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Hexarelin – evaluation of factors influencing oral bioavailability and ways to improve absorption

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Abstract

Hexarelin, a hexapeptide with growth hormone-releasing activity, has been found in man to have a biological bioavailability (estimated from growth hormone levels) of $0.3 \pm 0.1\%$ after oral administration. The cause of the low oral efficacy of hexarelin and means of improving its absorption have been evaluated. It was found that hexarelin was degraded in the presence of the contents of the intestine. The metabolite was identified as hexarelin deamidated at the lysine residue. The degradation of hexarelin in the contents of rat ileum was inhibited by the addition of chymostatin, Pefabloc SC, EDTA, and EGTA. Furthermore, the presence of pancreatic proteases from pancrease substitute drugs caused a degradation of hexarelin that could be inhibited by the addition of Pefabloc SC. The same hexarelin metabolite that was found with the contents of rat ileum was found in the presence of human, porcine and bovine trypsin. Hexarelin permeability across rat ileum and in Caco-2 cell monolayers was low. An increase in hexarelin permeability was observed in the presence of different permeability enhancing agents.

Introduction

There are an increasing number of peptides and proteins exhibiting therapeutic potential at the development and commercialization stages. However, formulating these new drugs for oral administration is often difficult due to physical and/or enzymatic degradation and poor membrane permeation (Lee & Yamamoto 1990; Aungst 1993; Fix 1996). The properties of peptide and protein drugs connected with poor membrane permeation are high molecular weight, low lipid partitioning, and the amount of hydrogen bonding (Aungst 1993; Fix 1996).

There are numerous enzymes or enzyme systems throughout the body which are able to degrade peptides and proteins. For example, the human pancreas possesses the capacity to produce vast amounts of different protein e.g. proteases (Rinderknecht 1986). It is a storage site for proteolytic serine proteases such as chymotrypsin, elastase and trypsin (Rinderknecht 1986; Lee et al 1991). Serine proteases, as described by Lee & Traver (1991), are endopeptidases and so cleave peptide bonds internally. The combined activity of α-chymotrypsin, elastase and trypsin is able to cleave almost all of the internal peptide linkages in peptides and proteins (Lee et al 1991).

Low permeability of orally administered peptide and protein drugs may be overcome with the use of enhancers. Enhancers, as described by Lee (1990), act by

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diverse mechanisms, including fluidization of the plasma membrane, loosening of the tight junctions, inhibition of proteases, and increasing the thermodynamic activity of proteins prone to self-association. Widely used methods of determining permeability are transport studies in in-vitro models such as excised intestinal epithelial tissue in Ussing chambers and the Caco-2 cell monolayers. In both models the accumulated amount of drug permeating the epithelium is analysed as a function of time and the results are the basis for calculating the apparent permeability (P_{app}) of the drug.

Hexarelin (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2) is a hexapeptide with growth hormone-releasing activity (Ghigo et al 1994; Imbimbo et al 1994) and its chemical structure is as described by Smith et al (1994). Biological bioavailability (estimated from growth hormone levels) was found to be 0.3 ± 0.1 % after oral administration (Ghigo et al 1994).

We have evaluated the cause of the low oral efficacy of hexarelin and the means of improving its absorption. We have investigated the stability of hexarelin in the intestine, and aimed to identify possible degradation mechanisms and to determine the apparent permeability (P_{app}) of hexarelin. Furthermore, by utilizing the results obtained, the possibility of enhancing the stability and/or permeability of hexarelin by formulation additives was investigated. Several protease inhibitors were evaluated for their ability to increase the stability of the peptide.

Materials and Methods

Materials

Hexarelin was obtained from Pharmacia & Upjohn's internal product supply. It has a molecular weight of 887 Da and pK_a values of 5.2, 7.2 and 10.1 at ionic strength 0.15 mM (Fagerholm et al 1998). The reference substance [^{14}C]mannitol was obtained from Dupont, NEN, Boston, MA. Additives were: α-cyclodextrin (Pharmacia & Upjohn internal product supply), methyl-β-cyclodextrin, taurocholic acid, L-phosphatidylcholine, sodium caprate, and palmitoyl carnitine (Sigma, St Louis, MO), ethylenediaminetetraacetic acid (EDTA) (Triplex III, Merck, Darmstadt, Germany), polycarbophil and Carbopol 934 (BF Goodrich, Cleveland, OH) and Biosomes, a mixture of soybean phosphatidylcholine and medium chain monoacylglycerol (Pharmacia & Upjohn/Scotia Lipidteknik AB, Sweden), hypromellose (hydroxypropylmethylcellulose, Pharmacia & Upjohn internal product supply), Pefabloc SC (Pentapharm AG, Basel, Switzerland), monoglycerides (Larodan AB, Malmö, Sweden), Phospholipon (Rhône-Poulenc Rorer, Cologne, Germany), oleic acid (Kebo Lab AB, Spånga, Sweden), medium chain triglyceride (MCT) oil (Pharmacia & Upjohn internal product supply, purified Miglyol from Karlshamn, Sweden), and Seacure G 210 (Pronova Biopolymer, Oslo, Norway).

Protease inhibitors: Pefabloc SC, chymostatin, bestatin, E64, antipain-dihydrochloride, EDTA-Na_2, leupeptin, phosphoramidon (all from Boehringer Mannheim, Mannheim, Germany), Triplex III and Na-citrate (from Merck, Darmstadt, Germany), polycarbophil and Carbopol 934P (BF Goodrich, Cleveland, OH), trypsin inhibitor soybean (Gibco BRL, Life Technologies, Paisley, UK), ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′,-tetraacetic acid (EGTA; Sigma, St Louis, MO).

Pancreatic protease substitute drugs: Combizym compositum (Luitpold Pharma, Munich, Germany), Pancrease (Janssen-Cilag, Sollentuna, Sweden).

Trypsin: bovine trypsin (Sigma, St Louis, MO), porcine trypsin (Gibco BRL, Life Technologies, Paisley, UK and Sigma, St Louis, MO), and human trypsin (Calbiochem Novbiochem, CA).

Gastrointestinal stability

Perfusion buffer pH 6.5 (Fagerholm et al 1996) adjusted to pH 2.2 and 7.4 or Tris (hydroxymethyl) aminomethane buffer (Tris-buffer) (0.1 M) with $CaCl_2$ (20 mM) at pH 7.8 were used for the experiments. All studies were carried out at a hexarelin concentration of 0.133 mg mL^{-1}.

Stability of hexarelin in luminal fluid and mucosal tissue from rat and dog gastrointestinal tract

The rats were anaesthetized with halothane and the gastrointestinal tract was removed. The contents and mucosal tissues of ventricle, duodenum, jejunum, ileum, and colon + caecum + rectum were diluted with perfusion buffer 1:5 (w/w), and homogenized. The homogenate was then centrifuged at 34000 *g* at 4°C. The protein concentration of the supernatant was determined (absorbance at 280 nm) and the final test solution was standardized to 6 mg protein (mL fluid)$^{-1}$. Hexarelin was added and the test incubate was slowly agitated at 37°C. Samples were immediately frozen for later hexarelin analysis. The stability of hexarelin in intestinal fluid (duodenum, ileum and colon) from dog was investigated also.

Stability of hexarelin in luminal fluid from rat ileum in the presence of protease inhibitors

Hexarelin and protease inhibitors were added to incubates with the luminal fluid from rat ileum: a mixture of Pefabloc SC (200 μg mL^{-1}), antipain-dihydrochloride (100 μg mL^{-1}), aprotinin (4 μg mL^{-1}), bestatin (80 μg mL^{-1}), EDTA-Na_2 (1330 μg mL^{-1}) leupeptin (2.5 μg mL^{-1}) and phosphoramidon (200 μg mL^{-1}); a combination of E64 and bestatin (50 μg mL^{-1} and 80 μg mL^{-1}, respectively); or Pefabloc SC (25 μg mL^{-1}), chymostatin (25 μg mL^{-1}), EDTA (20 mM), polycarbophil (0.4% w/w), Carbopol 934P (0.4% w/w), trypsin inhibitor soybean (2.5 mg mL^{-1}), Na-citrate (40 mM) and EGTA (50 mM) were evaluated for their individual effect on the stability of hexarelin.

Stability of hexarelin in the presence of pancreas substitute drugs

Combizym and Pancrease tablets were pulverized and dissolved in Tris-buffer to calculate final protease concentrations of 150 IU mL^{-1} (first study) or 117 IU mL^{-1} (second study). The solutions were then centrifuged at 14 000 *g* for 5 min. The supernatant was taken, buffered to pH 7.8, and used in the test incubates with hexarelin. A sample of each drug solution contained the protease inhibitor Pefabloc SC as a control. The solutions were kept at 37°C for 180 min and were continuously rotated. Samples were taken and placed in Pefabloc SC (8 mM) and then immediately frozen for later hexarelin analysis.

Hexarelin stability to trypsin with and without inhibitors

Hexarelin stability was investigated in the presence of bovine, porcine and human trypsin. The investigated relative concentrations of hexarelin and bovine or porcine trypsin were ($w_{hexarelin}$:$w_{trypsin}$): 1:50, 1:25, 1:10, 1:1, and 1:0.01. Hexarelin stability in porcine trypsin (2.5 mg mL^{-1}) was evaluated in the presence of protease inhibitors (same inhibitor concentration as for incubations in fluid from rat ileum): EDTA, EGTA, polycarbophil and Carbopol 934P. The test incubates were kept at 37°C and were continuously rotated. Samples were taken and placed in Pefabloc SC solution (8 mM) to stop any trypsin activity and then immediately frozen for later hexarelin analysis.

Permeability

The apical to basolateral intestinal epithelial permeability of hexarelin, at a donor concentration of 1.13 mM, was evaluated using an Ussing chamber and Caco-2 cell studies. [^{14}C]Mannitol was added to the hexarelin formulations in all experiments as a reference compound for intestinal epithelial integrity.

Ussing chamber experiments

Ussing-type diffusion chamber systems were obtained from Costar (Cambridge, MA). Male Sprague Dawley rats were obtained from Charles River. The animal studies were approved by the regional ethical board in Stockholm (Sweden), with an application number for approval of N314/95. Krebs bicarbonate Ringer (KBR) buffer pH 7.4 with glutamate (4.9 mM), fumarate (5.4 mM), pyruvate (4.9 mM) and HEPES (20 mM) were used for the experiments. The buffer was continuously gassed with O_2/CO_2 (95%/5%, v/v). For formulations containing the additives EDTA, Na-caprate, polycarbophil, and Carbopol, a KBR buffer without Mg^{2+} and Ca^{2+} was used. Experimental procedures were adapted from methods described by Grass & Sweetana (1988) and Ungell et al (1992).

The viability and integrity of the tissue was measured by monitoring electro-physiological parameters, current pulses (0, $\pm$15 and $\pm$30 mA) were applied and the voltage responses were recorded. The resistance, resting potential and short circuit current were calculated using an application of the computer program Labview, developed by Johan Gråsjö of the Department of Pharmaceutics, Uppsala University, Sweden.

Caco-2 cell monolayer experiments

Cell culture and permeability experiments were performed as described by Artursson et al (1996). Caco-2 cells (American Type Culture Collection), passages 36–46 at 14 to 28 days after seeding, were used for the experiments. Hank's balanced salt solution (HBSS) (Gibco BRL, Life Technologies, Paisley, UK), at pH 7.4 was used for the permeability experiments. HBSS pH 5.5 was used for the chitosan formulation.

Potential permeability enhancers were evaluated in Ussing chambers and Caco-2 cells and a selection of these was further evaluated. EDTA (10 mM), palmitoyl carnitine (0.2 mM), a combination of methyl-β-cyclodextrin and α-cyclodextrin (both at 5% w/v), taurocholic acid (20 mM), a combination of taurocholic acid and phosphatidylcholine (20 mM and 0.5% w/v, respectively), and sodium caprate (20 mM) were investigated in the Ussing chambers. EDTA (10 mM), palmitoyl carnitine (0.2 mM), polycarbophil (0.4% w/v), Carbopol (0.4% w/v), chitosan (50 μg mL^{-1}), Biosomes (0.6 and 6 mM) and monoglycerides, MG, C6 and C8–C10, (0.54 and 5.4 mM) were investigated in Caco-2

cell monolayers. Formulations containing hypromellose (10 %), MCT oil and Pefabloc SC (1.25 mM) were evaluated also in the Caco-2 cell model.

Calculations

The apparent permeability (P_{app}) of hexarelin and [^{14}C]-mannitol was calculated by the following equation:

$$P_{app} = dQ/dt \times 1/ACo \quad (1)$$

where dQ/dt is the steady state appearance of the compound on the acceptor side and Co is the initial concentration of the substance on the donor side. A is the surface area of exposed epithelium.

Analytical methods

Hexarelin analysis

Hexarelin concentrations were determined with isocratic reversed-phase HPLC. The system consisted of a Hewlett Packard chromatograph model 1090 M with a Jasco FP-920 Fluorescence Detector. A binary Solvent DR5 delivery system with a μBondapak C18 column (dp = 10 μm) and mobile phase of acetonitrile 27.7 % (v/v) and trifluoroacetic acid 1 % (v/v) was used. The samples were analysed at room temperature with an injection volume of 20 μL and a flow rate of 1 mL min^{-1}. The excitation and emission wavelengths were 280 nm and 340 nm, respectively. Retention time using this system was 9–11 min. Limit of quantity (LOQ) was 0.050 μg mL^{-1} and the standard deviation of the method was 5 %.

Metabolite identification by LC-MS and LC-MS/MS

Reversed-phase chromatography in gradient mode with mass spectrometric detection was applied for the separation of hexarelin and its metabolite from the matrix compounds. The separation system consisted of a Zorbax SB-C_{18} column, 0.8 × 150 mm with 5-μm particles, 80 Å (LC-Packings, Amsterdam, The Netherlands). The mobile phases used were (A) 15 % acetonitrile in 1 % formic acid and (B) 1 % formic acid in acetonitrile, pumped at a flow rate of 30 μL min^{-1}. Gradient program: 0 % B to 100 % B in 50 min. The injection volume was 10 μL.

The mass spectrometer used was a magnetic sector instrument model VG Autospec-TOF (Micromass Ltd, Manchester, UK) equipped with an electrospray ion source. The scanned mass range was 200–2000 Da at a resolution (10 % valley) of 1500 and the scan speed was 4 s/scan. The MS/MS experiments were performed with the following settings: scanned mass range was 0–2000 Da, methane gas pressure in the collision cell was 8.5E-6 torr and collision energy was 400 V. Mass spectra of positive ions were recorded in the profile data mode.

Results and Discussion

Gastrointestinal stability

Results indicated that the stability of hexarelin was marginally affected by intestinal brush border membrane enzymes or by interaction with mucus; 70–80 % of initial hexarelin was recovered after 180-min incubation with intestinal mucosal tissue from rat.

The data showed a decrease in hexarelin concentration in incubates with contents from the rat intestine, predominantly the ileum (Figure 1). In the preparations with luminal fluid from rat ileum, approximately 24 % of the initial hexarelin dose was recovered after 180 min (Table 1). Preliminary results indicated that hexarelin was degraded in the contents of the dog jejunum and duodenum (data not shown).

The addition of a mixture of protease inhibitors (inhibiting aminopeptidase, cysteine proteases, serine proteases and metalloendopeptidases) to the contents of rat ileum retained 95 % of the initial hexarelin concentration, indicating a proteolytic degradation mechanism. Chymostatin, Pefabloc SC, EDTA and EGTA inhibited the disappearance of hexarelin in the contents of rat ileum ($\geq$ 80 % recovery after 3 h). Soybean trypsin inhibitor, Na-citrate, bestatin with E-64, polycarbophil, and Carbopol stabilized hexarelin to a lesser extent (44–56 % was recovered after 3 h) (Table 1).

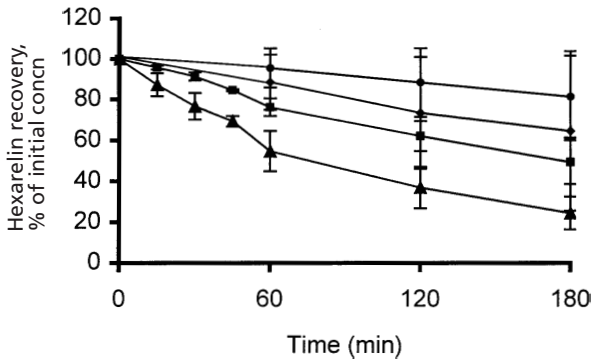

Figure 1 Hexarelin stability in contents of different parts of the rat gastrointestinal tract: ventricle (◆), duodenum (■), jejunum (▲), ileum (●). Each time point is the mean and standard deviation of two or more rats.

Table 1 Hexarelin stability (% recovery of initial concentration) in the contents of rat ileum and porcine trypsin, with and without the addition of different protease inhibitors.

Protease inhibitor	Rat distal jejunum contents		Porcine trypsin	
	60 min	180 min	60 min	180 min
Rat ileum contents[a]	55	24		
Porcine trypsin[b]			62	39
Mixture of inhibitors		95		
Chymostatin		107		
Pefabloc SC		93		
E-64/Bestatin		56		
EGTA	96	91	96	74
EDTA	91	79	104	89
Trypsin inhibitor soybean	84	51	37	13
Na-citrate	72	48	65	36
Carbopol	68	46	79	59
Polycarbophil	69	44	63	56

[a]Mean of a number of preparations from six rats. [b]Mean of five experiments.

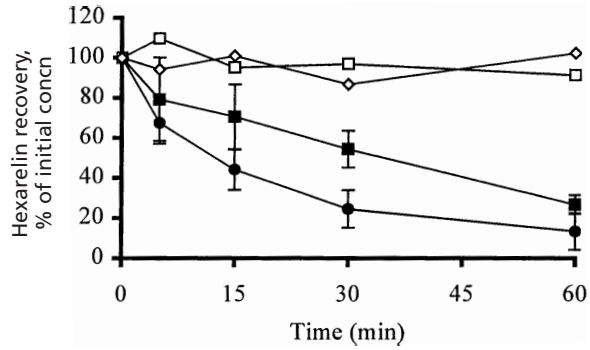

Figure 2 Hexarelin stability in the presence of pancrease substitute drugs (filled symbol) and in presence of these drugs and the protease inhibitor Pefabloc SC (open symbol), Combizym (■ and □) and Pancrease (● and ◇). For the test solutions without Pefabloc SC, each time point is the mean and standard deviation of two experiments.

Combizym and Pancrease contain pancreatic proteases and are used therapeutically as pancrease substitute drugs. In the presence of these enzymes, 25–35% of initial hexarelin was recovered after 60 min. Upon adding Pefabloc SC, the enzymatic degradation of hexarelin in the presence of the pancrease substitute drugs was inhibited (Figure 2). These results add weight to the hypothesis that hexarelin was degraded in the presence of pancreatic proteolytic enzymes.

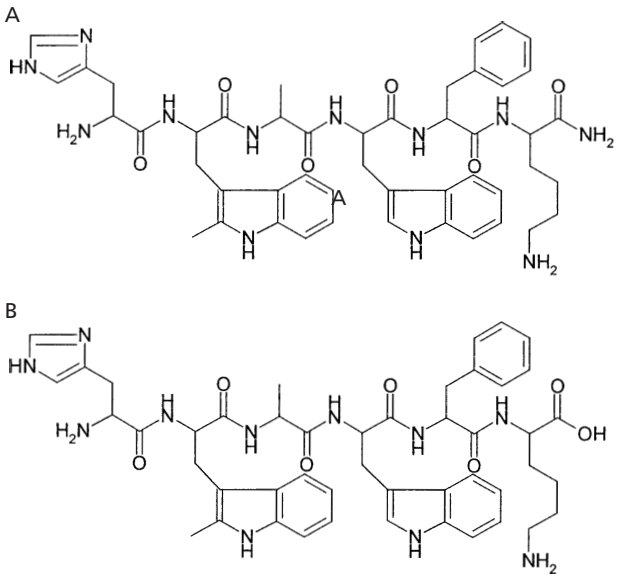

Figure 3 The structure of A hexarelin and B the major metabolite, the deamidated form of hexarelin.

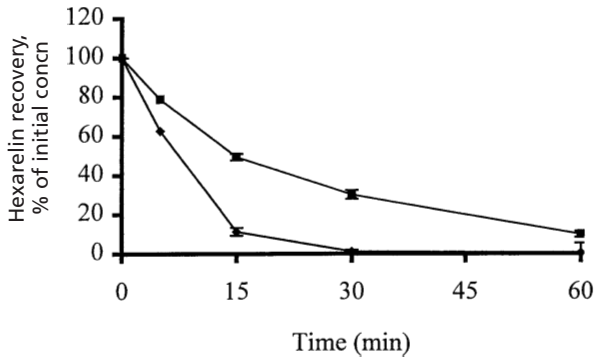

Figure 4 Hexarelin stability in the presence of bovine (■) and porcine (◆) trypsin ($w_{hexarelin}:w_{trypsin}$/1:10). Each time point, apart from the 5-min sample, is the mean and standard deviation of two experiments.

A protease that cuts with high specificity at arginine and lysine residues and is abundant in the small intestine is the serine protease trypsin (Rinderknecht 1986; Oeswein & Shire 1991; Krishnamoorthy & Mitra 1995). After 180-min incubation with porcine trypsin, 39% of the initial hexarelin was recovered (Table 1). When EDTA and EGTA were added, the degradation of hexarelin was inhibited, and the addition of polycarbophil and Carbopol increased the stability of hexarelin.

After incubation of hexarelin in the contents from rat ileum, as well as in porcine, bovine and human trypsin,

a deamidated form of hexarelin was identified as the major metabolite (Figure 3). These results indicated that trypsin was the cause of the degradation of hexarelin by deamidation at the lysine residue. The degradation of hexarelin in the presence of bovine and porcine trypsin ($w_{hexarelin}:w_{trypsin}/1:10$) is shown in Figure 4.

Permeability

Hexarelin P_{app} in rat ileum was $1.3 \pm 0.9 \times 10^{-6}$ cm s^{-1} in the apical to basolateral direction. In the Caco-2 cells, a less leaky model, the amount of hexarelin that permeated the epithelium was below the detection limit i.e. hexarelin P_{app} was less than 1.5×10^{-7} cm s^{-1}. Low permeability across the intestinal epithelial membrane and therefore low bioavailability is commonly observed upon oral administration of peptide and protein drugs.

The effect of hexarelin on cell integrity was investigated in rat intestinal tissue and Caco-2 cells. The apparent permeability of [^{14}C]mannitol was similar in rat intestinal tissue exposed to hexarelin (1.13 mM) compared with tissue not exposed to hexarelin. This was also the case for experiments with Caco-2 cells. However, at a concentration of 11.3 mM hexarelin, the permeability of [^{14}C]mannitol in Caco-2 cells increased (by a factor of 50). Therefore, the cell toxicity of hexarelin was investigated using an MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide) method with Caco-2 cells. Results showed that the enzyme activity was approximately 100 % for hexarelin concentrations up to approximately 3.5 mM; at higher hexarelin concentrations the enzyme activity decreased, indicating toxicity to the cells. These results were consistent with the mannitol permeability observed at different concentrations of hexarelin.

Table 2 Apparent permeability (P_{app}) of hexarelin across Caco 2-cell monolayers.

Additive	Hexarelin P_{app} (10^{-7} cm s^{-1})
None	< 1.5[a]
Palmitoyl carnitine	34 ± 4
Chitosan	19 ± 2
Biosomes (6 mM)	42 ± 26
Biosomes (6 mM), Pefabloc SC	278 ± 22
MG C8–C10 (5.4 mM)	253 ± 26
MG C8–C10 (5.4 mM), Pefabloc SC	313 ± 16
MG C8–C10 (5.4 mM), Pefabloc SC, hypromellose	1.9 ± 0.4
MG C8–C10 (5.4 mM), hypromellose	< 1.5[a]
MG C8–C10 (5.4 mM), hypromellose, Tween 80	< 1.5[a]
MG C8–C10 (5.4 mM), hypromellose, taurocholate	< 1.5[a]
MG C6–C10, oleic acid, MCT oil	6 ± 4

[a]Acceptor concentration was below detection limit i.e. $P_{app} < 1.5 \times 10^{-7}$ cm s^{1}.

Several permeability enhancers, with diverse mechanisms of action, were evaluated. Paracellular permeability, governed by the tight junctions, may be affected using palmitoyl carnitine (Hochman et al. 1994), sodium caprate (Lee & Yamamoto 1990; Tomita et al 1996), EDTA (Aungst 1993; Tomita et al 1996), or chitosan (Artursson et al 1994; Schipper et al 1997). Enhanced transcellular permeability may be achieved by including, for example, fatty acid derivatives (Lee 1991).

In the Ussing chamber studies, EDTA, sodium caprate and palmitoyl carnitine enhanced the P_{app} of hexarelin (by factors of 2.5, 1.8 and 1.6, respectively) across rat distal small intestine. In the presence of EDTA and sodium caprate the P_{app} of [^{14}C]mannitol increased also.

In the Caco-2 cell studies palmitoyl carnitine and chitosan increased the P_{app} of hexarelin (Table 2). EDTA was observed to have a small effect (data not shown).

Biosomes were found by Fagerholm et al (1998) to increase the rat jejunal permeability of hexarelin. Medium chain monoglycerides have been suggested to enhance epithelial permeability by dissolving and thereby extracting cholesterol from the cell (Mulligan & Corrigan 1986; Lohikangas et al 1994). To investigate whether the monoglycerides (MG) were the components, in the Biosome formulations, responsible for the absorption-enhancing mechanism, formulations with MG C8–C10 were evaluated. Biosomes (6 mM) and MG C8–C10 (5.4 mM) increased the P_{app} of hexarelin across Caco-2 cells. Our results indicated that the permeability-enhancing effect in Biosomes seemed mainly to stem from their content of MG C8–C10.

In all Caco-2 cell studies the permeability of the reference compound [^{14}C]mannitol increased when the permeability of hexarelin increased.

Formulations containing enhancers/protease inhibitors

Based on the results obtained, several formulations were suggested for further in-vivo studies and hence evaluated in the Caco-2 cell model. The presence of Pefabloc SC may inhibit metabolic degradation in the intestine. Including hypromellose in the formulations may reduce dilution and thereby obtain higher concentrations of

hexarelin and the additives nearby the intestinal epithelium.

Hexarelin permeability increased in the presence of Biosomes + Pefabloc SC. When compared with Biosomes only, results suggested an additional enhancing effect of Pefabloc SC in this case. However, MG C8–C10 + Pefabloc SC showed an increase in hexarelin similar to MG C8–C10 only. This observed increase in permeability was also comparable with the results obtained with Biosomes + Pefabloc SC (Table 2).

The P_{app} of hexarelin with MG C8–C10 with Pefabloc SC and hypromellose gel was only $1.9 \pm 0.4 \times 10^{-7}$ cm s^{-1} (compared with $313 \pm 16 \times 10^{-7}$ without hypromellose). The permeability of hexarelin with MG C8–C10 in hypromellose without Pefabloc SC was not increased to a detectable level. Apparently the presence of hypromellose gel in formulations with MG C8–C10 decreased the permeability of hexarelin. This could possibly be the result of the gel hindering hexarelin from reaching the cell monolayers, and/or a hindrance of the diffusion of the enhancing agents C8–C10 to reach the cell monolayers. To overcome the obstruction of hypromellose gel, a study was performed comparing the enhancing effects of C8–C10 in hypromellose, adding the surface active agent Tween 80 (0.1 mg mL^{-1}) and the bile salt taurocholate (6.6 mM). However, the hexarelin P_{app} results were in the same range as without Tween 80 and taurocholate (Table 2).

The alternative of oil based as opposed to aqueous based formulation was evaluated using MCT oil. The formulation, containing MG C6–C10, was evaluated as an emulsion since the oil could not be applied directly to the cells. Hexarelin permeability increased to $6 \pm 4 \times 10^{-7}$ cm s^{-1} (Table 2).

Similar to the previous Caco-2 cell permeability studies, an increase in [^{14}C]mannitol permeability was observed with all formulations that had an enhancing effect on hexarelin permeability.

Conclusions

Hexarelin was degraded in the presence of the contents of rat ileum. The metabolite was identified as hexarelin deamidated at the lysine residue. These findings together with data obtained in studies with serine protease inhibitors suggested that hexarelin was degraded by trypsin. This hypothesis was supported further by the results showing that the same metabolite was identified in incubations with human, porcine and bovine trypsin. Formulations with absorption enhancing potential were evaluated; palmitoyl carnitine, chitosan, EDTA, sodium caprate, MG C6–C10/oleic acid/MCT oil and Biosomes increased hexarelin intestinal epithelial permeability in in-vitro models. The permeability of hexarelin was also increased by the addition of MG C8–C10; this enhancing effect was shown to be inhibited by including hypromellose in the formulation.

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