temperature, the hydrolysis displayed strict first-order kinetics over several half-lives (cf. Fig. 1). No general acid-base catalytic effect was noted by the buffers used to maintain a constant pH at concentrations up to 0.05 M.

The influence of pH on the rate of hydrolysis is shown for a number of compounds in Figs 2 and 3 where the logarithms of the observed pseudo-first-order rate constants (k_{obs}) are plotted against pH. Except for the 4-imidazolidinone derived from propionaldehyde (**IV**), the derivatives showed pH-rate profiles having a sigmoid shape with maximum and constant rates at pH above about 4. These sigmoidal pH-rate profiles

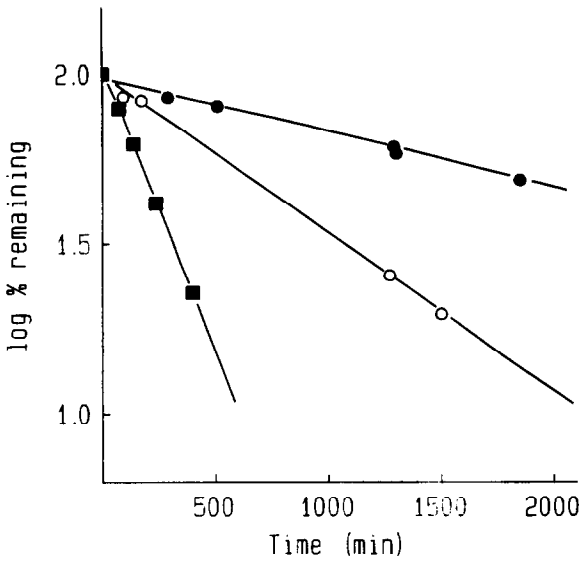


Fig. 1. First-order plots for the hydrolysis in 0.02 M phosphate buffer pH 7.4 of compounds **III** (●), **V** (○) and **VIII** (■).

are similar to those for the hydrolysis of 4-imidazolidinones derived from acetone and various di- and tripeptides (Klixbüll and Bundgaard, 1984;

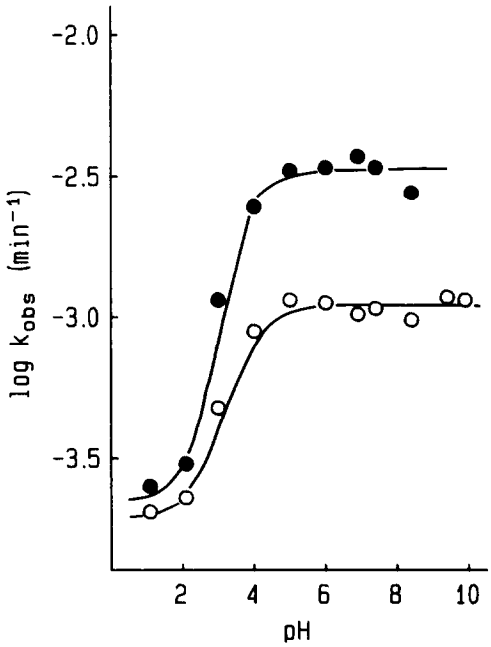


Fig. 2. The pH-rate profile for the hydrolysis of the 4-imidazolidinones **V** (○) and **VIII** (●) in aqueous solutions at 37°C.

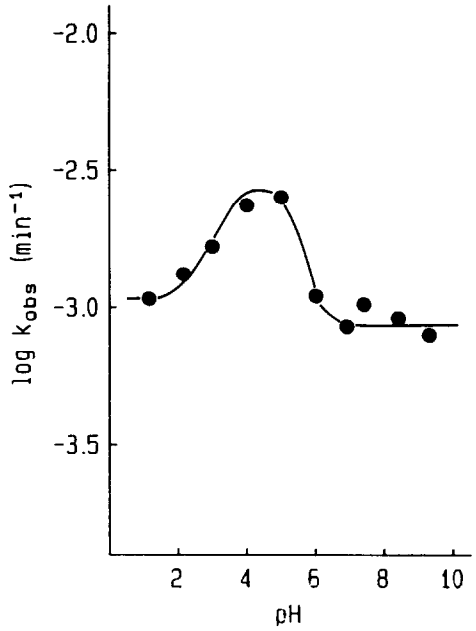


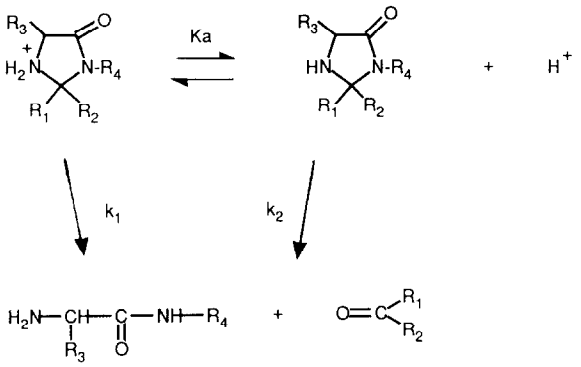
Fig. 3. The pH-rate profile for the hydrolysis of the 4-imidazolidinone **IV** in aqueous solution at 60°C.

Rasmussen and Bundgaard, 1991) and can be accounted for, as described in these previous papers, by the following rate expression:

$$k_{obs} = \frac{k_1 a_H}{a_H + K_a} + \frac{k_2 K_a}{a_H + K_a} \quad (2)$$

where a_H is the hydrogen ion activity, K_a is the ionization constant of the protonated imidazolidinone and k_1 and k_2 are apparent first-order rate constants for the spontaneous or water-catalyzed degradation of the protonated or unprotonated species, respectively, of the 4-imidazolidinone (Scheme 2). The values of k_1, k_2 and pK_a derived from the pH-rate profiles and Eqn 2 are listed in Table 1. The half-lives of hydrolysis of all the derivatives studied at pH 7.4 and 37°C are given in Table 2.

As previously discussed (Klixbüll and Bundgaard, 1984, 1985), the hydrolysis of 4-imidazolidinones most likely proceeds through a Schiff base intermediate being in equilibrium with the imidazolidinone. A change in the rate-determining step of the overall hydrolysis of compound **IV**



Scheme 2.

TABLE 1

Rate data for the hydrolysis of various 4-imidazolidinone derivatives of Leu-enkephalin and Met-enkephalin in aqueous solution at 37°C

4-Imidazolidinone	k_1 (min^{-1})	k_2 (min^{-1})	pK_a
V	2.0×10^{-4}	9.4×10^{-4}	3.3
VIII	2.2×10^{-4}	3.4×10^{-3}	3.4
IX	2.5×10^{-4}	2.7×10^{-3}	3.3
XI	1.6×10^{-4}	8.8×10^{-4}	3.3

with pH may account for its bell-shaped pH-rate profile (cf. Fig. 3).

The rate data obtained show that the nature of the 2-substituents (R_1 and R_2) in the 4-imidazolidinones and hence the structure of the carbonyl compound used in preparation of the derivatives has a pronounced influence on the stability at physiological pH where the k_2 reaction is predominant. The compounds derived from acetaldehyde (**III**) and propionaldehyde (**IV**) are seen to be much more stable than those derived from ketones. This indicates that the steric properties of the 2-substituents may be a predominant factor. This apparent relationship of increased reactivity with increasing steric properties within the 2-substituents is also seen for the compounds **III**, **V**, **VI**, and **VII** (all containing one methyl substituent), where the order of reactivity parallels the steric properties of the R_1 substituent as shown in Fig. 4 where the logarithm of the half-life of hydrolysis at pH 7.4 at 37°C has been plotted against Charton's steric parameter (ν). The most reactive derivatives are those derived from the cyclic ketones cyclopentanone (**VIII**) and cyclohexanone (**IX**). 4-Imidazolidinones can be considered as cyclic *N*-Mannich bases in which the amide and amino functions are placed in the same molecule. The reactivity of acyclic *N*-Mannich bases (*N*-(aminomethyl)amides) in neutral and basic solutions has been shown to increase with increasing steric effects within the amine moiety, probably through decreased solvation of

TABLE 2

Half-lives of hydrolysis of enkephalins and their 4-imidazolidinone derivatives in various media (all containing 0.02–0.05 M phosphate buffer of pH 7.40) at 37°C

Compound	Half-life					
	pH 7.4 buffer	80% human plasma	10% rabbit intestinal homogenate	Amino-peptidase (20 U ml^{-1})	Carboxypep-tidase A (1.0 U ml^{-1})	α-Chymotryp-sin (70 U ml^{-1})
I (Leu-enkephalin)		6.0 min	1.2 min	10.0 min	2.0 min	8.3 h
II (Met-enkephalin)		5.7 min	3.8 min	5.3 min	6.2 min	9.0 h
III	30 h					
IV	149 h					
V	10.9 h	23.5 h	5.5 h	11.9 h	0.9 min	14.6 h
VI	7.6 h					
VII	5.9 h					
VIII	3.1 h	3.1 h	1.7 h	3.0 h	0.7 min	
IX	5.5 h	5.7 h	2.4 h	4.8 h		
X	11.8 h					
XI	12.4 h	21.3 h	1.3 h	17.3 h	10 min	8.2 h

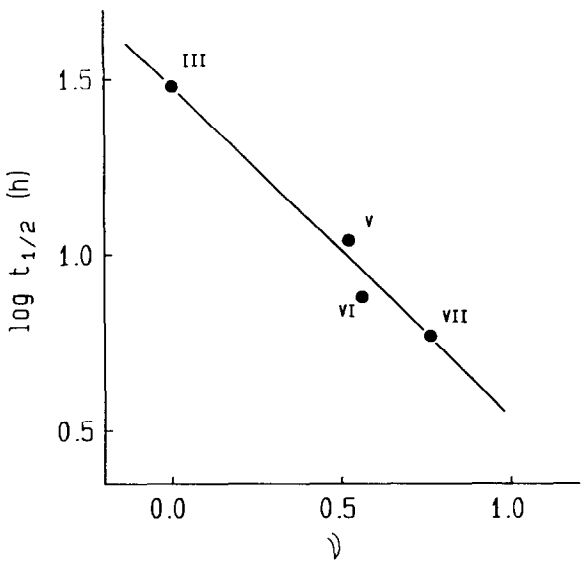


Fig. 4. Plot of the logarithm of the half-life of hydrolysis of the 4-imidazolidinones **III**, **V**, **VI** and **VII** at pH 7.4 and 37°C against the steric parameter ν. The regression equation for the plot is $\log t_{1/2} = 1.48 - 0.95\nu$ ($n = 4$; $r = 0.988$). The values of ν were taken from Charton (1977).

the amine nitrogen resulting in increased nucleophilicity (Bundgaard and Johansen, 1980a,b). The steric effect seen with the 2-substituents in the 4-imidazolidinones may be similarly explained.

Hydrolysis of enkephalins and their 4-imidazolidinone derivatives in the presence of enzymes

The enkephalins are rapidly degraded in the organism by various enzymes as illustrated in Fig. 5, the dominating route of metabolism being dependent on both species and tissue type (Schwartz et al., 1981; Geary et al., 1982; Hersh, 1982; Roscetti et al., 1985; Venturelli et al., 1985; Roda et al. 1986; Shibanoki et al., 1990, 1991; Friedman and Amidon, 1991).

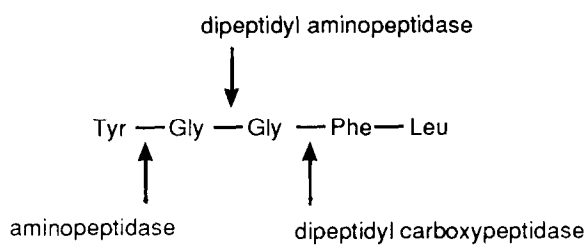


Fig. 5. Enzymatic degradation pathways of Leu-enkephalin.

The major route (> 85%) of degradation in homogenates of ileal, nasal, rectal, vaginal and buccal mucosae from the rabbit has been shown to be cleavage of the Tyr-Gly bond by aminopeptidases (Kashi and Lee, 1986a). The same has been found for the degradation of Leu- or Met-enkephalin in the nasal tissue of the rat (Hussain et al., 1985; Faraj et al., 1990), guinea-pig ileum (Geary et al., 1982), eye tissue homogenates of the rabbit (Kashi and Lee, 1986b) and in cultured keratinocytes and mouse skin (Choi et al., 1990; Shah and Borchardt, 1991). In human plasma or blood the predominant (> 90%) route of degradation of Leu-enkephalin has also been reported to be hydrolysis of the N-terminal Tyr-Gly bond by aminopeptidases (Roscetti et al., 1985; Venturelli et al., 1985). Thus, aminopeptidases play the major role in the enzymatic inactivation of enkephalins and circumvention of aminopeptidase-catalyzed metabolism therefore seems to be mandatory in order to improve the delivery of these peptides.

In agreement with these previous investigations, we found that the enkephalins were rapidly degraded in 80% human plasma solutions, 10% rabbit intestinal homogenate and in a 0.05 M phosphate buffer solution containing leucine aminopeptidase at a concentration of 20 U ml^{-1}. Under the experimental conditions used, the degradation of the peptides proceeded according to first-order kinetics. Examples of typical first-order plots are shown in Fig. 6. The half-lives of hydrolysis observed are listed in Table 2. The values in human plasma are similar to those found by other investigators (Roscetti et al., 1985; Boarder and McArdle, 1986). HPLC analysis of the plasma solutions revealed the almost complete (> 95%) conversion of Leu-enkephalin to Gly-Gly-Phe-Leu as seen from Fig. 7. In Fig. 7, the full curve for Gly-Gly-Phe-Leu was constructed on the basis of complete conversion of Leu-enkephalin to this compound and a rate constant of 2.3×10^{-2} min^{-1} for the subsequent degradation of the peptide as determined in a separate experiment.

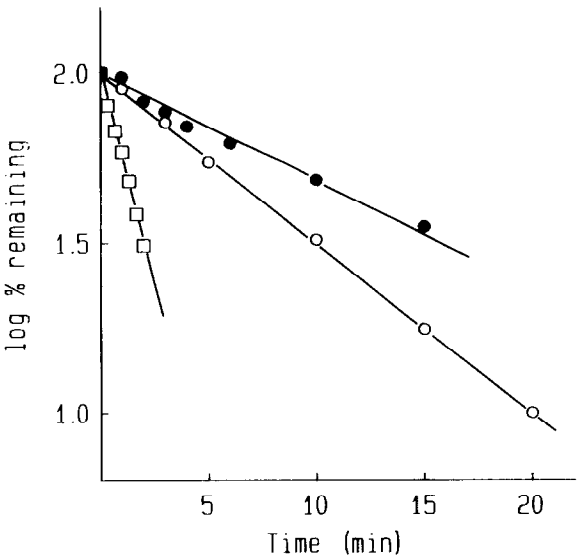


Fig. 6. First-order plots for the hydrolysis of Leu-enkephalin (I) in 80% human plasma (○), 10% rabbit intestinal homogenate (□) and 0.05 M phosphate buffer (pH 7.4) containing leucine aminopeptidase (20 U ml^{-1}) (●) at 37°C.

The kinetics of degradation of the 4-imidazolidinones **V**, **VIII**, **IX** and **XI** was determined under similar conditions. These compounds also degraded according to first-order kinetics, the half-lives obtained being listed in Table 2. As seen from the data, the rates of degradation of the 4-imidazolidinones do not increase in human plasma or aminopeptidase solutions but either remain the same or become even slower than the rates of hydrolysis in buffer solutions of the same pH and temperature. Binding of the compounds to proteins may be responsible for the slower hydrolysis of compounds **V** and **XI** in plasma than in buffer solution.

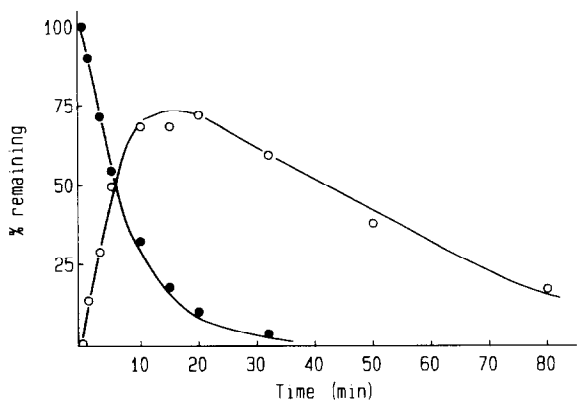


Fig. 7. Time courses for Leu-enkephalin (●) and Gly-Gly-Phe-Leu (○) during the degradation of Leu-enkephalin in 80% human plasma at 37°C.

These data demonstrate that it is feasible to protect fully the enkephalins against aminopeptidase-catalyzed cleavage by forming a 4-imidazolidinone derivative at their N-terminal α-aminoamide moiety. The consequences of this derivatization are that the primary amino group is transformed into a secondary amino group and the vulnerable Tyr-Gly bond becomes alkylated, both modifications making the peptide a poor substrate for aminopeptidase and other exopeptidase-cleaving peptides at their N-terminals (Delange and Smith, 1971).

As further seen from Table 2, the 4-imidazolidinone derivatives are also much more stable than their parent enkephalins in a rabbit intestinal homogenate. Complete protection against enzymatic degradation, however, is not achieved as the half-lives in the homogenate are slightly shorter than those in the buffer solutions. This may be due to the presence of either a dipeptidyl carboxypeptidase activity in the gut homogenate against which the 4-imidazolidinone derivatization may not afford protection or, more likely, the luminal proteolytic enzyme carboxypeptidase A in the homogenate. As can be seen from Table 2, this exopeptidase rapidly degrades the enkephalins. Carboxypeptidase A is a pancreatic proteolytic enzyme that catalyzes the hydrolysis of almost any peptide having a terminal free carboxyl group and a C-terminal residue of the L-configuration (Hartsuck and Lipscomb, 1971). The catalytic effect is enhanced if the terminal residue is aromatic or, as in the enkephalins, branched aliphatic (Hartsuck and Lipscomb, 1971). The data in Table 2 show that 4-imidazolidinone derivatization at the N-terminal part of the enkephalins offers no protection against this C-terminal exopeptidase as is also to be expected.

The facile degradation of the enkephalins by carboxypeptidase A has apparently not been described before, and yet, based on the present data, such degradation may be of at least equal

importance to that by brush border enzymes (Friedman and Amidon, 1991) for the poor oral absorption of these peptides. Conversion of the C-terminal free carboxyl groups in the enkephalins to esters may be a simple bioreversible means to protect the C-terminal peptide bond against carboxypeptidase A.

The finding with carboxypeptidase A led us to examine the stability of the compounds against another pancreatic proteolytic enzyme, α-chymotrypsin. At a concentration (70 U ml^{-1}) twice that normally prevailing in the gut (Goldberg et al., 1968), this enzyme was found to catalyze the hydrolysis of the enkephalins, the half-lives of hydrolysis being 8–9 h (Table 2). The half-lives of hydrolysis of the derivatives **V** and **XI** were found to be about the same in the presence of this enzyme and in buffer solutions without the enzyme (Table 2). These data show that the enkephalins are poor substrates for α-chymotrypsin and that degradation of the peptides by this protease does not play any important role in their oral absorption.

Lipophilicity of the 4-imidazolidinones

The apparent partition coefficients (P) for the two enkephalins and some 4-imidazolidinone prodrugs were measured using an octanol-aqueous buffer system (pH 7.4). The values determined for log P were −0.99 (**I**), −1.57 (**II**), −1.23 (**V**), −0.58 (**VII**), 0.07 (**IX**), 0.23 (**X**) and −1.65 (**XI**). Obviously, the type of carbonyl component used in the imidazolidinone formation greatly influences the lipophilicity exhibited by its substituents. The increased lipophilicity of the derivatives relative to the parent peptides also results from the large decrease in basicity of the N-terminal amino group brought about by the derivatization. Whilst the pK_a of the amino group in enkephalins is in the range 7–8 it is only about 3.3 (cf. Table 1) in the derivatives. It is interesting to note, however, that the imidazolidinones derived from acetone (**V** and **XI**) both show a slightly lower log P value than the unmodified enkephalins. This may indicate that the partitioning of the enkephalins at pH 7.4 has some contribution from ion-pair formation of their protonated forms with the phosphate anions present in the aqueous phase.

Conclusions

Along with previously described findings (Rasmussen and Bundgaard, 1991), the results of the present study show that 4-imidazolidinone derivatization may be a useful prodrug approach to protect the N-terminal amino acid residue of enkephalins and other peptides against enzymatic cleavage by aminopeptidases such as those present in plasma and at various mucosal absorption sites. Such cleavage has been reported to be the principal route of metabolism of enkephalins in various mucosae. The derivatization is readily bioreversible, the parent peptide being formed by spontaneous hydrolysis at physiological pH and temperature at a rate that is influenced by the steric properties of the 2-substituents and hence the carbonyl component used in forming the imidazolidinone.

For the enkephalins, a half-life of 4-imidazolidinone hydrolysis of 3.1 h at pH 7.4 and 37°C can be achieved by using cyclopentanone for the derivatization whereas aldehydes afford much longer half-lives. As shown by octanol-buffer partitioning experiments, the type of carbonyl component used in the preparation of the prodrug further influences the lipophilicity exerted by its substituents. It is readily feasible to obtain an enkephalin prodrug with increased lipophilicity and an appropriate rate of conversion to the parent peptide.

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