ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 841–850

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Comparison of sandwich enzyme-linked immunoadsorbent assay and radioimmunoassay for determination of exogenous glucagon-like peptide-1(7–36)amide in plasma

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Received for review 19 September 1994; revised manuscript received 28 November 1994

Abstract

A sensitive sandwich enzyme-linked immunoadsorbent assay (ELISA) for determination of exogenous glucagon-like peptide-1(7–36)amide (GLP-1(7–36)amide) in plasma samples from pharmacokinetic studies is described. The assay employs an N-terminally directed antibody and a C-terminally directed antibody. The ELISA has a working range from 10 to 500 pmol l^{-1}, and can be applied to plasma samples from humans, dogs, pigs, minipigs, cats, rabbits, and rats. The assay was compared to a validated radioimmunoassay (RIA), employing an antibody directed against the mid-region of GLP-1. After s.c. administration of GLP-1(7–36)amide, the plasma immunoreactivity of GLP-1 (P-GLP-1-IR) measured by ELISA was markedly lower than P-GLP-1-IR measured by RIA. After HPLC fractionation of plasma samples with subsequent RIA and ELISA analyses of the fractions, this difference was shown to be due to cross reaction with biologically inactive fragments of GLP-1(7–36)amide in the RIA but not in the ELISA.

Keywords: Glucagon-like peptide-1; GLP-1(7–36)amide; HPLC fractionation RIA; Sandwich ELISA

1. Introduction

Glucagon-like peptide-1, GLP-1, is a recently discovered gut hormone [1,2]. Two biologically active forms of GLP-1 are formed by post-translational processing of the precursor peptide pro-glucagon: GLP-1(7–36)amide and GLP-1(7–37), corresponding to proglucagon (78–107)amide and proglucagon(78–108), respectively [3]. Both peptides are present in plasma. However, in humans GLP-1(7–36)amide is by far the most abundant form [3]. The amino acid sequence of GLP-1 and some related peptides are shown in Fig. 1 [4].

GLP-1(7–36)amide is secreted from the distal ileum as a response to the oral intake of e.g. carbohydrates [1,2,5]. The peptide has several biological functions, with its main target in the islets of Langerhans in the endocrine pancreas. In β-cells, it enhances the glucose-stimulated insulin secretion [1,2] and the biosynthesis of insulin [6]. In D-cells, it enhances the secretion of somatostatin [1,2], and in α-cells glucagon secretion is inhibited, either directly or through an intraislet pathway involving somatostatin [1,2]. In the gastro-intestinal tract it inhibits gastric emptying and secretion [7].

Deletion studies have shown that the biologically active forms of GLP-1 are GLP-1(7–37), GLP-1(7–36)amide, GLP-1(7–35) and GLP-

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SSDI 0731-7085(95)01312-1

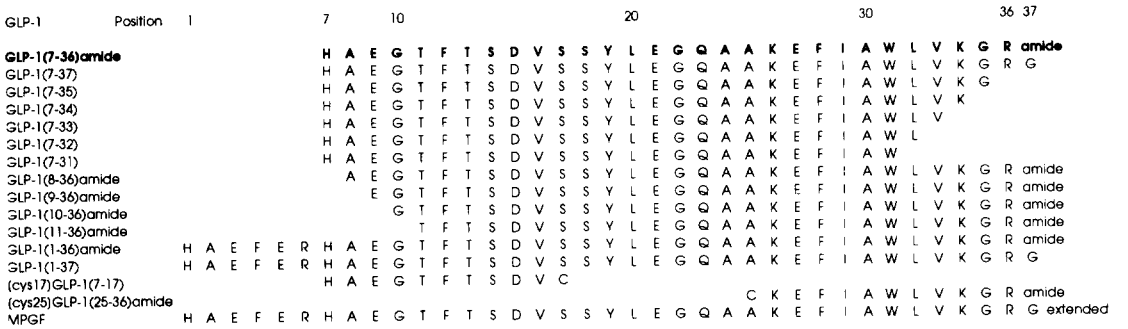

Fig. 1. Aminoacid sequence of GLP-1(7–36)amide and selected fragments, and extended forms of GLP-1(7–36)amide.

1(7–34) [8]. If the N-terminal histidine in position 7 or the lysine in position 34 is removed, the biological activity decreases by at least three orders of magnitude [8].

Recently, several studies have shown exogenous GLP-1(7–36)amide to exhibit a potential blood glucose lowering effect in patients with non-insulin dependent diabetes mellitus (NIDDM). In these studies, GLP-1(7–36)amide was infused intravenously to give plasma concentrations of 100–200 pmol l^{-1}. This corresponds to three to five times the increase in plasma concentrations of GLP-1(7–36)amide observed following a meal [5,9,10]. These results hold promise for GLP-1 as a potential therapeutic drug in the management of NIDDM.

A sandwich enzyme-linked immunoadsorbent assay (ELISA) for the determination of GLP-1(7–36)amide in dog, human, rat, rabbit, pig, minipig and cat plasma is described here. This ELISA employes two different antibodies, directly against two different epitopes: a monoclonal mouse antibody directed against the (26–33) region and a polyclonal rabbit antibody directed against the (7–14) region. The ELISA was compared to a radioimmunoassay (RIA) employing a polyclonal rabbit antibody directed against an epitope in the mid-region of the peptide. Both assays were investigated for use in pharmacokinetics studies.

2. Materials and methods

2.1. Chemicals and reagents

Aprotinin and mouse IgG (A-TNP) were from Novo Nordisk A/S, Bagsvaerd, Denmark. Bovine serum albumin (BSA), bovine gamma globulin, sodium caseinate, and 3,3′,5,5′-tetramethyl benzidine (TMB) were from Sigma (St. Louis, MO, USA). Human serum albumin (HSA) was from Behringwerke (Marburg, Germany). Tween 20, Imidazol, glycerol and DMSO were from Merck (Darmstadt, Germany). Keyhole limpet haemocyanin (KLH) and Biotin-X-NHS were from Calbiochem (La Jolla, CA, USA). Horseradish peroxidase avidin D was from Vector (Burlingame, CA, USA). CNBr-activated Sepharose 4B and Protein-A Sepharose 4 Fast Flow were from Pharmacia (Uppsala, Sweden). Deionized water was purified using a Milli-Q plant (Millipore, Milford, MA, USA).

2.2. Synthesis of peptides and conjugates

Peptides were prepared by solid phase synthesis and purified by preparative reversed-phase HPLC as previously described [11]. The purity, determined by capillary electrophoresis and analytical HPLC, was >95%. MPGF (major prolucagon fragment corresponding to proglucagon(72–158)) was isolated from human pancreas as described previously [12,13]. [cys^{17}]GLP-1(7–17) and [cys^{25}]GLP-1(25–36)amide were coupled to KLH as described by Dyrberg and Kofod [14], with the ratio of peptide to KLH being 1 mg to 1 mg in the reaction mixture. GLP-1(7–36)amide was coupled to BSA as described previously [15].

2.3. Preparation of tracer

[^{125}I-Tyr^{19}]GLP-1(7–36)amide was prepared by the lactoperoxidase method [16] and purified by reverse-phase chromatography. The specific radioactivity was 60–80 MBq $nmol^{-1}$.

2.4. Preparation of polyclonal antibodies

Rabbit no. 2135 was immunized with GLP-1(7–36)amide coupled to BSA, and rabbit no.

91022 was immunized with [cys[17]]GLP-1(7–17) coupled to KLH as described previously [12,13].

2.5. Preparation of monoclonal antibodies

RBF-mice [17] were immunized every second week with three subcutaneous injections of [cys[25]]GLP-1(25–36)amide coupled to KLH (45 μg peptide/mouse per dose). The first injection was made with Freunds Complete adjuvant, the two subsequent injections with incomplete adjuvant. Three months after the last injection and three days before fusion, a mouse was boosted with 45 μg antigen intravenously and spleen cells were prepared. Fusion was carried out using a previously described method [17], except for the use of the myeloma cell line FOX-NY as fusion partner and the corresponding selective components (Taggart Hybidoma Technology). Hybridoma supernatants were screened for antigen binding antibodies in a direct ELISA with either the GLP-1(26–36)amide[25]cys-KLH or KLH as coat. Selected clones were stabilized by subcloning, and immunoglobin purified using Protein-A Sepharose 4 Fast Flow. One antibody, GLP1-F5, was chosen for development of the sandwich ELISA.

2.6. Preparation of GLP1-F5 Sepharose

25 mg of the antibody GLP1-F5 was coupled to 5 ml washed CNBr-activated Sepharose 4B according to the manufacturer's instructions. 0.2 M glycine (pH 8.0) was used for blocking.

2.7. Preparation of biotinylated 91022-antibody

Antibody from rabbit no. 91022 was purified using Protein-A Sepharose 4 Fast Flow according to the manufacturer's instructions. 900 μg Biotin-X-NHS in 450 μl DMSO was added dropwise to 3 mg purified antibody in 4.5 ml phosphate buffered saline (PBS) and incubated overnight at 4 °C. Thereafter, 1050 μl of 0.5 M imidazol and finally 6 ml glycerol were added. The solution was kept at −20 °C.

2.8. Blood sampling

Blood samples were collected in ice-chilled polyethylene vials (Minisorp, Nunc, Roskilde, Denmark) containing 20 μl 0.3 M EDTA, and 3.7 mg ml^{-1}aprotinin (pH 7.4) per ml blood. The samples were kept on ice and centrifuged (2000 *g*, 10 min, 4 °C) within 30 min. Plasma was transferred to Minisorp vials and stored at −20 °C until analyzed.

2.9. ELISA procedure

The ELISA setup was a sandwich ELISA.

PBS

10 mM sodium phosphate and 145 mM sodium chloride (pH 7.2).

Buffer A

PBS containing in addition 35 mM ammonium sulphate, 0.024% thiomersal, 10 g l^{-1} BSA, 1 g l^{-1} bovine gamma globulin and 0.1 g l^{-1} mouse IgG (A-TNP).

Buffer B

PBS containing in addition 250 mM ammonium sulphate, 1 g l^{-1} BSA, 1 g l^{-1} sodium caseinate, and 0.1 g l^{-1} methyl-4-hydroxybenzoate.

Substrate buffer

40 mM sodium acetate/acetic acid (pH 5.0) containing 3.2 mM sodium borate.

Stop buffer

4 M phosphoric acid and 10 mM ethylene diamine tetraacetic acid (EDTA).

Standards

2, 5, 12.5, 30, 75, 180, 413, 1025 and 2475 pmol l^{-1}GLP-1(7–36)amide were prepared in homone free plasma (plasma immunosorbed in order to remove endogenous GLP-1-IR: 10 ml plasma and 100 μl Sepharose-F5 were rotated slowly for 1 h, centrifuged (10 min, 2000 *g*), decanted, and filtered (0.45 μm Millipore)).

Assay procedure

Microtiter plates (96-well, Immunoplate Maxisorp, Nunc, Roskilde, Denmark) were coated with 100 μl catching antibody (GLP1-F5, 10 μg ml^{-1} in PBS buffer) and incubated overnight at 4 °C. The following day the plates were washed once with washing solution (PBS containing in addition 1 ml l^{-1} of Tween 20) and blocked by incubation for 1 h with 200 μl blocking buffer (PBS containing in addition 5 g l^{-1} of BSA), and washed a further three times with washing solution. 25 μl standard or

plasma sample and 75 µl detecting antibody (biotinylated 91022-antibody, 4 µg ml^{-1} in buffer A) were added and the plates incubated for 2 h at room temperature with continuous shaking. The plates were then washed three times with washing solution, and incubated for 1 h at room temperature under continuous shaking with 100 µl avidin-peroxidase (diluted 1:35 000 in buffer B). The plates were again washed three times with washing solution and once with substrate buffer. Finally, the plates were developed by incubation for 10 min at room temperature with 100 µl substrate (one tablet, containing 1 mg of TMB, dissolved in 7 ml substrate buffer), and the reaction was stopped by addition of 100 µl stop buffer. Absorbance values were determined by an ELISA reader (Easy reader 340 AT, SLT Labinstruments, Grödig, Austria) at 450 nm with 620 nm as reference. The raw data were transferred on-line to a computer (IBM, PS/2, Model 30) and processed using the software ELISA+ version 3.0 (Meddata Inc., NY, USA). Standard curves and unknown samples were calculated using a four parametric logistic curvefit.

2.10. RIA procedure

Assay buffer

100 mM tris(hydroxymethyl)aminomethane (TRIS), 50 mM sodium chloride, 20 mM disodium EDTA, 0.02% sodium azide, 0.2% HSA, and 60 mg l^{-1} aprotinin, pH 8.5 was used for analyzing rabbit, rat and minipig plasma samples. When used for analyzing dog and human plasma samples, 100 ml ethanol were added to 900 ml of assay buffer. The RIA assay has not been validated for use with pig and cat plasma samples.

Standards

0, 30, 45, 70, 110, 176, 250, 400, 650, 1000, 1500, and 2000 pmol l^{-1} of GLP-1(7–36)amide were prepared in hormone free plasma (charcoalabsorbed plasma in order to remove endogenous peptides) [18].

Assay procedure

100 µl standard or 100 µl plasma sample and 300 µl antibody (serum from rabbit 2135 diluted 1:30 000 in assay buffer, final dilution 1:50 000) were incubated for 40–72 h at 4 °C in Minisorp polyethylene vials. 100 µl tracer (10 000–20 000 cpm dissolved in assay buffer) were added and the samples incubated for 40–72 h at 4 °C, before the bound and free peptides were separated by addition of 1.5 ml of activated charcoal suspension (4.5 g activated charcoal, 50 ml pig plasma and 250 ml assay buffer), centrifugation (30 min, 4 °C, 3000*g*) and decantation. The radioactivity of the supernatant was counted in a gamma counter (Packard Cobra II Auto-Gamma). The raw data were processed using the Packard Cobra II Auto Gamma software. Standard curves and unknown samples were calculated using a four parametic logistic curvefit.

2.11. HPLC

Extraction procedure

0.5 ml plasma was extracted on a Sep-Pak C_{18} plus cartridge (No 23635, Millipore, Milford, Mass, USA). The columns were activated by 2 ml methanol followed by 2 ml acetate buffer (0.1 M acetic acid mixed with 0.1 M sodium acetate to pH 5.0). 0.5 ml plasma was mixed with 2.5 ml acetate buffer and applied to the column. The column. The column was washed with 2 ml acetate buffer, followed by 2 ml Milli-Q water. GLP-1(7–36)amide was eluted from the column by 4 ml 0.1% (v/v) trifluoroacetic acid in 20% (v/v) methanol. The eluates were evaporated in a SpeedVac SC200 (Savant, NY, USA), and redissolved in 125 µl eluent A and 100 µl injected onto the HPLC column.

HPLC procedure

HPLC column: Superspher 100 RP-18 endcapped (E. Merck, Darmstadt, Germany). Eluents: (A) 0.1% trifluoracetic acid in Milli-Q water and (B) 0.1% trifluoracetic acid in acetonitrile. The mobile phase was changed using linear gradients from 41% (v/v) B to 43% B between 0 and 35 min, from 43 to 80% B between 35 and 40 min, whereafter it was changed back to the initial 40% B between 40 and 45 min. The wavelength used was 280 nm. HPLC system: two Shimadzu LC-6A pumps, a Shimadzu SCL-6B controller, a Shimadzu SPD-6A UV-detector, a Shimadzu SIL-6A autosampler and Shimadzu C-R5A integrator. Fractions were collected each 0.5 min from 5–35 min post injection. The fractions were divided for ELISA and RIA assays and freeze dried. For ELISA the fractions were dissolved in immunosorbed dog plasma before assay and for RIA the fractions were dissolved in assay buffer.

2.12. Pharmacokinetics

Immediately before use, 360 μg GLP-1(7–36)amide was dissolved in 0.975 ml Actrapid medium (16 mg ml^{-1} glycerol and 3 mg ml^{-1}-cresol, pH 7.4). One Beagle dog (♂, weight 18.5 kg, age 4 years) was restrained, and a teflon catheter (Venflon 2, 1.2 mm°, 32 mm, Viggo-Spectramed, Helsingborg, Sweden) was inserted into a vein in one foreleg. The dog was injected with 500 μl subcutaneously in the neck, and blood samples were collected at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min after injection.

3. Results and discussion

3.1. Immuno assays

The ELISA was applied to plasma samples for dogs, humans, minipigs, pigs, cats, rabbits, and rats. The RIA was only applied to plasma samples from dogs, humans, minipigs, rabbits, and rats. In order to compare the two assays, only results for dog plasma are shown, as both assays were developed for use in pharmacokinetic studies primarily in dogs.

3.2. Standard curves

The standard curves can be characterized by the four parametric equation

$$y = (a - d)/\{1 + [\log(x)/c]^b\} + d$$

where a = response at zero concentration, b = slope factor, c = log concentration mid-point ≈ log IC_{50} (IC_{50} is the concentration that gives the response halfway between the response of zero concentration and the response at infinite concentration of the compound) and d = response at infinite concentration. Fig. 2 shows MEAN ± SD of eight standard curves in dog plasma measured by ELISA and RIA, respectively.

3.3. Assay specificity

A dilution curve with 11 concentrations, ranging from 15 to 150 000 pmol l^{-1}, of each peptide were measured in triplicate in both RIA and ELISA. Cross reactivity was calculated using the ratio between estimated IC_{50} between GLP-1(7–36)amide and the other peptide.

Several GLP-1 fragments were detected by the sandwich ELISA, with cross reactivities of: GLP-1(7–37) 130%, GLP-1(7–35) 88%, GLP-1(7–34) 92%, GLP-1(7–33) 17%, GLP-1(7–

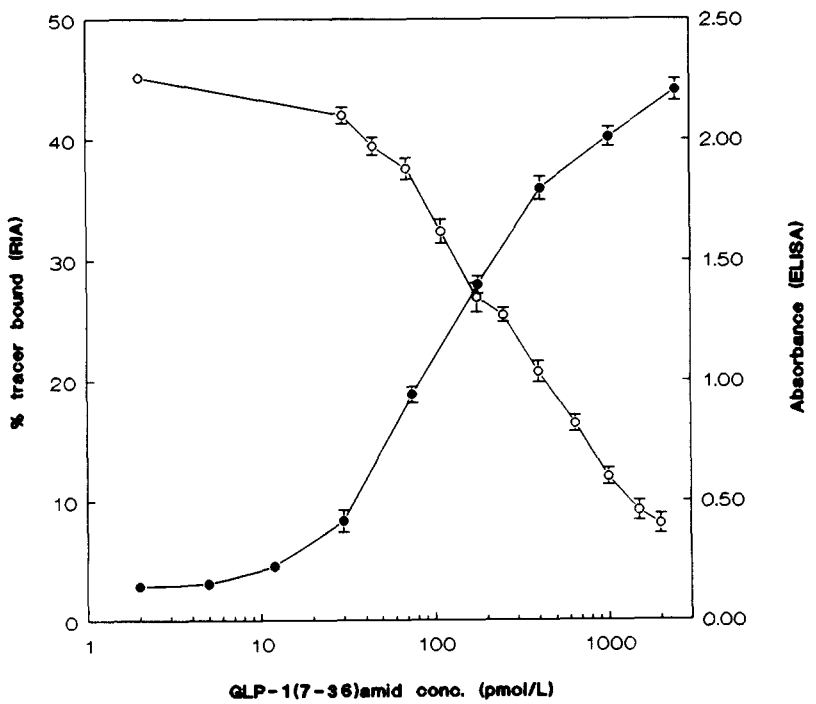

Fig. 2. Standard curves of GLP-1(7–36)amide in dog plasma measured by RIA (○) and ELISA (●), respectively. Results are MEAN ± SD. $N = 8$.

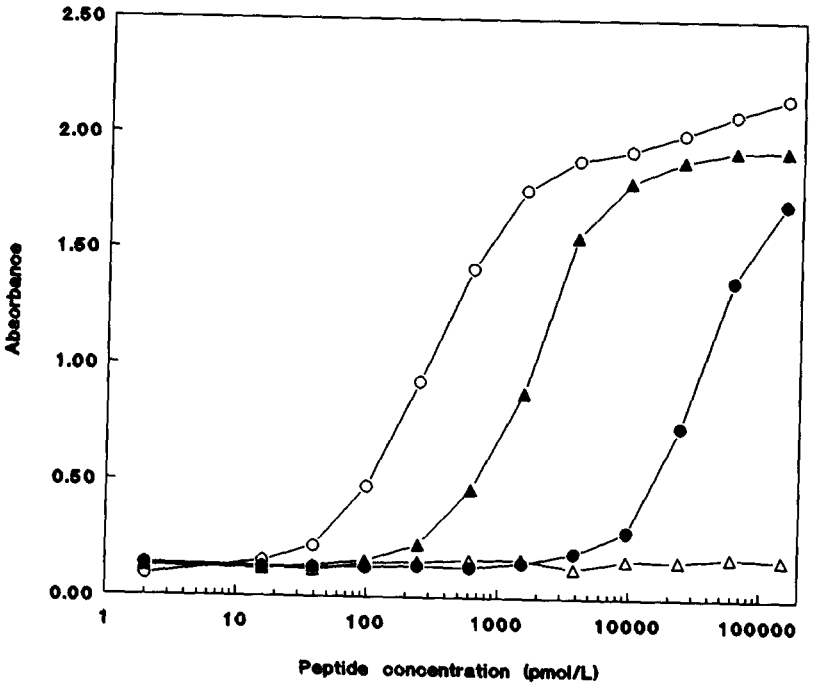

Fig. 3. Dilution curves in dog plasma measured by ELISA of GLP-1(7–36)amide (○), GLP-1(7–33) (▲), GLP-1(8–36)amide (●) and GLP-1(7–32)/GLP-1(10–36)amide (they give identical curves) (△).

32) < 0.01%, GLP-1(7–31) < 0.01%, GLP-1(8–36)amide 1%, GLP-1(9–36)amide 1%, GLP-1(10–36)amide < 0.01% and GLP-1(11–36) amide < 0.01%. The precursors to GLP-1(7–36)amide were also detected in this assay, with cross reactivities of: GLP-1(1–37) (proglucagon (72–108)) 79%, GLP-1(1–36)amide (proglucagon(72–107)amide) 65% and MGPF (proglucagon(72–158)) 71%. Other peptide hormones of the glucagon family did not interfere in this assay, with cross reactions: glucagon, GIP, VIP, PHM and secretin, all <0.01%. The cross reactivity of the fragments of GLP-1 and their biological activity corresponds very well in this assay. Fig. 3 illustrates the cross reaction with different GLP-1 fragments in the ELISA.

The following GLP-1 fragments were detected by the RIA, with cross reactivities of: GLP-1(7–37) 151%, GLP-1(7–35) 138%, GLP-1(7–34) 159%, GLP-1(7–33) 103%, GLP-1(7–32) 269%, GLP-1(7–31) 307%, GLP-1(8–36)amide 105%, GLP-1(9–36)amide 113%, GLP-1(10–36)amide 92%, and GLP-1(11–36)amide 40%. The precursors to GLP-1(7–36)amide were also detected in this assay with cross reactivities: GLP-1(1–37) 126% and GLP-1(1–36)amide 141%. Glucagon was not detected in this assay, having a cross reaction of <0.01%. It should, though, be noted that the cross reactivities of C-terminal extended and truncated forms of GLP-1 were calculated to be above 100%, although none of the fragments at any concentration displaced the tracer better than GLP-1(7–36)amide at the same concentration. This was due to the displacement curves for C-terminally extended or truncated form of GLP-1(7–36)amide not being parallel to the displacement curve for GLP-1(7–36)amide, and they did not displace the tracer 100% at any concentration. The reason for this might be the polyclonal nature of the antibody. The cross reactivity of the fragments of GLP-1 and their biological activity does not correspond. In this assay, the antibody did cross react with all the tested fragments of GLP-1. This is illustrated in Fig. 4.

The cross reactivity of GLP-1(7–36)amide fragments was markedly lower in the ELISA compared to the RIA. The tested fragments were possible metabolites of GLP-1(7–36)amide and, thus, the ELISA was preferable to the RIA for estimation of exogenous GLP-1(7–36)amide in pharmacokinetic studies.

3.4. Limit of quantification and accuracy

Plasma pooled from six different dogs was spiked with GLP-1(7–36)amide, and the plasma concentrations was determined (Table 1). The accuracy could not be determined when

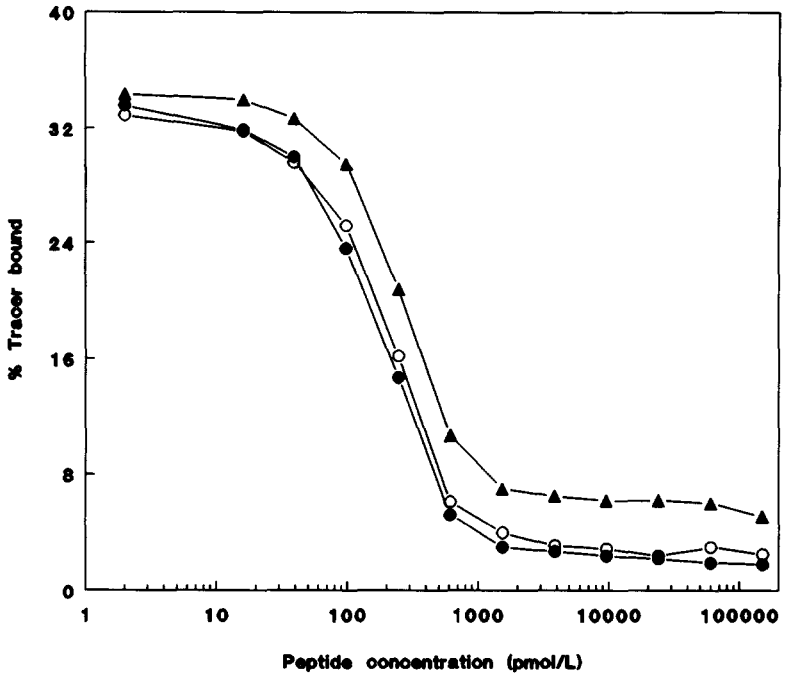

Fig. 4. Dilution curves in dog plasma measured by RIA of GLP-1(7–36)amide (○), GLP-1(7–33) (▲) and GLP-1(9–36)amide (●).

the plasma samples contained endogenous GLP-1. Instead, the recovery was used. As seen in Table 1, the limit of quantification (LOQ) was defined as the lowest concentration where the accuracy was 80–120% and the CV < 20% was 20 pmol l^{-1} for the ELISA and 50 pmol l^{-1} for the RIA. From Table 1, and Figs. 2 and 3, the working range was estimated to be 20–500 pmol l^{-1} for the ELISA and 50–1000 pmol l^{-1} for the RIA.

3.5. Intraassay and interassay precision

Three plasma samples with P-GLP-1 concentrations at different levels were measured eight times each in one assay (intraassay), and the same samples were measured in triplicate in six different assays (interassay) (Table 2). From Table 2, the intraassay precision of ELISA and RIA was 6–14% and 9–11%, respectively, whereas the interassay precision was 15–19% in ELISA compared to 8–13% in RIA. Thus, RIA had a better interassay precision than ELISA.

3.6. Pharmacokinetics

Fig. 5 shows the plasma concentrations obtained by RIA and ELISA after s.c. administration of 10 μg kg^{-1} of GLP-1(7–36)amide to a dog. The concentration increment over the

Table 1
Limit of quantification and accuracy

	ELISA			RIA		
	Mean (pmol l^{-1})	Recovery (%)	CV (%)	Mean (pmol l^{-1})	Recovery (%)	CV (%)
+0 pmol l^{-1}	19	–	9	66	–	18
+10 pmol l^{-1}	26	75	7	45	–	33
+20 pmol l^{-1}	35	80	11	62	–	19
+50 pmol l^{-1}	65	94	14	120	108	18
+100 pmol l^{-1}	128	109	10	182	116	19
+200 pmol l^{-1}	250	116	10	317	126	10
+500 pmol l^{-1}	529	102	17	658	118	11
+800 pmol l^{-1}	1162	143	47	994	116	5
+1000 pmol l^{-1}	1335	132	45	1137	107	6

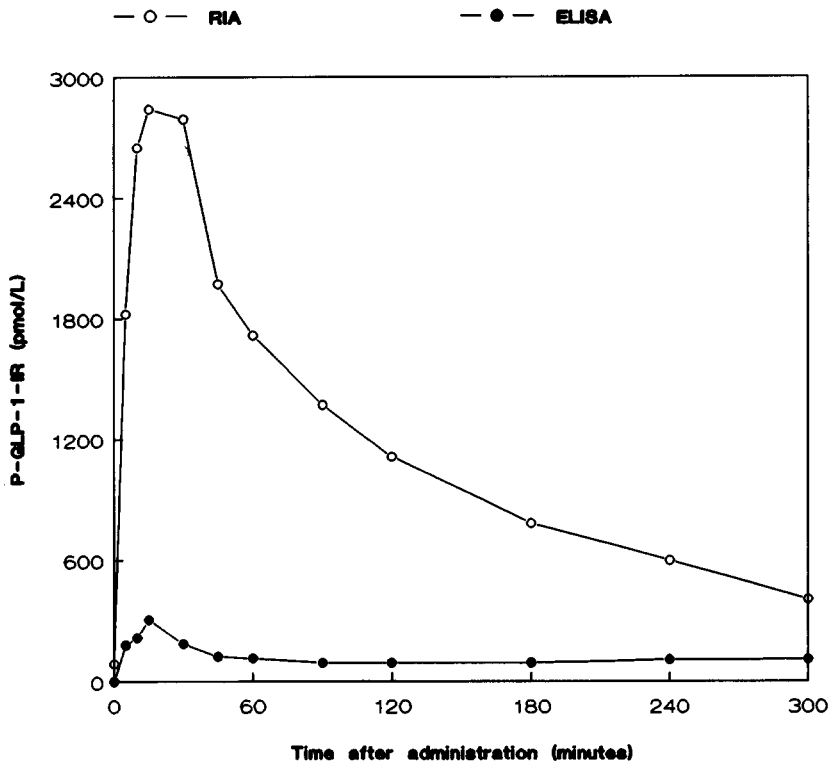

Fig. 5. Concentration–time profiles of GLP-1 in plasma obtained after s.c. administration of 10 μg kg^{-1} of GLP-1(7–36)amide to one Beagle dog measured by RIA (○) and ELISA (●).

baseline of GLP-1 measured by RIA was 3–15 times higher than by the use of ELISA. The reason for this could be that RIA crossreacts almost 100% with the tested metabolites of GLP-1(7–36)amide contrary to ELISA, which only crossreacts with biologically active fragments of GLP-1(7–36)amide. It has been suggested previously [19] that in plasma samples GLP-1(7–37) is degraded to GLP-1(9–37) with a $t_{1/2}$ of 19.9 ± 6.6 min at 37 °C in human plasma, but circulating concentration of GLP-1 fragements have not yet been studied extensively.

3.7. HPLC fractionation of plasma samples

GLP-1(7–36)amide and fragments were injected into the column both separately and in a mixture. The reaction time was GLP-1(7–36)amide 7.0 min, GLP-1(8–36)amide 9.7 min, GLP-1(9–36)amide 9.8 min, GLP-1(7–35) 15.4 min, GLP-1(7–34) 13.6 min, and GLP-1(7–33) 16.3 min, respectively. Thus, GLP-1(7–36)amide, GLP-1(7–35), GLP-1(7–34) and GLP-1(7–33) could be separated on this column. GLP-1(8–36)amide and GLP-1(9–36) amide could not be separated from each other,

Table 2
Intra-assay and interassay precision of ELISA and RIA

	ELISA				RIA			
	Intra-assay		Interassay		Intra-assay		Interassay	
	Mean (pmol l^{-1})	CV (%)	Mean (pmol l^{-1})	CV (%)	Mean (pmol l^{-1})	CV (%)	Mean (pmol l^{-1})	CV (%)
Sample 1	16	6	16	19	108	9	97	13
Sample 2	57	9	54	17	203	11	210	11
Sample 3	249	14	226	15	450	11	432	8

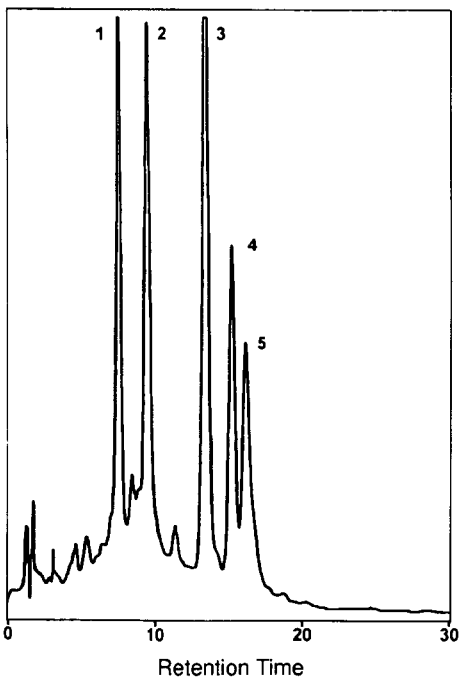

Fig. 6. HPLC chromatogram of a mixture of six GLP-1 fragments. (1) GLP-1(7–36)amide, $t_R = 7.8$ min; (2) GLP-1(8–36)amide/GLP-1(9–36)amide, $t_R = 9.7$ min; (3) GLP-1(7–34), $t_R = 13.6$ min; (4) GLP-1(7–35), $t_R = 15.4$ min; (5) GLP-1(7–33), $t_R = 16.4$ min. A mixture containing 10–20 μg of each fragment was injected onto the column.

but could be separated from the other tested peptides. This is illustrated in Fig. 6 showing a HPLC chromatogram, obtained by injecting a mixture of GLP-1(7–36)amide, GLP-1(8–36)amide, GLP-1(9–36)amide, GLP-1(7–35), GLP-1(7–34) and GLP-1(7–33) on the column.

Fig. 7 shows the HPLC fractions from the 10 min post-injection plasma sample following s.c. administration to a dog, measured by ELISA and RIA, respectively. As illustrated, the HPLC fractionation of the GLP-1-IR contained in the 10 min post-dose plasma sample when measured by ELISA resulted in three peaks, corresponding to GLP-1(7–36)amide (≈35%), GLP-1(7–34) (≈45%) and GLP-1(7–35) (≈20%). The same HPLC-fractions measured by RIA contained more than four main peaks, GLP-1(7–36)amide (≈15%), GLP-1(8/9–36)amide (≈30%), GLP-1(7–34) (≈35%) and GLP-1(7–35) (≈20%). This study indicated that GLP-1(7–36)amide was degraded both from the N-terminus and from the C-terminus when administered s.c. to a dog. Thus, when developing an assay to measure exogenous GLP-1(7–36)amide, the specificity of the assay should be examined and considered carefully.

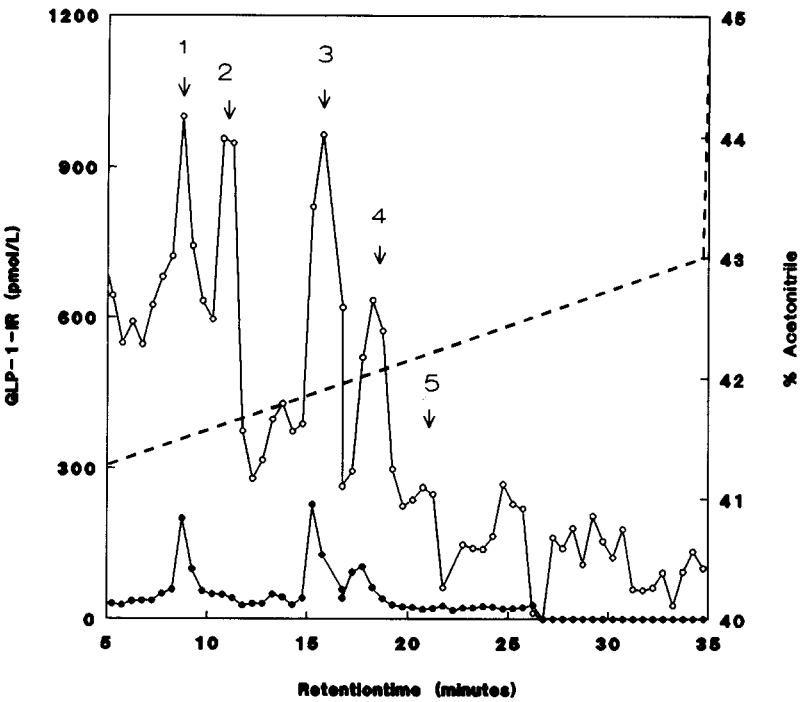

Fig. 7. HPLC fractionation with subsequent RIA and ELISA of one plasma sample, from one Beagle dog collected 10 min after administrating 10 μg kg^{-1} of GLP-1(7–36)amide s.c. (1) GLP-1(7–36)amide; (2) GLP-1(8–36)amide/GLP-1(9–36)amide; (3) GLP-1(7–34); (4) GLP-1(7–35); and (5) GLP-1(7–33). The HPLC gradient is indicated by the dashed line.

4. Conclusions

In conclusion the ELISA described here was not specific to exogenous GLP-1(7–36)amide, but *was* specific to the biologically active fragments of GLP-1(7–36)amide. The ELISA was superior to the RIA for estimation of exogenous GLP-1(7–36)amide in pharmacokinetic studies, owing to the reduced cross reactivity with fragments of GLP-1 in the ELISA. The results suggest that GLP-1(7–36)amide was degraded both from the N-terminus and from the C-terminus when administered s.c. to one dog. When developing an assay for measurement of exogenous GLP-1(7–36)amide, the specificity of the assay should be examined and considered carefully. The specificity of the present assay might be preferable to a strictly specific GLP-1(7–36)amide assay, when the biological activity is the parameter of interest in clinical studies.

Acknowledgements

Frits Poulsen and Lennart Andersen are thanked for helpful advice, and Ulla Dahl Larsen and Christian Foged for labelling and purifying the tracer. For technical assistance; Linda Clemmensen, Merete Hansen, Alex Jensen and Mette Winther are thanked.

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