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The major glucagon-like peptide-1 metabolite, GLP-1-(9–36)-amide, does not affect glucose or insulin levels in mice

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Abstract

Glucagon-like peptide-1 (GLP-1), a future treatment for type 2 diabetes, is efficiently degraded by the enzyme dipeptidyl peptidase IV (DPP IV), yielding the major metabolite GLP-1-(9–36)-amide. In this study, we examined the potential glucose lowering effect of GLP-1-(9–36)-amide in mice and found that GLP-1-(9–36)-amide (3 and 10 nmol/kg) did not affect insulin secretion or glucose elimination when administered intravenously together with glucose (1 g/kg). This was observed both in normal mice and in transgenic mice having a complete disruption of the signalling from the GLP-1 receptor. Furthermore, after blocking insulin secretion, using diazoxide (25 mg/kg), no effect on insulin-independent glucose disposal of GLP-1-(9–36)-amide was observed. Therefore, GLP-1-(9–36)-amide does not affect glucose disposal in mice either in the presence or absence of intact GLP-1-receptors or in the presence or absence of stimulated insulin levels. This suggests that the GLP-1 metabolite is not involved in the regulation of glucose homeostasis.

Keywords: Glucagon like peptide 1; Dipeptidyl peptidase IV; Transgenic animal model; Diabetes

1. Introduction

Glucagon-like-peptide 1 (GLP-1) is an incretin hormone, which is secreted from the intestinal L-cells and efficiently lowers blood glucose in type 2 diabetic patients (Ørskov, 1992; Gutniak et al., 1992; Nathan et al., 1992; Nauck et al., 1993; Rachman et al., 1997; Kieffer and Habener, 1999). The antihyperglycaemic effects of GLP-1 are multifactorial, involving the pancreas, gastrointestinal tract and brain. In islets, GLP-1 potently stimulates insulin secretion (Ørskov et al., 1988, 1996; Elliott et al., 1993), and β-cell proliferation (Edvell and Lindström, 1999; Xu et al., 1999; Perfetti et al., 2000) and inhibits apoptosis (Li et al., 2003; Farilla et al., 2003) and glucagon secretion (Ørskov et al., 1988). GLP-1 also decreases gastric emptying (Wettergren et al., 1993) and reduces appetite (Flint et al., 2001). GLP-1 is a substrate for the enzyme dipeptidyl peptidase IV (DPPIV) (Mentlein et al., 1993; Deacon et al., 1995; Kieffer et al., 1995) and is rapidly broken down to form the truncated metabolite GLP-1-(9–36)-amide. In fact, already immediately following its release, GLP-1 is largely metabolized to GLP-1-(9–36)-amide (Hansen et al., 1999), giving a very short half-life for the intact peptide and resulting in the bulk of the circulating GLP-1 pool consisting of GLP-1-(9–36)-amide (Deacon et al., 1995). This raises the issue of a possible function of this metabolite. In order to establish this, studies have shown that GLP-1-(9–36)-amide behaves as an antagonist in vitro using the cloned human pancreatic GLP-1 receptor (Knudsen and Pridal, 1996). Furthermore, an in vivo study has shown that GLP-1-(9–36)-amide antagonizes the inhibitory effect of GLP-1 on antral motility (Wettergren et al., 1998). Recently, it was also suggested that GLP-1-(9–36)-amide lowers blood glucose in anaesthetised pigs without showing any insulinotropic action (Deacon et al., 2002). One possibility explaining this action could be that GLP-1-(9–36)-amide cross-reacts with GLP-1 receptors, as a low affinity for these receptors has been proposed (Knudsen and Pridal, 1996) and an insulin-independent antihyperglycaemic effect has been reported for

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GLP-1 (Villaneuva-Penacarillo et al., 1994, Redondo et al., 2003). Another possibility could be that GLP-1-(9–36)-amide activates another receptor, resulting in an insulin-independent glucose disposal.

In this study, we examined the potential glucose lowering effect of GLP-1-(9–36)-amide in mice. In order to avoid misinterpretation of data due to a potential cross-reactivity with GLP-1 receptors, we also examined its effects in GLP-1 receptor gene-deleted mice having a complete disruption of the signalling from the GLP-1 receptor (Scrocchi et al., 1996). To further assess whether any antihyperglycaemic action was insulin-dependent or insulin-independent, diazoxide, a known potassium channel opener, which potently inhibits insulin secretion from pancreatic β-cells in vivo (Pacini et al., 2001) was added in the experiment.

2. Materials and methods

2.1. Animals

Female GLP-1-receptor −/− mice, 4–6 weeks of age, were obtained from Taconic A/S, Ry, Denmark, bred for Novo Nordisk A/S, Bagsvaerd, Denmark under a license kindly provided by Dr. D.J. Drucker, Toronto, Canada. Wild-type mice (CD-1) were obtained from Charles River, Sulzfeld, Germany. The mice were fed a standard diet (Research Diets, New Brunnswick, NJ) and had free access to food and drinking water throughout the study period. The mice were kept five mice per cage in a temperature-controlled room (22 °C) with a 12 h light:12 h darkness cycle with lights on at 6 a.m. The study was approved by the animal Ethics Committee at Lund University.

2.2. Experiments

Two to 3 weeks after arrival, the mice were fasted for 4 h, divided into groups and anaesthetised with an i.p injection of 0.14 mg/mouse midazolam (Dormicum, Roche, Basel, Switzerland) combined with 0.9 mg/mouse fluanison and 0.02 mg/mouse fentanyl (hypnorm, Janssen, Beerse, Belgium). This mode of anesthesia in mice provides a safe and stable anesthesia and does not alter baseline levels of insulin or glucose or the insulin or glucose response to an intravenous glucose load (unpublished data). At time 0, a baseline blood sample of 75 μl for determination of plasma glucose and plasma insulin was taken from the retrobulbar, intraorbital capillary plexus. Thereafter, D-glucose (British Drug Houses, Poole, UK) was injected intravenously over 3 s at the dose of 1 g/kg in a tail vein without flushing of the 27-gauge needle after injection, either alone or together with synthetic GLP-1-(9–36)-amide. This peptide was synthesised by Dr. Kim Adelhorst (Novo Nordisk A/S). Its authenticity was confirmed by amino acid analysis, analytical reversed phase high-pressure liquid chromatography (HPLC) and plasma desorption mass spectrometry, and its purity shown to be greater than 99% by HPLC with detection at 214 nm. In the initial experiment, GLP-1-(9–36)-amide was administered at a dose of 3 nmol/kg, which is equimolar with a maximal insulinotropic dose of GLP-1 in mice (Ahrén and Pacini, 1999). In a subsequent experimental series, this dose was raised to 10 nmol/kg. In one experimental series, diazoxide (Sigma, St Louis, MO) was injected at a dose of 25 mg/kg together with glucose or glucose + GLP-1-(9–36)-amide. The volume load was 10 μl/g body weight. Additional blood samples (75 μl each) were taken at 1, 5, 20, and 50 min. Plasma was immediately separated and stored at −20 °C until analyses.

2.3. Analyses

Insulin concentration was determined by a double-antibody radioimmunoassay using guinea pig anti rat insulin antibodies, ^{125}I-labelled human insulin and, as standard, rat insulin (Linco Res., St. Charles, MO, USA). Glucose was measured by the glucose oxidase technique.

2.4. Calculations and statistics

The acute insulin response (AIR) to intravenous glucose with or without GLP-1-(9–36)-amide was calculated as the mean of suprabasal 1 and 5 min values. The glucose elimination was quantified as the K_G, i.e., the glucose elimination constant, as the reduction in circulating glucose between 1 and 20 min after intravenous administration after logarithmic transformation of the individual plasma glucose values and expressed as percentage elimination of glucose per minute. Data and results are reported as means ± S.E.M. Statistical comparisons between two groups were made with a Student's *t*-test.

3. Results

In the first series of experiments, we studied the glucose disposal and the insulin secretory response upon an intravenous glucose load, comparing wild-type mice with GLP-1 receptor −/− mice. In both types of animals, the acute glucose challenge elicited a rapid and marked increase in insulin levels, seen after 1 and 5 min. Insulin levels then declined, and was lower than baseline after 20 and 50 min, showing the rapid turnover of insulin after its acute release in mice. We found that the glucose disposal rate and the insulin response were reduced in GLP-1 receptor −/− mice compared to wild-type mice (Fig. 1). Thus, the AIR was 340 ± 54 pmol/l in wild-type mice but only 148 ± 67 pmol/l in GLP-1 receptor −/− mice ($P = 0.039$). Furthermore, the K_G was 2.9 ± 0.3%/min in wild-type mice but only 2.1 ± 0.1%/min in GLP-1 receptor −/− mice ($P = 0.011$).

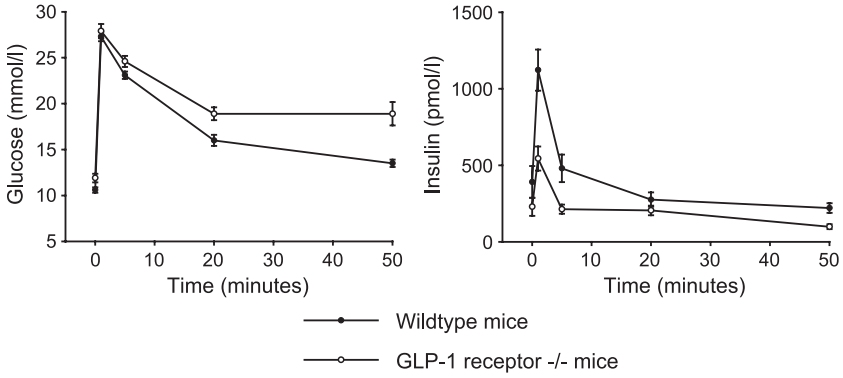

Fig. 1. Glucose and insulin levels during the intravenous glucose test (1 g/kg) in wild-type ($n = 12$) and GLP-1 receptor $-/-$ mice ($n = 12$). Means ± S.E.M. are shown.

In the next experimental series, we coadministered GLP-1-(9–36)-amide at a dose of 10 nmol/kg with glucose (1 g/kg). We found that there was no additional effect of GLP-1-(9–36)-amide on either plasma insulin or plasma glucose levels as compared to animals given glucose alone in either wild-type or GLP-1-receptor $-/-$ mice (Fig. 2). Thus, in wild-type mice, AIR was 365 ± 96 pmol/l after glucose alone and 363 ± 99 pmol/l after glucose + GLP-1-(9–36)-amide (NS) and the corresponding figures in GLP-1 receptor $-/-$ mice were 97 ± 100 and 94 ± 69 pmol/l, respectively (NS). Furthermore, K_G in wild-type mice injected with glucose was 2.8 ± 0.4%/min and in mice injected with glucose + GLP-1-(9–36)-amide was 2.4 ± 0.4%/min (NS) and the corresponding figures in GLP-1 receptor $-/-$ mice were 2.1 ± 0.2 and 1.9 ± 0.2%/min, respectively (NS). A similar lack of effect of GLP-1-(9–36)-amide was also observed when administered at a lower dose of 3 nmol/kg together with glucose (data not shown).

In the third experimental series, the animals were also given diazoxide (25 mg/kg i.v.) together with glucose (1 g/kg), and with or without GLP-1-(9–36)-amide (10 nmol/kg), in order to inhibit insulin secretion from pancreatic β-cells and exclude that the lack of effect of GLP-(9–36)-amide was due to counteraction or masking by insulin. Following diazoxide, the insulin response to glucose was

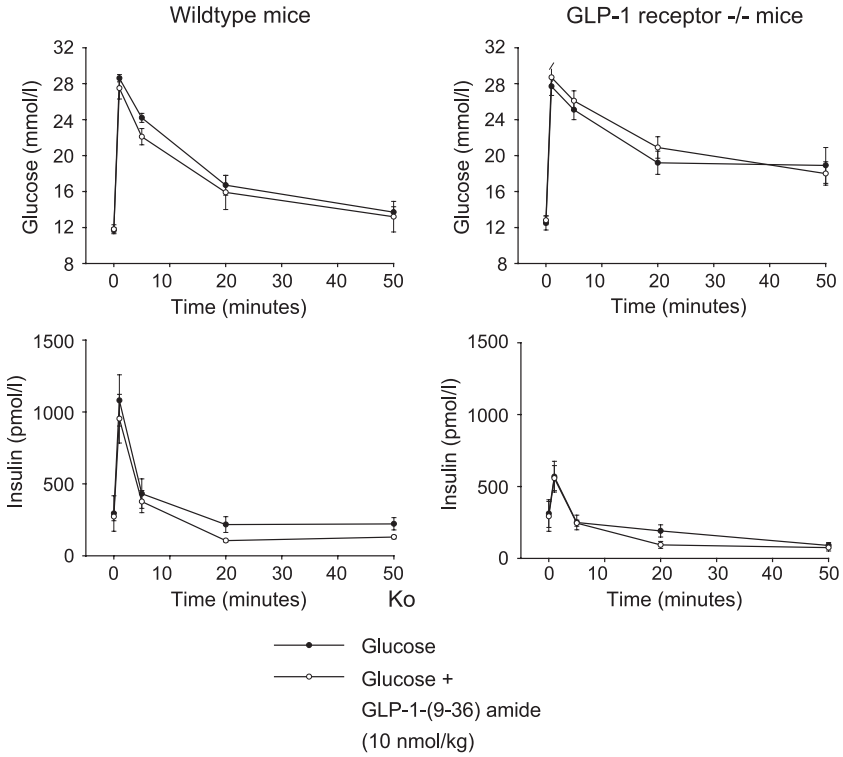

Fig. 2. Glucose and insulin levels during the intravenous glucose test (1 g/kg) with or without coadministration of GLP-1-(9–36)-amide at 10 nmol/kg in wild-type and GLP-1-receptor $-/-$ mice. There were six animals in each of the four groups. Means ± S.E.M. are shown.

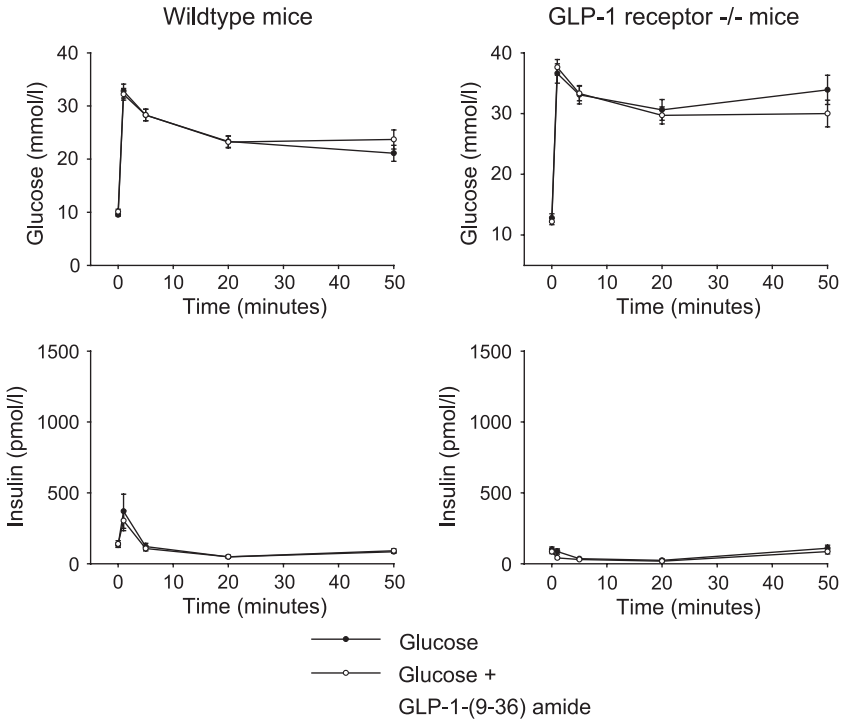

Fig. 3. Glucose and insulin levels during the intravenous glucose test (1 g/kg) with coadministration of diazoxide (25 mg/kg) with or without coadministration of GLP-1-(9–36)-amide at 10 nmol/kg in wild-type and GLP-1 receptor −/− mice. There were six animals in each of the four groups. Means ± S.E.M. are shown.

totally absent and the glucose disposal retarded, but, again, GLP-1-(9–36)-amide had no effect (Fig. 3).

4. Discussion

In the present study, we found that the GLP-1 receptor −/− mice responded poorly to intravenously administered glucose. Thus, the acute insulin response to glucose was reduced by more than 50% in the GLP-1 receptor −/− mice compared to the wild-type mice and subsequently, glucose was eliminated considerably more slowly from the plasma as evident by the lower K_G. This shows that not only disruption of the enteroinsular axis seems to be important for glucose metabolism in the mice lacking the GLP-1 receptor, as suggested earlier (Scrocchi et al, 1998, 2000), but that also the insulin response to intravenous glucose is disrupted. This suggests that intact GLP-1 receptors are required for a normal glucose-stimulated insulin secretion. Previous studies have shown that disruption of GLP-1 receptor signalling in mice lacking the GLP-1 receptor is associated with glucose intolerance with a reduced insulin secretory response to orally administered glucose, most probably due to the elimination of the incretin effect of GLP-1 (Scrocchi et al., 1996, 1998). It has been shown that GLP-1 receptor −/− mice exhibit a compensatory increase in glucose-dependent insulinotropic polypeptide (GIP) secretion and action, which may provide the explanation as to why the mice phenotypically appear normal, apart from displaying modest glucose intolerance (Pederson et al., 1998). However, our present finding suggests that GLP-1 receptor gene deletion does also seem to lead to perturbation of islet function. This is consistent with findings that pancreatic islets from GLP-1 receptor −/− mice have an altered topography of α- and β-cells, indicating an essential role for GLP-1 in normal cellular organisation of the endocrine pancreas (Ling et al., 2001). Furthermore, although it has been shown that perifused pancreatic β-cells from GLP-1-receptor −/− mice have a preserved insulin response to glucose (Flamez et al., 1998), abnormalities in the intracellular signalling pathways in β-cells from GLP-1-receptor −/− mice seem to exist (Flamez et al., 1999). Taken together these studies show that elimination of GLP-1 receptor signalling leads to a mild disturbance in insulin secretion from β-cells leading to modest glucose intolerance after orally or intraperitoneally administered glucose, without affecting body weight or food intake.

The main finding in the present study was that no antihyperglycaemic effect was observed for the GLP-1 metabolite GLP-1-(9–36)-amide, and that this was observed both in GLP-1 receptor −/− mice and in wild-type mice. Therefore, GLP-1-(9–36)-amide does not seem to affect glucose disposal in mice, either by affecting the GLP-1 receptor or by affecting any other receptor. In order to exclude that the failure of GLP-1-(9–36)-amide to affect glucose disposal would be due to the high insulin levels

induced by the iv glucose injection, either by counteracting or masking any effect, we also performed a series of experiments in which diazoxide was given together with glucose and GLP-1-(9–36)-amide. Diazoxide is a potassium channel opener, capable of inhibiting insulin secretion from pancreatic β-cells (Pacini et al., 2001) and, consequently, the insulin response to glucose in mice given diazoxide was severely suppressed. However, also after almost complete blocking of the glucose-stimulated insulin secretion, no effect of GLP-1-(9–36)-amide was observed. Therefore, we have shown that in mice, GLP-1-(9–36)-amide does not affect glucose disposal either in the presence or absence of intact GLP-1-receptors or in the presence or absence of stimulated insulin levels. Our results seem to be in contrast to previous findings in normal pigs (Deacon et al., 2002), where an antihyperglycaemic effect was observed without a concomitant increase in insulin secretion. This was suggested to be mediated by a receptor distinct from the GLP-1 receptor and one explanation for the different results might be species dependent expression of this putative novel receptor. Other explanations exist also, however, such as different experimental conditions, and further studies are therefore required to explain the different results obtained in the two studies.

We conclude that it is highly unlikely that the metabolite of GLP-1, GLP-1-(9–36)-amide, exerts any antihyperglycaemic action in mice. This finding may be of relevance for the inhibition of the metabolism of GLP-1 using DPP-IV-inhibitors, which is currently considered a new treatment modality for type 2 diabetes (Holst and Deacon 1998). The inhibition of DPPIV reduces the formation of GLP-1-(9–36)-amide, as the degradation of GLP-1 is inhibited. In view of the present results, the antihyperglycaemic effect of DPPIV inhibitors would most likely not be affected by the reduced formation of GLP-1-(9–36)-amide, as this peptide, at least in mice, does not seem to exert any antihyperglycaemic action of its own.

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