KINETICS AND DISPOSITION OF HEXARELIN, A PEPTIDIC GROWTH HORMONE SECRETAGOGUE, IN RATS

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(Received June 9, 1999; accepted October 1, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:

To document the disposition of hexarelin, a peptidyl growth hormone secretagogue, male Sprague-Dawley rats received a 5-50 µg/kg bolus i.v. dose or three single s.c. doses of 5, 10, and 50 µg/kg. To assess hexarelin tissue distribution and excretion, rats were given 1 µg/kg of [3H]hexarelin (9.4 Ci/mmol). Metabolism of [3H]hexarelin was assessed in bile duct-exteriorized rats given 50 µg/kg where radiolabeled hexarelin biliary and urinary excretion was quantitated. After its i.v. injection, hexarelin displayed a half-life of 75.9 ± 9.3 min, a systemic clearance of 7.8 ± 0.7 ml/min/kg, and a volume of distribution at steady state of 744 ± 81 ml/kg. After s.c. administration, the area under the curve (477–3828 pmol-min/ml) estimated with increasing doses confirmed the absence of hexarelin accumulation. Clearance/F (12–15 ml/min/kg) and volume of distribution/F (1208–1222 ml/kg) were dose independent. Hexarelin bioavailability given s.c. was 64%. The highest radioactivity levels were detected in the kidney, liver, and duodenum. The pattern of hexarelin excretion was similar after i.v. or s.c. administrations. Total radioactivity in bile, urine, and feces corresponded to 60, 22, and 10% of the dose, respectively. Of the radioactivity excreted in bile and urine, 90 and 71% was unchanged hexarelin, respectively. These results suggest that: 1) the kinetics of hexarelin appear to be first order up to 50 µg/kg; 2) hexarelin is rapidly absorbed after s.c. administration; 3) biliary excretion is the primary route of hexarelin elimination; and 4) the high recovery of unchanged peptide in bile and urine demonstrates hexarelin stability toward proteolytic enzymes.

Materials and Methods

Chemicals. Hexarelin (EP 23905) was provided by Europeptides (Argenteuil, France). [3H]Hexarelin (9.4 Ci/mmol, 348 GBq/mmol), code TRQ7484, was custom synthesized (Amersham International, Buckinghamshire, England).
by reductive dehalogenation of the dichloro-\(\alpha\)-phenylalanine precursor peptide with tritium gas using a palladium catalyst. The tritium label was incorporated at the 3- and 4-positions of the \(\alpha\)-phenylalanine residue. Radiochemical purity, determined using HPLC, was 95%. For metabolism and disposition studies, the radiolabeled peptide was further purified as described previously (Roumi et al., 1997). A solution of \([\text{H}]\)hexarelin was mixed with unlabeled hexarelin in 0.9% NaCl to the desired specific activity. Aprotinin was purchased from Boehringer Mannheim (Laval, Quebec). Triton X-100 was obtained from Sigma (St. Louis, MO). Trifluoroacetic acid (TFA) was supplied by Pierce (Rockford, IL). Unless otherwise specified, all reagents and materials were of analytical grade and were purchased from Fisher Scientific (Montreal, Quebec).

**Animal Studies.** Animals. Male Sprague-Dawley rats (380–420 g) were obtained from Charles River (St. Constant, Quebec) and acclimated in metabolic cages for 3 to 5 days before use. The rats were maintained on standard laboratory chow pellets and water ad libitum.

**Kinetics of hexarelin after its i.v. (bolus) or s.c. injection.** The kinetics of hexarelin were evaluated after i.v. and s.c. administration of the peptide to conscious rats (\(n = 3/dose\)). Hexarelin was injected i.v. into the jugular vein of rats at the dose of 5 \(\mu\)g/kg. The solution used to administer hexarelin contained 10 \(\mu\)g/ml in 0.9% NaCl. Blood (0.2 ml) was drawn from the tail vein before and after the injection of hexarelin. Hexarelin was injected s.c. into the intrascapulae region of the rat after the injection of the peptide. Each blood sample was collected in Microtainer tubes coated with dipotassium EDTA (Becton Dickinson & Co., Lincoln Park, NJ) to which 1 mM aprotinin was added. The plasma was immediately separated by centrifugation at 2400g for 15 min and stored at \(-20^\circ\)C until analysis.

**Tissue distribution of hexarelin.** Rats (\(n = 4/dose/time point\)) received 1 \(\mu\)g/kg \([\text{H}]\)hexarelin (\(-20 \mu\)Ci/\(\mu\)g) and were sacrificed 5 or 60 min after the i.v. injection or 60 or 180 min after the s.c. administration. The following tissues were rapidly excised: adrenals, duodenum, heart, jejunum, kidneys, liver, lungs, muscle, pancreas, pituitary gland, salivary glands, spleen, and stomach. Replicate samples of each organ (except for the pituitary gland, which was analyzed whole because of the small amount of tissue available) were immediately pretreated for radiometric analysis, while the remaining tissues were stored at \(-20^\circ\)C.

**Biliary excretion of hexarelin.** To study hexarelin biliary excretion, rats were anesthetized with sodium pentobarbital (1 \(\text{ml/kg}\)) and their common bile duct surgically implanted with polyethylene cannulas externalized at the back of the neck 1 day before dosing. Rats were housed individually in metabolic cages to allow for separate collections of urine and feces, with free access to food and water containing 0.9% NaCl, 5% dextrose, and 0.05% KCl during the 24-h collection period to supply adequate electrolyte replacement (Cocchetto and Bjornsson, 1983). Hexarelin was injected i.v. through the lateral tail vein or s.c. between the scapula at the dose of 5 \(\mu\)g/kg \([\text{H}]\)hexarelin (\(-20 \mu\)Ci/\(\mu\)g). Bile was quantitatively collected on ice at 2-h intervals for the first 8 h and then from 8 to 24 h. Urine and feces were collected at 8-h intervals. After the study, cages were rinsed with a known volume of water and aliquots of it were analyzed for radioactivity. In an attempt to identify potential hexarelin metabolites in bile, the study was repeated at the dose of 50 \(\mu\)g/kg hexarelin containing tritiated derivative as tracer at the specific activity of 0.75 \(\mu\)Ci/\(\mu\)g.

**Urinary excretion of hexarelin.** While the rats were under pentobarbital anesthesia, the urinary bladder was cannulated and externalized at the back of the neck 1 day before the experiment. After surgery, the rats received 300,000 I.U. of penicillin (Ayerst Laboratories, Montreal, Canada) i.m. to prevent infection. Food and water were provided ad libitum throughout the study. The rats received i.v. or s.c. 1 \(\mu\)g/kg \([\text{H}]\)hexarelin (\(-20 \mu\)Ci/\(\mu\)g). Both urine and feces were collected in cooled polypropylene tubes over the following periods: 0 to 8, 8 to 24, 24 to 48, and 48 to 72 h. After the study, cages were rinsed with a known volume of water and aliquots of it were analyzed for radioactivity. For the identification of potential hexarelin metabolites in urine, the study was repeated at the dose of 50 \(\mu\)g/kg hexarelin containing tritiated derivative as tracer at the specific activity of 0.75 \(\mu\)Ci/\(\mu\)g.

**Analytical Methods.** *Quantitation of hexarelin in plasma.* Concentrations of hexarelin in rat plasma were measured using a radioimmunoassay method as described by Roumi et al. (1995) with the only modification in the assay procedure being the addition of 25 \(\mu\)l of either hexarelin-free rat plasma or rat plasma sample to the incubation mixture. The standard curves for hexarelin, set in the range of 0.1 to 30,000 fmol/assay, featured the following characteristics: an \(ED_{50}\) of 10.8 \pm 0.34 fmol/assay, a slope factor of 1.08 \pm 0.02, a limit of detection of 0.84 fmol/assay, and a limit of quantification of 2.55 fmol/assay. The accuracy for three control plasma-spiked samples consisting of 5, 15, and 50 fmol/assay was 97.6, 98, and 88.6%, respectively. *Intra- and interassay coefficients of variation were less than 2 and 4%, respectively. Cross-reactivity with hexarelin analogs was less than 1% on modification of positions 3, 4, or 5 of the peptide. The potential cross-reactivity with endogenous substances or metabolites of hexarelin in plasma was assessed by establishing HPLC immuno-nograms of a pool of plasma extracts from rats receiving hexarelin. The results confirmed the presence of a single peak of immunoreactivity corresponding to the elution position of hexarelin, which was 36.7% actoninre (with the HPLC conditions described previously (Roumi et al., 1995). *Radioactivity in solutions, bile, and urine.* Radioactivity was measured in a LKB Wallac 1217 liquid scintillation counter (Fisher Scientific, Montreal, Quebec). The radioactivity in solutions, bile, urine, cage rinse, and HPLC fractions were determined by direct liquid scintillation counting (LSC) in triplicate aliquots (0.01–0.2 ml) mixed with 4 or 10 ml of a liquid scintillator (Ecolume; ICN, Aurora, OH) in propylene vials. Hexarelin in feces was extracted with a solution of 0.5% Triton X-100 in water. After centrifugation for 20 min at 2400g, the radioactivity in the supernatant was calculated. To assess hexarelin in tissues, triplicate aliquots of blood samples (20 \(\mu\)l) and tissues (50 mg) were solubilized in 0.5 ml hyamine hydroxide (ICN, Costa Mesa, CA) and incubated for 24 h at 50°C. The samples were then bleached with 0.25 ml of 30% hydrogen peroxide (VWR Scientific, Mon-Royal, Quebec) and further incubated for 3 h before counting in 15 ml of a scintillation liquid (CytoScint; ICN, Costa Mesa, CA).

**HPLC for hexarelin and metabolite radiochemical profiling in bile and urine.** Radiochemical profiling of pooled bile and urine samples from each collection time interval was performed by gradient reversed phase HPLC with a Waters automated gradient controller equipped with M510 pumps (Waters Assoc., Milford, MA) followed by LSC. The mobile phase consisted of 0.1% TFA in water (A) and acetonitrile in 0.1% TFA (B). No sample preparation was necessary for bile sample analysis. Bile was resolved on a Vydac C18 column (4.6 \(\times\) 250 mm, \(5 \mu\)m, 300 Å) by running a linear gradient of B from 18 to 42% in 80 min at a flow rate of 1 ml/min. Sample pretreatment was required to assess hexarelin in urine. Urine was extracted by solid phase extraction using Sep-Pak light C18 cartridges (Waters, Milford, MA) then loaded on an Ultrasphere cyano column (4.6 \(\times\) 250 mm, 5 \(\mu\)m). Separation was achieved by running a linear gradient of B from 2 to 18% in 16 min then 18 to 33% in 50 min at a flow rate of 1 ml/min. Elute was collected as 0.5-ml fractions with a programmable fraction collector (Pharmacia LKB Frac-100, Uppsala, Sweden) and the radioactivity counted.

**Liquid chromatography with tandem mass spectrometry analysis.** Mass spectral analysis of bile and urine samples was performed on a Finnigan-MAT TSQ 7000 (San Jose, CA) mass spectrometer using electrospary ionization in positive ion mode with on-line radiochemical detection (\(\beta\)-RAM Mode 2A, LabLogic, Sheffield, UK). The HPLC system consisted of a Shimadzu SCL10A controller equipped with two LC-10AD pumps and SIL-10A autosamplers (Dysons Instruments Ltd, Tyne & Wear, UK). Chromatography was achieved with a HiChrom HIRPB-100AS (100 \(\times\) 3.2 mm i.d.) column using a mobile phase consisting of 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). Elution proceeded at 1 ml/min with a linear gradient of 5 to 50% B in 21 min. An approximate 9:1 split of the HPLC eluent reduced the flow into the mass spectrometer to \(\sim 7\) \(\mu\)l/min. Spray voltage and the capillary temperature were set at 4.5 kV and 250°C, respectively. Nitrogen was used as sheath gas at a head pressure of 55 psi. Mass spectrometric data was acquired over an appropriate mass range at a scan rate of 1 s/scan.

**Data Analysis.** *Pharmacokinetic parameters.* Pharmacokinetic parameters after i.v. and s.c. administration were analyzed by noncompartmental methods using a pharmacokinetic software package (WinNonlin; Scientific Consulting Inc., Apex, NC). The area under the curve (AUC\(_{0-\infty}\), where \(t\) is the time of the last measurable plasma concentration) was estimated from the log-linear
Hexarelin Kinetics. Mean plasma concentration-time profiles of hexarelin after i.v. injection of 5 \( \mu g/kg \) and s.c. injection of 5, 10, and 50 \( \mu g/kg \) in male rats are shown in Fig. 1. After i.v. administration, the mean maximal hexarelin plasma concentration observed was 27 ± 3 pmol/ml. The decline in plasma hexarelin was characterized by a terminal half-life of 76 ± 9 min and a \( CL_p \) of 7.6 ± 0.7 ml/min/kg. The volume of distribution at steady state was estimated to be 744 ± 81 ml/kg. The kinetic parameters of hexarelin injected s.c. are summarized in Table 1. Maximal hexarelin plasma levels were attained approximately 10 to 20 min after administration. A 10-fold increase in the doses up to 50 \( \mu g/kg \) did not induce any accumulation of hexarelin as observed with AUC (477–3826 minpmol/ml). Furthermore, dose-normalized AUC values tended \( (P = .093) \) to decrease with the increase in dose (0.22, 0.19, and 0.17 for the 5, 10, and 50 \( \mu g/kg \) dose, respectively). Hexarelin \( CL_p \) values (12–15 ml/min/kg) and \( V_d \) (1208–1222 ml/kg) were not significantly different over the dose range studied, as were the terminal half-life values (57–71 min). Finally, comparison of the AUC values obtained after i.v. and s.c. administration of 5 \( \mu g/kg \) hexarelin indicated that the s.c. bioavailability was 64%.

Hexarelin Tissue Distribution. Five minutes after the i.v. injection of 1 \( \mu g/kg \) [\( ^3 \)H]hexarelin, significant levels of radioactivity were observed in the liver, kidneys, and duodenum (Fig. 2). One hour after its injection, the concentration of hexarelin radioactivity decreased in the liver, but increased in the duodenum. Heart and muscle contained the lowest concentrations of radioactivity. A similar profile was observed in radioactivity distribution after the s.c. injection of 1 \( \mu g/kg \) [\( ^3 \)H]hexarelin (Fig. 3). At 1 and 3 h, radioactivity levels in the liver, kidneys, and duodenum were significantly greater than in other tissues. Radioactivity did not accumulate in any endocrine tissues.

Hexarelin Excretion. The biliary and urinary excretion profiles of hexarelin after the i.v. injection of [\( ^3 \)H]hexarelin to bile-cannulated rats was very similar to those observed after s.c. injection (Table 2). Around 60% of the total radioactivity was recovered in bile within 24 h, indicating that hexarelin elimination occurs primarily via biliary excretion. In addition, excretion into the bile was rapid as 50% of the radioactivity was recovered within the first 2 h. Total urinary excretion accounted for 22% of the administered radioactivity, and most of the radioactivity was eliminated within the first 8 h. The amount of radioactivity recovered in feces after 3 days, from the urinary excretion study, was about 10%. Total mass balance over 72 h was 94% for both the i.v. and s.c. routes of administration. Carcasses and soft tissues were not analyzed and hence may account for the 6% loss in total radioactivity. To identify potential metabolites of hexarelin in bile and urine, the excretion study was repeated at a higher dose of [\( ^3 \)H]hexarelin (50 \( \mu g/kg \)). The excretion radioactivity profiles were identical with those observed after the 1 \( \mu g/kg \) [\( ^3 \)H]hexarelin regardless of the dosing route (data not shown).

Hexarelin Metabolism. Radiochemical profiling of bile and urine samples was performed to determine the amount of intact hexarelin and possible metabolites recovered. Samples of bile and urine collected from the 0- to 2- and 0- to 8-h time intervals, respectively, were pooled for each group of rats and analyzed by HPLC and LSC. Hexarelin was not metabolized in rats. After s.c. injection of hexarelin, the radiochemical profile of bile (Fig. 4) displayed a single peak corresponding to the elution position of the parent compound (~36 min). It was estimated that 92% of the radioactivity recovered in bile during the first 2 h was intact hexarelin and accounted for 50% of the administered dose. Intact hexarelin was the major radioactive entity observed in urine samples as shown in Fig. 5(A), and...
represented 71% of the radioactivity excreted in urine within the first 8 h, i.e., approximately 14% of the dose administered. Several minor peaks with shorter retention times than hexarelin were detected at 8, 31, and 33 min, which accounted for about 3 to 5% of the radioactivity in the urine sample, and hence, 2% of the administered dose. The analysis of bile and urine samples obtained after the i.v. injection of hexarelin showed identical profiles.

To confirm the identity of the major peak in bile and urine as intact hexarelin, an aliquot of bile sample containing a known amount of radioactivity was spiked with an equivalent amount of pure [3H]hexarelin standard and analyzed by HPLC. The major peak observed in the profiles coeluted with the reference standard. The radiochemical profile displayed one single radioactive peak at ~36 min containing approximately twice the original amount of radioactivity. Analyses of the samples by liquid chromatography with tandem mass spectrometry further confirmed the identity of hexarelin. Figure 5B shows the mass spectrum of the major compound found in rat urine after the s.c. injection of hexarelin. The spectrum shown displays an ion at m/z 444, which corresponds to the [M + 2H]^{2+} species of hexarelin.

**Discussion**

Although its pharmacological and pharmacodynamic effects have thoroughly been studied, the disposition and metabolism of hexarelin had never been assessed until now. In the present study, the pharmacokinetics of hexarelin was examined in rats. The dose range studied was selected after preliminary clinical studies whereby satisfactory therapeutic GH-releasing activity was observed in humans after i.v. administration of 1 to 2 μg/kg and s.c. administration of 1.5 to 3 μg/kg hexarelin (Ghigo et al., 1994).

The present results demonstrate that the peptide is rapidly absorbed from the site of injection after its extravascular administration because maximal levels are attained in less than 20 min. After s.c. injection, hexarelin AUC values tend to decrease with increasing dose, indicating a lack of accumulation of hexarelin up to 50 μg/kg. In addition, both clearance and Vd values are independent of the dose. These results suggest that hexarelin may obey first order kinetics between the doses of 5 and 50 μg/kg. Hexarelin was characterized by having a longer terminal half-life (approximately 70 min) than hormone peptides. This long half-life is inherent with peptides that have been modified by replacement of the naturally occurring L amino acids for the D-configuration. The pharmacokinetics of hexarelin correlates well with the pharmacodynamic findings reported by Deghenghi et al. (1994) who observed that in the adult male rat, GH peak levels were apparent 20 min after the s.c. administration of hexarelin. The authors observed that GH secretion induced by hexarelin was prompt and sustained as it was still highly stimulated at 30 min. Comparison of hexarelin with GHRP-6 revealed that the former peptide was more effective as slightly higher GH levels were observed. This might be explained by the higher lipophilicity and/or higher resistance to metabolic degradation of hexarelin.

After an i.v. and a s.c. dose of [3H]hexarelin, the amount of
radioactivity found in most tissues was relatively low. No accumulation of hexarelin for up to 3 h was detected in the tissues analyzed. Levels of radioactivity were found in the organs of elimination, that is, the liver and the kidneys. Significant levels of hexarelin were also observed in the duodenum, a result in agreement with the excretion study whereby it was demonstrated that the peptide was mostly eliminated via biliary excretion. Hexarelin excreted into bile was conveyed into the intestine and consequently high levels were observed in the duodenum.

The actual results show that hexarelin excretion is quite rapid because most of the radioactivity is observed in the bile within the first 2 h. There is good correlation between the pharmacokinetic and the bile excretion studies as the terminal half-life of the peptide, determined from the rate of hexarelin elimination from bile over a period of 8 h, was estimated to be about 65 min. Furthermore, because the urine excretion profiles of hexarelin of bile-cannulated rats were similar to those of bladder-cannulated rats, it may be assumed that hexarelin is not subjected to an enterohepatic circulation. Almost identical excretion profiles were observed after the i.v. and the s.c. administration of \([\text{H}]\)hexarelin as more than 50% of the dose is excreted within 4 h after both routes of administration. This confirms the rapid absorption of hexarelin when given s.c.. In addition, hexarelin mass balance for both i.v. and s.c. routes of administration was approximately 94% and as a consequence, it may be concluded that after s.c. injection of the peptide, 100% of the radioactivity is recovered after 3 days. The estimation of the absolute bioavailability at 64% might, however, suggest some potential degradation of hexarelin at the site of injection after its s.c. administration.

### TABLE 2

<table>
<thead>
<tr>
<th>Collection Period</th>
<th>Bile (%)</th>
<th>Urine (%)</th>
<th>Feces (%)</th>
<th>Cage Rinse (%)</th>
<th>Total (%)</th>
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<td>h</td>
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<td>i.v.</td>
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<tr>
<td>0–2 h</td>
<td>46.4 ± 0.24</td>
<td>68.43 ± 1.87</td>
<td>3.40 ± 1.05</td>
<td>0.92 ± 0.45</td>
<td>59.75 ± 1.23</td>
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<tr>
<td>2–4 h</td>
<td>8.43 ± 1.87</td>
<td>0.60 ± 0.58</td>
<td>6.06 ± 0.58</td>
<td>0.38 ± 0.19</td>
<td>21.95 ± 3.54</td>
</tr>
<tr>
<td>4–6 h</td>
<td>3.40 ± 1.05</td>
<td>0.60 ± 0.58</td>
<td>6.06 ± 0.58</td>
<td>0.38 ± 0.19</td>
<td>21.95 ± 3.54</td>
</tr>
<tr>
<td>6–8 h</td>
<td>0.92 ± 0.45</td>
<td>0.60 ± 0.58</td>
<td>6.06 ± 0.58</td>
<td>0.38 ± 0.19</td>
<td>21.95 ± 3.54</td>
</tr>
<tr>
<td>8–24 h</td>
<td>0.60 ± 0.58</td>
<td>0.38 ± 0.19</td>
<td>6.06 ± 0.58</td>
<td>0.38 ± 0.19</td>
<td>21.95 ± 3.54</td>
</tr>
<tr>
<td>0–24 h</td>
<td>60.82 ± 1.9</td>
<td>10.38 ± 1.39</td>
<td>1.30 ± 0.48</td>
<td>93.38 ± 3.49</td>
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<td>s.c.</td>
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<tr>
<td>0–2 h</td>
<td>47.06 ± 1.5</td>
<td>9.47 ± 1.17</td>
<td>1.10 ± 0.22</td>
<td>1.32 ± 0.32</td>
<td>21.96 ± 1.67</td>
</tr>
<tr>
<td>2–4 h</td>
<td>9.47 ± 1.17</td>
<td>1.85 ± 0.25</td>
<td>1.10 ± 0.22</td>
<td>1.32 ± 0.32</td>
<td>21.96 ± 1.67</td>
</tr>
<tr>
<td>4–6 h</td>
<td>1.85 ± 0.25</td>
<td>1.10 ± 0.22</td>
<td>1.32 ± 0.32</td>
<td>1.30 ± 0.32</td>
<td>21.96 ± 1.67</td>
</tr>
<tr>
<td>6–8 h</td>
<td>1.10 ± 0.22</td>
<td>1.32 ± 0.32</td>
<td>1.30 ± 0.32</td>
<td>1.30 ± 0.32</td>
<td>21.96 ± 1.67</td>
</tr>
<tr>
<td>8–24 h</td>
<td>1.32 ± 0.32</td>
<td>1.30 ± 0.32</td>
<td>1.30 ± 0.32</td>
<td>1.30 ± 0.32</td>
<td>21.96 ± 1.67</td>
</tr>
<tr>
<td>0–24 h</td>
<td>60.82 ± 1.9</td>
<td>10.22 ± 0.22</td>
<td>0.64 ± 0.08</td>
<td>93.76 ± 1.65</td>
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</tr>
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</table>

* Percent dose recovered from the urinary bladder-cannulated rat study.

* Values represent the mean ± S.E. of three rats.

* Values represent the mean ± S.E. of four rats.

The major peak corresponds to intact hexarelin and represents 92% of the radioactivity recovered in bile.
Interestingly, hexarelin seems to exhibit some resistance toward hepatic proteases because the metabolism of hexarelin was minimal as 92 and 71% of the radioactivity, excreted in bile and urine, respectively, were found to be intact peptide. Only trace levels of metabolites were detected in urine, hence, identification of these metabolites was not possible. These results are comparable to those observed with GHRP-6 (Davis et al., 1994) and indicative of high stability in the liver. We have previously reported that the hepatic extraction of hexarelin was low (20%) and that its clearance could be limited by its binding to plasma proteins (Roumi et al., 1997).

The results from the present study support the term “impervious peptides” proposed to describe the metabolic stability characteristic of hexarelin (Deghenghi, 1997). The increased in vivo stability of hexarelin governed by its modified structure has been previously observed with other small synthetic peptides such as GHRP-6, the LHRH antagonist RS-26306 (Chan et al., 1991), and Sandostatin, a long-acting octapeptide analog of somatostatin (Lemaire et al., 1989). This latter peptide was reported to display remarkable stability in the rat. Interestingly, whereas the natural endogenous peptide somatostatin has a half-life of 20 min, Sandostatin has a half-life of 2 h due to the presence of D amino acid residues in its structure. In addition to its stability imparted by the D-configuration of the tryptophan and phenylalanine residues, increased resistance of hexarelin toward proteases may also be explained by its conformational structure. Minimal energy conformation study of this peptide indicates a folded structure, almost cyclic, with the carbonyls of the peptide bonds on the inside (Deghenghi, 1998). The peptide bonds are presumably less accessible to peptidases and consequently the likelihood for proteolysis is diminished.

In summary, the disposition of hexarelin is characterized by a lack of accumulation up to 50 μg/kg and long terminal half-life. Biliary excretion is the primary route of hexarelin elimination, where the intact peptide is predominant. The high recovery of intact peptide in bile and urine suggests a relatively significant in vivo stability that may represent an advantage for its potential oral administration.

References


