ELSEVIER

European Journal of Pharmacology 318 (1996) 429–435

ejp

Glucagon-like peptide-1-(9–36) amide is a major metabolite of glucagon-like peptide-1-(7–36) amide after in vivo administration to dogs, and it acts as an antagonist on the pancreatic receptor

Lotte Bjerre Knudsen *, Lone Pridal

Novo Nordisk, Novo Allé, DK-2880 Bagsværd, Denmark

Received 1 October 1996; accepted 4 October 1996

Abstract

This study assesses the importance of metabolites formed following exogenous administration of glucagon-like peptide-1-(7–36) amide (GLP-1). After subcutaneous (s.c.) administration of GLP-1 to dogs the plasma immunoreactivity of GLP-1 measured by two different radioimmunoassays (RIAs) were higher than that measured by a sandwich enzyme-linked immunosorbent assay (ELISA). This discrepancy was due to the formation of the metabolites GLP-1-(9–36) amide, GLP-1-(7–35) and GLP-1-(7–34). Receptor binding studies using baby hamster kidney cells expressing the human pancreatic GLP-1 receptor showed that the affinity of GLP-1-(9–36) amide, GLP-1-(7–35) and GLP-1-(7–34) was 0.95%, 12% and 2.8%, respectively, of the affinity of GLP-1-(7–36) amide. Furthermore, GLP-1-(9–36) amide was shown to be an antagonist to adenylyl cyclase activity, whereas GLP-1-(7–35) and GLP-1-(7–34) were shown to be agonists. GLP-1-(9–36) amide was shown to be present in vivo in amounts up to 10-fold that of GLP-1-(7–36) amide. Due to its low binding affinity, this antagonistic metabolite does not seem to be able to cause physiological antagonism upon s.c. administration of the peptide.

Keywords: GLP-1 (glucagon-like peptide-1); GLP-1-(7–36) amide; GLP-1-(9–36) amide; GLP-1-(7–35); GLP-1-(7–34); Receptor binding; Receptor activation; (Metabolite)

1. Introduction

Discrepancies between the plasma concentrations of glucagon-like peptide-1 (GLP-1) measured by different immunoassays have been reported before (Pridal et al., 1995, 1996; Deacon et al., 1995a). These discrepancies were shown to be due to different specificity of the antibodies used in the assays and accordingly different cross-reactivity with the in vivo formed circulating metabolites of GLP-1.

The metabolism of GLP-1-(7–37) to GLP-1-(9–37) and GLP-1-(7–36) amide to GLP-1-(9–36) amide by a proline specific peptidase dipeptidyl peptidase IV in vitro has recently been reported (Mentlein et al., 1993; Deacon et al., 1995b). In addition, in vivo studies in humans and dogs revealed that GLP-1-(9–37) and GLP-1-(9–36) amide were major metabolites (Deacon et al., 1995b; Pridal et al., 1996). So far, neither GLP-1-(9–36) amide nor GLP-1-(9–37) have been characterized with respect to their biological action.

Structure-activity studies of GLP-1 (Adelhorst et al., 1994; Gallwitz et al., 1994, 1995) have shown the N-terminus to be essential for signal transduction as replacement of the N-terminal histidine with alanine or tyrosine results in a peptide with a markedly reduced ability to activate the receptor. The C-terminus mainly served to keep the peptide in the right conformation (Adelhorst et al., 1994). In the glucagon/secretin family of which GLP-1 is a member, some of the peptides such as glucagon, vasoactive intestinal peptide (VIP) and secretin have a serine in the penultimate position and a histidine in the N-terminal position. Deletion of His^1 in these peptides (Unson et al., 1987; Rorstad et al., 1990; Robberecht et al., 1988) have long been known to result in peptides with a markedly reduced binding affinity. Also, in the case of glucagon the resulting product is an antagonist (Unson et al., 1987). However, it is not known if such products are

* Corresponding author. Department of Molecular Pharmacology, F9.S.14, Novo Nordisk A/S, Novo Park, DK-2760 Måløv, Denmark. Tel.: (45-44) 434-788; Fax: (45-44) 662-980.

PII S0014-2999(96)00795-9

formed as a part of the physiological metabolism of these hormones. The other part of the family has alanine in the penultimate position and histidine or tyrosine in the N-terminal position. These peptides are potentially metabolized by dipeptidyl peptidase IV, which gives rise to products truncated by two amino acids in the N-terminal position (Mentlein et al., 1993; Frohman et al., 1989). Gastrointestinal inhibitory peptide (GIP) and growth hormone-releasing factor (GRF) have been described to be metabolized by dipeptidyl peptidase IV to GIP-(3–42) and GRF-(3–44) resulting in loss of activity (Frohman et al., 1989; Jörnvall et al., 1981; Schmidt et al., 1986). As the receptor for GLP-2 is not known, no structure-activity studies have been performed.

Here, we report that GLP-1-(9–36) amide is a major metabolite when exogenous administrated GLP-1-(7–36) amide is metabolized in vivo in the dog and that it acts as an antagonist to the human pancreatic GLP-1 receptor. Also, we report the receptor affinities and potencies of two other metabolites, GLP-1-(7–35) and GLP-1-(7–34).

2. Materials and methods

2.1. Peptide synthesis

The peptides were prepared by solid phase synthesis and purified by preparative high performance liquid chromatography (HPLC) as previously described (Adelhorst et al., 1994). The purity, determined by analytical HPLC and capillary electrophoresis was > 95%.

2.2. Iodination of tracer

Iodination of GLP-1-(7–36) amide was performed by the lactoperoxidase method (Thorell and Johansson, 1971). Purification by HPLC as previously described (Drejer et al., 1991) yielded (^{125}I-Tyr^{19})GLP-1-(7–36) amide with a specific activity of 80 kBq/pmol.

2.3. Receptor binding

Receptor binding was carried out as previously described (Adelhorst et al., 1994) with few changes. In brief, baby hamster kidney (BHK) cells expressing the human pancreatic GLP-1 receptor were used. Plasma membranes were prepared by homogenization in buffer (10 mmol/l Tris-HCl and 30 mmol/l NaCl pH 7.4, containing, in addition, 1 mmol/l dithiothreitol, 5 mg/l leupeptin (Sigma, St. Louis, MO, USA), 5 mg/l pepstatin (Sigma), 100 mg/l bacitracin (Sigma), and 16 mg/l aprotinin (Novo Nordisk A/S, Bagsværd, Denmark)). The homogenate was centrifuged on top of a layer of 41% (w/v) sucrose. The white band between the two layers was diluted in buffer and centrifuged. Plasma membranes were stored at −80°C until used. The assay was performed in 96-well filter microtiter plates. The buffer used was 50 mmol/l Hepes, pH 7.4, with the addition of 2.5% human serum albumin grade V (Sigma). Peptide, tracer and plasma membranes were incubated for 30 min at 30°C.

2.4. Adenylyl cyclase experiments

Plasma membranes were prepared as above. Incubations were carried out in 96-well microtiter plates in a total volume of 150 μl. The buffer used was 50 mmol/l Tris-HCl, pH 7.4 with the addition of 1 mmol/l EGTA, 1.5 mmol/l $MgSO_4$, 1.7 mmol/l ATP, 20 μmol/l GTP, 2 mmol/l 3-isobutyl-1-methylxanthine (IBMX), 0.01% Tween-20 and 0.1% human serum albumin (HSA) (Reinst, Behringwerke, Marburg, Germany). GLP-1 or analogues were diluted in buffer, added to the membrane preparation and the mixture was incubated for 2 h at 37°C. The reaction was stopped by the addition of 25 μl of 0.05 mol/l HCl. Samples were diluted 5-fold before analysis for cAMP formed by a scintillation proximity assay (RPA 538, Amersham, UK).

2.5. Pharmacokinetics

Two Beagle dogs one of each gender weighing between 16 and 17 kg were restrained in a harness. Venipuncture was performed in a foreleg using a teflon catheter (Venflon 2, 1.2 mm diameter, 32 mm, Viggo-Spectramed, Helsingborg, Sweden). 100 μl GLP-1-(7–36) amide dissolved in saline were injected subcutaneously (s.c.) in the neck, resulting in a dose of 2 μg/kg (600 pmol/kg). Blood samples were collected pre-dose and 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min post-dose. Blood samples were collected into ice-chilled polyethylene vials (Minisorp, Nunc, Roskilde, Denmark) containing 20 μl of anti-coagulant/enzyme inhibitor (0.3 mol/l EDTA, 3.7 mg/ml 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 polyethylene vials and stored at −20°C until analyzed.

2.6. Immunoassays

Plasma samples were analyzed with three different immunoassays. The first assay was a mid-terminal RIA that employs antibody 2135, which cross-reacts with all molecules containing a central sequence of GLP-1-(7–36) amide. The cross-reactivities of GLP-1-(7–33) and GLP-1-(10–36) amide were 103% and 92%, respectively. This assay was carried out as previously described (Pridal et al., 1995). The second assay was a C-terminal specific RIA that employs a C-terminally directed antibody 89390, which cross-reacts less than 0.4% with GLP-1-(7–35) and 100% with GLP-1-(9–36) amide. This assay was carried out as previously described (Hvidberg et al., 1994) except that

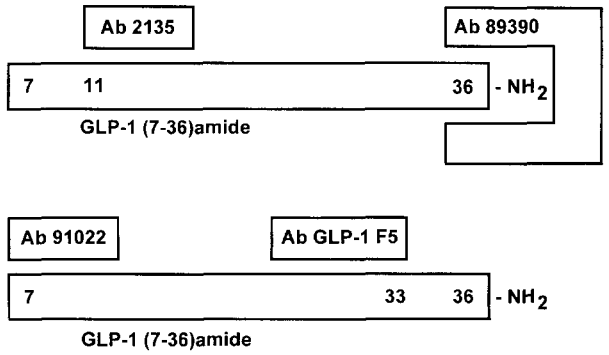

Fig. 1. The specificity of the antibodies used in this study. The upper drawing shows the antibodies used in the two radioimmunoassays. The antibody 2135 cross-reacts with all peptides containing a central sequence of GLP-1-(7–36) amide. The cross-reactivities of GLP-1-(7–33) and GLP-1-(11–36) amide were 103% and 40%, respectively. The antibody 89390 cross-reacts less than 0.4% with GLP-1-(7–35) and 100% with GLP-1-(9–36) amide. The lower drawing shows the two antibodies used in the sandwich ELISA. This combination of antibodies results in cross-reactivities of 17% with GLP-1-(7–33), less than 0.1% with GLP-1-(7–32) and 1% with GLP-1-(8–36) amide.

the assay was performed on non-extracted plasma and the assay buffer was supplemented with 10% (v/v) ethanol. The third assay was a sandwich enzyme-linked immunosorbent assay (ELISA) measuring full length and some C-terminally shortened fragments. The assay employs both an N-terminally directed antibody 91022 and a C-terminally directed antibody GLP1-F5. The cross-reactivity with C-terminal fragments of GLP-1-(7–36) amide is 17% with GLP-1-(7–33), less than 0.01% with GLP-1-(7–32) and 1% with GLP-1-(8–36)amide. The ELISA has been described and validated elsewhere (Pridal et al., 1995). Fig. 1 shows the specificity of the antibodies used in this study.

2.7. HPLC fractionation

HPLC fractionation of plasma samples with subsequent analysis of the fractions with the mid-terminal RIA has been described in detail elsewhere. Also, the identity of the different peaks has been confirmed by HPLC separation of a mixture of pure GLP-1-(7–36) amide and analogues (Pridal et al., 1995).

3. Results

3.1. Pharmacokinetics

GLP-1-(7–36) amide was injected s.c. to Beagle dogs. The plasma immunoreactivity of GLP-1 measured by the mid-terminal RIA, C-terminal specific RIA and ELISA, respectively, are outlined in Fig. 2. The area under the curves (AUC) for the two dogs, respectively, were calculated to be 62 nmol/min per l and 72 nmol/min per l when measured by the mid-terminal RIA, 33 nmol · min/l and 36 nmol · min/l when measured by C-terminal specific RIA, 6.9 nmol/min per l and 7.5 nmol/min per l when measured by ELISA.

In order to account for these differences in plasma GLP-1 immunoreactivity as measured by the three different immunoassays, plasma samples were subjected to HPLC fractionation with subsequent measurement by the mid-terminal RIA. The GLP-1 immunoreactivity after HPLC fractionation of a plasma sample is shown in Fig. 3. Three of the major metabolites have been identified both by their retention time and by their reaction with the different immunoassays. The metabolites were, GLP-1-(9–

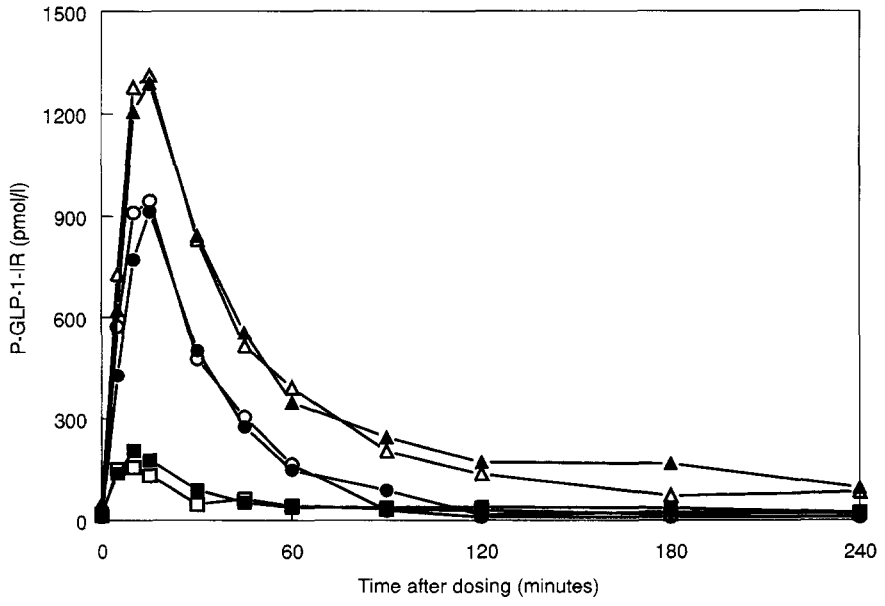

Fig. 2. Concentration-time profile of GLP-1 in plasma obtained after s.c. administration of 2 μg/kg of GLP-1-(7–36) amide to two Beagle dogs (open markers male, closed markers female). Results measured by mid-terminal RIA (▲, △) by C-terminal specific RIA (●,○) and by ELISA (■,□).

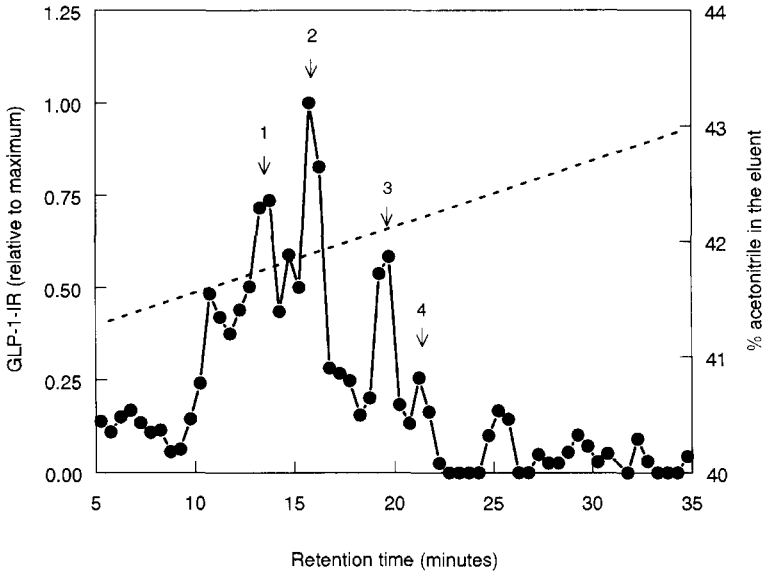

Fig. 3. HPLC fractionation with subsequent measurement by mid-terminal RIA (●) of a representative plasma sample, from a Beagle dog collected 15 min after s.c. administration of GLP-1-(7–36) amide. (1) GLP-1-(7–36) amide, (2) GLP-1-(9–36) amide, (3) GLP-1-(7–34), and (4) GLP-1-(7–35). The HPLC gradient is indicated by the dashed line. The profile represents one of three experiments.

36) amide, GLP-1-(7–34) and GLP-1-(7–35), ranked after their peak area from the HPLC fractionation, which is equivalent to their contribution to the immunoreactivity from the mid-terminal RIA.

3.2. Metabolite characterization

To study the biological significance of the metabolites, GLP-1-(9–36) amide, GLP-1-(7–35) and GLP-1-(7–34)

Table 1
Binding affinity and adenylyl cyclase activities using the cloned human pancreatic GLP-1 receptor expressed in BHK cells. Results are given as IC_{50} or EC_{50}, respectively, relative to GLP-1-(7–36) amide

Compound	Binding affinity (%)	Adenylyl cyclase activity (%)
GLP-1-(7–36) amide	100	100
GLP-1-(9–36) amide	0.95 ± 0.25	Not measured
GLP-1-(7–35)	12 ± 1	8.1 ± 3.9
GLP-1-(7–34)	2.8 ± 0.3	1.1 ± 1.0

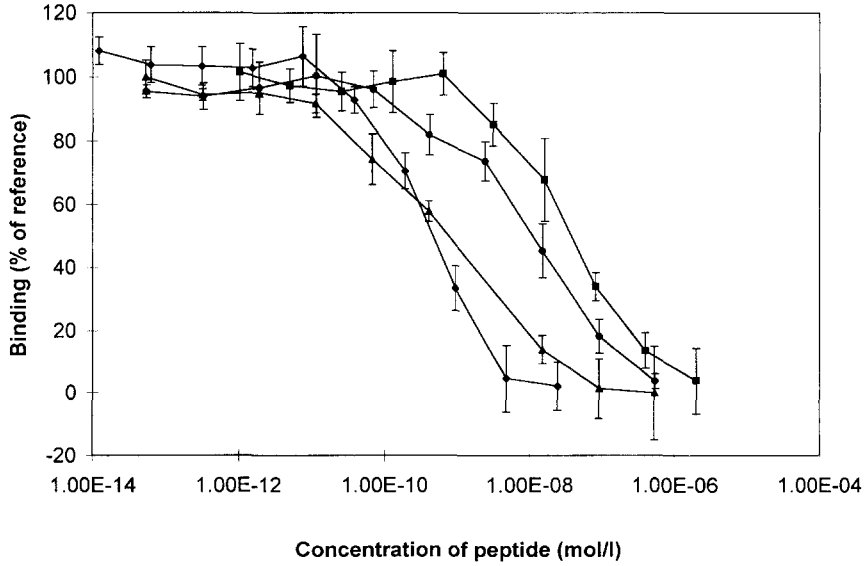

Fig. 4. Competition with binding of (^{125}I-Tyr^{19})-GLP-1-(7–36) amide to plasma membranes from BHK cells expressing the cloned human pancreatic receptor. GLP-1-(7–36) amide (◆), GLP-1-(9–36) amide (■), GLP-1-(7–34) (●) and GLP-1-(7–35) (▲). Curves represent one experiment with samples measured in triplicate.

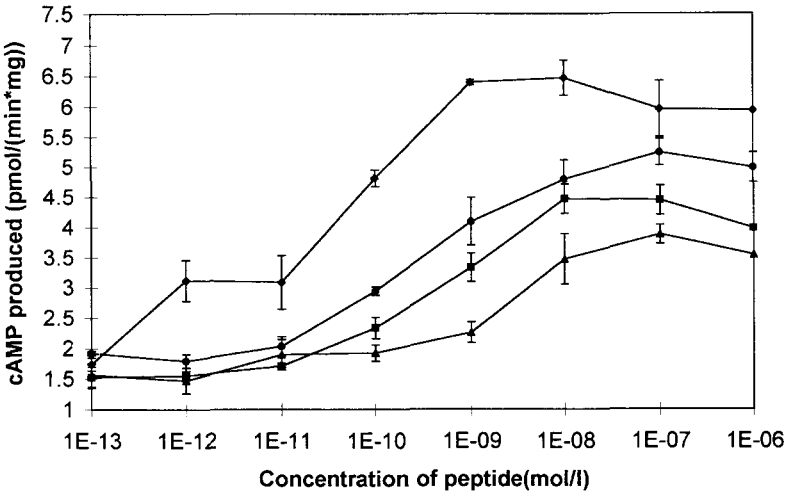

Fig. 5. Adenylyl cyclase activity using plasma membranes from BHK cells expressing the cloned human pancreatic receptor. Dose-response curves for GLP-1-(7–36) amide in the presence of increasing concentrations of GLP-1-(9–36) amide. GLP-1-(7–36) amide (♦), GLP-1-(7–36) amide and 1.25 μmol/l GLP-1-(9–36) amide (●), GLP-1-(7–36) amide and 2.5 μmol/l GLP-1-(9–36) amide (■) and GLP-1-(7–36) amide and 5.0 μmol/l GLP-1-(9–36) amide (▲). Curves represent one experiment with samples measured in triplicate.

were analyzed for receptor binding and activation of the adenylyl cyclase. Fig. 4 and Table 1 show the binding affinity of GLP-1-(9–36) amide, GLP-1-(7–34) and GLP-1-(7–35) to the cloned human GLP-1 receptor. The affinities were, respectively, 0.95 ± 0.25% ($n = 4$), 2.8 ± 0.28% ($n = 3$) and 12 ± 0.65% ($n = 3$) compared to GLP-1-(7–36) amide. The value for GLP-1-(7–36) amide was 0.19 ± 0.045 nmol/l ($n = 4$).

GLP-1-(9–36) amide was analyzed for its ability to antagonize GLP-1-(7–36) amide activation of the cloned human GLP-1 receptor (Fig. 5). GLP-1-(9–36) amide was shown to be an antagonist. GLP-1-(9–36) amide at 1.25 μmol/l was able to shift the dose-response curve for GLP-1-(7–36) amide approximately 10-fold. With increasing concentrations of antagonist, the maximally attainable response was lowered.

Both GLP-1-(7–34) and GLP-1-(7–35) were shown to be full agonists. As shown in Table 1, relative activities were found to be 100% for GLP-1-(7–36) amide (0.020 ± 0.0082 nmol/l, $n = 4$); 1.1 ± 0.98% for GLP-1-(7–34) ($n = 4$) and 8.1 ± 3.9% for GLP-1-(7–35) ($n = 3$), respectively, in this study (curves are not shown).

4. Discussion

AUC calculated after measurement by the mid-terminal RIA was 1.9- and 2.0-fold higher for the two dogs, respectively, compared to measurement by the C-terminal specific RIA. This indicates that approximately 50% of the GLP-1-IR measured by the mid-terminal RIA originates from metabolites lacking at least one amino acid in the C-terminus, as the antibody used in the C-terminal RIA recognizes the intact C-terminus, only, in contrast to the antibody used in the mid-terminal RIA, which recognizes truncated forms. AUC measured by the mid-terminal RIA compared to ELISA was 9.0- and 9.6-fold higher for the two dogs, respectively. This indicates that only 10% of the GLP-1-IR measured by the mid-terminal RIA contains both an intact N-terminus and a C-terminus that is not truncated beyond Val^{33} according to the specificity of the antibodies used in the two assays. The discrepancy between the AUC calculated after measurement of plasma immunoreactivity by the C-terminal specific RIA and the ELISA indicates that less than 20% of the GLP-1-IR measured by the C-terminal specific RIA has an intact N-terminus.

In agreement with this difference between the AUC calculated after measurement by the mid-terminal RIA and the ELISA the results in Fig. 3 indicate that GLP-1-(7–36) amide contributes approximately 10% to the immunoreactivity measured by the mid-terminal RIA. Accordingly, the difference in plasma concentration measured by the two RIAs can be explained by cross-reaction with the C-terminally truncated metabolites GLP-1-(7–34) and GLP-1-(7–35) in the mid-terminal RIA, but not in the C-terminally-specific RIA.

Prior studies have shown GLP-1-(8–37), GLP-1-(7–34) and GLP-1-(7–35) to have 0.3%, 1% and 20% receptor affinity compared to GLP-1-(7–37), respectively, on the rat insulinoma RIN1046-23 cell line (Mojsov, 1992). Accordingly, the relative affinities of the C-terminal metabolites obtained in this study are thus in the same order of magnitude as those reported for the RIN1046-23 cell line. Also, it does not seem to make a major difference for the affinity whether one or two amino acids are deleted from the N-terminus.

Earlier studies have suggested GLP-1-(9–36) amide to be an antagonist as measured by its ability to lower the insulin release from isolated perfused rat pancreas but

without further elucidation of the mechanism (Grandt et al., 1994). We report that this compound is indeed an antagonist at the human receptor. Fig. 5 shows at shift in the dose-response curve as well as a decrease in the maximally attainable response. Thus, GLP-1-(9–36)amide is characterized as an insurmountable antagonist. However, a 100-fold excess of antagonist is needed to compete with the natural ligand.

Both GLP-1-(7–34) and GLP-1-(7–35) were shown to be full agonists in agreement with structure-activity studies (Adelhorst et al., 1994; Gallwitz et al., 1994, 1995) and earlier published effects on insulin secretion from both isolated rat pancreas and the βTC1 cell line (Suzuki et al., 1989; Gefel et al., 1990).

With respect to relative activities, our results are not entirely in agreement with those obtained in the βTC1 cell line. Relative activities on insulin secretion from the βTC1 cell line of GLP-1-(7–36) amide, GLP-1-(7–34) and GLP-1-(7–35) were 100% (0.9 nmol/l), 30% and 90%, respectively (Gefel et al., 1990). However, the agonist functional data presented here is in agreement with the binding data as well as the binding studies using the RIN1046-23 cell line.

Several groups have studied GLP-1-(7–36) amide/GLP-1-(7–37) as a potential drug for patients suffering from non-insulin dependent diabetes mellitus (Nauck et al., 1993; Gutniak et al., 1992; Elliot et al., 1993; Cottrell et al., 1992). Pharmacokinetic studies showed that GLP-1-(9–36) amide/GLP-1-(9–37) is a physiological metabolite of this hormone (Pridal et al., 1995, 1996; Deacon et al., 1995a,b). Studies from our group have shown that GLP-1-(7–36) amide was metabolized very fast with a total plasma clearance of 25 ± 3 ml/kg per min measured by the sandwich ELISA, whereas the total plasma clearance measured by the mid-terminal RIA was 3.4 ± 0.3 ml/kg per min after i.v. administration to dogs ($n = 4$) (Pridal et al., 1996 and unpublished results). Thus after i.v. administration to dogs the ratio between GLP-1-(9–36) amide and GLP-1-(7–36) amide was shown to be below 10 ($25/3.4 = 7.4$), which was confirmed after s.c. administration in this study. If the total plasma clearance for the antagonist is significantly lower than that of the parent compound, the antagonist will be accumulated. This could cause tachyphylaxis after chronic treatment. However, to obtain a 50% decrease in receptor activation the ratio between GLP-1-(7–36) amide and GLP-1-(9–36) amide must be at least 1:100 as judged from the binding experiments (Fig. 4). Thus, a ratio of 1:10, the maximally estimated ratio from the in vivo experiments presented here does not reduce the receptor activation significantly. Also, other studies have shown that the effect of GLP-1 was maintained during treatment over a period of 4 h in patients suffering from non-insulin dependent diabetes mellitus (Nauck et al., 1993). Further studies with chronic treatment are required in order to assess the problem of potential tachyphylaxis.

In conclusion, we have shown by HPLC, RIA and ELISA on samples from a pharmacokinetic study in dogs that exogenous GLP-1-(7–36) amide is metabolized rapidly. The major metabolites identified after s.c. administration to dogs were GLP-1-(9–36) amide, GLP-1-(7–34) and GLP-1-(7–35), respectively, ranked after the amount found in plasma. Metabolism at the N-terminus results in an antagonist. We propose that this metabolite, due to the low binding affinity compared to the parent compound, does not seem to cause physiological antagonism of GLP-1. Furthermore, we have confirmed, by in vitro receptor studies that C-terminal metabolites are agonists.

Acknowledgements

Professor Jens J. Holst and Dr. Birgitte Wulff are thanked for their guidance and helpfulness. Dr Kim Adelhorst is thanked for synthesizing the peptides used in this study. The technical assistance of Mette Frost, Alex Jensen, Vibeke Lykke and Mette Winther is gratefully acknowledged.

References

Adelhorst, K., B.B. Hedegaard, L.B. Knudsen and O. Kirk, 1994, Structure-activity studies of GLP-1, J. Biol. Chem. 269, 6275.

Cottrell, D., R. Gelfand, S. Cataland, T. O'Dorisio, C. Casey and K. Osei, 1992, Insulinotropic effect of GLP-1-(7–37) in non-insulin dependent diabetes mellitus patients: a study of dose and glucose dependency, Clin. Res. 40, 734A.

Deacon, C.F., A.H. Johnsen and J.J. Holst, 1995a, Degradation of GLP-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo, J. Clin. Endocrinol. Metab. 80, 952.

Deacon, C.F., M.A. Nauck, M. Toft-Nielsen, L. Pridal, B. Willms and J.J. Holst, 1995b, Both subcutaneous and intravenously administered GLP-1 are rapidly degraded from the N-terminus in type-2 diabetic patients and in healthy subjects, Diabetes 44, 1126.

Drejer, K., V. Kruse, U.D. Larsen, P. Hougaard, S. Bjørn and S. Gammeltoft, 1991, Receptor binding and tyrosine kinase activation by insulin analogues with extreme affinities studied in human hepatoma HepG2 cells, Diabetes 40, 1588.

Elliot, R.M., L.M. Morgan, J.A. Tredger, S. Deacon, J. Wright and V. Marks, 1993, GLP-1-(7–36) amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretionresponse to nutrient ingestion in man: Acute post-prandial and 24-h secretion pattern, J. Endocrinol. 138, 179.

Frohman, L.A., T.R. Downs, E.P. Heimer and A.M. Feli, 1989, Dipeptidylpeptidase IV and trypsin-like enzymatic degradation of human growth hormone-releasing hormone in plasma, J. Clin. Invest. 83, 1533.

Gallwitz, B., M. Witt, G. Paetzold, C. Morys-Wortman, B. Zimmermann, K. Eckart, U.R. Fölsch and W.E. Schmidt, 1994, Structure-activity characterization of glucagon-like peptide-1, Eur. J. Biochem. 225, 1151.

Gallwitz, B., M. Witt, G. Paetzold, C. Morys-Wortman, U.R. Fölsch and W.E. Schmidt, 1995, Binding characteristics of N-terminal GIP/GLP-1 hybrid peptides, Endocrinol. Metab. 2, 39.

Gefel, D., G.K. Hendrick, S. Mojsov, J.F. Habener and G.C. Weir, 1990, GLP-1 analogs: effects on insulin secretion and cAMP formation, Endocrinology 126, 2164.

Grandt, D., B. Sieberg, J. Sievert, M. Schimiezek, U. Becker, G. Holtmann, P. Layer, J.R. Reeve, Jr., V.E. Eysselein, H. Goebell and M. Müller, 1994, Is GLP-1-(9-36) amide an endogenous antagonist at GLP-1 receptors?, Digestion 55, 302.

Gutniak, M.D, C. Ørskov, J.J. Holst, B. Ahrén and S. Efendic, 1992, Antidiabetic effect of GLP-1-(7–36) amide in normal subjects and patients with diabetes mellitus, New Engl. J. Med. 326, 1316.

Hvidberg, A., M.T. Nielsen, J. Hilsted, C. Ørskov and J.J. Holst, 1994, Effect of GLP-1 on hepatic glucose production in healthy man, Metabolism 43, 104.

Jörnvall, H., M. Carlquist, S. Kwauk, S.C. Otte, C.H. McIntosh, J.C. Brown and W. Mutt, 1981, Amino acid sequence and heterogeneity of gastric inhibitory polypeptide, FEBS Lett. 123, 205.

Mentlein, R., B. Gallwitz and W.E. Schmidt, 1993, DPP IV hydrolyses gastric inhibitory peptide, GLP-1-(7–36) amide, peptide histidine methionine and is responsible for their degradation in human serum, Eur. J. Biochem. 215, 829.

Mojsov, S., 1992, Structural requirements for biological activity of GLP-1, Int. J. Pept. Protein Res. 40, 333.

Nauck, M.A., N. Kleine, C. Ørskov, J.J. Holst, B. Willms and W. Creutzfeldt, 1993, Normalization of fasting hyperglycemia by exogenous GLP-1-(7–36) amide in type 2 diabetic patients, Diabetologia 36, 741.

Pridal, L., S.H. Ingwersen, F.S. Larsen, J.J. Holst, K. Adelhorst and O. Kirk, 1995, Comparison of sandwich ELISA and RIA for detection of exogenous GLP-1-(7–36) amide in plasma, J. Pharm. Biomed. Anal. 13, 841.

Pridal, L, C.F. Deacon, O. Kirk, J.V. Christensen, R.D. Carr and J.J. Holst, 1996, Glucagon-like peptide-1-(7–37) has a larger volume of distribution than glucagon-like peptide-1-(7–36) amide in dogs and is degraded more quickly in vitro by dog plasma, Eur. J. Drug Metab. Pharmacokin. 21, 51.

Robberecht, P., P. De Neef, M. Waelbroeck, J.-C. Camus, J.-L. Scemema, D. Fourmy, M. Pradayrol, N. Vaysse and J. Christophe, 1988, Secretin receptors in human pancreatic membranes, Pancreas 3, 529.

Rorstad, O.P., I. Wanke, D.H. Coy, A. Fournier and M. Huang, 1990, Selectivity for binding of peptide analogs to vascular receptors for vasoactive intestinal peptide, Mol. Pharmacol. 37, 971.

Schmidt, W.E., E.G. Siegel, B. Gallwitz, H. Kümmel, R. Ebert and W. Creutzfeldt, 1986, Characterization of the insulinotropic activity of fragments derived from gastric inhibitory polypeptide, Diabetologia 29, 591A.

Suzuki, S., K. Kawai, S. Ohashi, H. Mukai and K. Yamashita, 1989, Comparison of the effects of various C-terminal and N-terminal fragment peptides of GLP-1 on insulin and glucagon release from isolated rat pancreas, Endocrinology 125, 3109.

Thorell, J.F. and B.G. Johansson, 1971, Enzymatic iodination of polypeptide with (^{125}I) to high specific activity, Biochim. Biophys. Acta 251, 363.

Unson, C.G., D. Andreu, E.M. Gurzenda and R.B. Merrifield, 1987, Synthetic peptide antagonists of glucagon, Proc. Natl. Acad. Sci. USA 84, 4083.