Peptides 60 (2014) 95–101

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Ablation of glucagon receptor signaling by peptide-based glucagon antagonists improves glucose tolerance in high fat fed mice

Laura M. McShane, Zara J. Franklin, Finbarr P.M. O'Harte, Nigel Irwin *

SAAD Centre for Pharmacy and Diabetes, School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK

ARTICLE INFO

Article history:
Received 4 July 2014
Received in revised form 6 August 2014
Accepted 6 August 2014
Available online 18 August 2014

Keywords:
Glucagon antagonist
Diabetes
Glucose tolerance
Insulin sensitivity

ABSTRACT

Modification to the structure of glucagon has provided a number of glucagon receptor antagonists with possible therapeutic application for diabetes. These novel peptide analogs include desHis^{1}Pro^{4}Glu^{9}-glucagon and desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon. This study has evaluated the metabolic benefits of once daily administration of desHis^{1}Pro^{4}Glu^{9}-glucagon and desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon in high fat (45%) fed mice for 15 days. Administration of desHis^{1}Pro^{4}Glu^{9}-glucagon and desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon had no significant effect on body weight, food intake or circulating glucose concentrations during the treatment period. However, both peptides significantly ($P<0.05$ to $P<0.01$) reduced circulating plasma insulin concentrations from day 6 onwards. Oral glucose tolerance and insulin sensitivity, as assessed by exogenous insulin administration, were significantly ($P<0.01$ to $P<0.001$) improved by both desHis^{1}Pro^{4}Glu^{9}-glucagon and desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon. These metabolic benefits were accompanied by significantly ($P<0.01$) increased pancreatic insulin stores. No significant differences in blood triacylglycerol or cholesterol levels were noted with desHis^{1}Pro^{4}Glu^{9}-glucagon, however desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon treatment significantly ($P<0.01$) increased HDL-cholesterol levels. Glucagon-mediated elevations of glucose and insulin were effectively ($P<0.01$ to $P<0.001$) annulled in both treatment groups on day 15. Interestingly, glucose levels during an intraperitoneal glucose tolerance test were not altered by either desHis^{1}Pro^{4}Glu^{9}-glucagon or desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon treatment. These data provide further evidence that glucagon antagonism could provide an effective means of treating T2DM.

Introduction

The worldwide explosion of type 2 diabetes (T2DM) represents a major public health burden [15]. As such, there is a wide range of treatment options available for T2DM patients, with complimentary modes of action [18,23]. Despite this, 50% of T2DM patients still fail to achieve adequate glycemic goals [2]. Therefore, there is clearly an unmet need for new therapeutic options for T2DM. In this regard, approaches to correct or reverse the inherent pathophysiological problems associated with T2DM are of considerable interest. Based on this premise, there is now accumulating evidence to suggest that inhibition of glucagon receptor signaling is an ideal method to tackle the T2DM epidemic [9,12,16,26].

For instance, physiological blood glucose control is maintained within strict boundaries through coordinated release of the pancreatic hormones glucagon and insulin [30,35]. Circulating glucagon concentrations are generally down-regulated by nutrient absorption, but this does not occur appropriately in T2DM, and promotes hyperglycemia [12]. Thus, elevated blood glucose levels in T2DM are associated with excessive glucagon secretion, as well as a deficiency in insulin production. Moreover, excessive circulating glucagon levels can act as forewarning to the development of T2DM [17]. Therefore, strategies to suppress glucagon receptor signaling have significant therapeutic promise [37], especially since this represents correction of a key pathophysiological issue in T2DM. In this regard, we have recently described and fully characterized two novel and specific peptide-based glucagon receptor antagonists, namely desHis^{1}Pro^{4}Glu^{9}-glucagon and desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon [26].

Both desHis^{1}Pro^{4}Glu^{9}-glucagon and desHis^{1}Pro^{4}Glu^{9}(Lys^{30}PAL)-glucagon possess significant glucagon receptor antagonistic properties. As such, the peptides specifically and potently inhibit glucagon-mediated elevations of insulin release and cAMP generation *in vitro* [26]. In addition, *in vivo* studies confirm antagonistic actions of both novel analogs against glucagon-induced elevations of blood glucose and plasma insulin levels in normal

* Corresponding author. Tel.: +44 0 28 70 124754; fax: +44 0 28 70 123939.
E-mail address: n.irwin@ulster.ac.uk (N. Irwin).

http://dx.doi.org/10.1016/j.peptides.2014.08.002
0196-9781/

and high fat fed mice [26]. Furthermore, a prolonged duration of biological action of desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon was confirmed [26], likely due to increased plasma protein binding and reduced elimination of this fatty acid dervatised peptide analog [22].

In the present study we have investigated the ability of once daily injection of desHis^{1}Pro^{4}Glu^9-glucagon or desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon to alleviate the glycemic dysregulation, insulin resistance and metabolic disarray associated with mice fed a high fat diet. This was achieved by evaluation of oral and intraperitoneal glucose tolerance and peripheral tissue insulin sensitivity. As such, the acute biological actions of desHis^{1}Pro^{4}Glu^9-glucagon or desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon have already been assessed [26], with the current study addressing potential sub-chronic antidiabetic effects. Importantly, our previous studies have confirmed the excellent safety profile of prolonged administration of peptide-based desHis^{1}Pro^{4}Glu^9 modified glucagon receptor antagonists in mice [9]. The current results provide experimental evidence that novel peptide-based glucagon receptor antagonists may provide an effective means of treating T2DM.

Materials and methods

Peptide synthesis

desHis^{1}Pro^{4}Glu^9-glucagon and desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon were obtained from GL Biochem Ltd. (Shanghai, China). All peptides were characterized using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, as previously described [26].

Animals

NIH Swiss male mice (Harlan Ltd., Oxon, UK) were used at 16 weeks of age. The animals were housed individually in an air-conditioned room at 22 ± 2 °C with a 12 h light:12 h dark cycle (lights off between 20:30 and 08:30 h). All animals had free access to drinking water and a high fat (45% fat, 35% carbohydrate and 20% protein, Dietex International Ltd. Witham, Essex, UK) diet for 100 days prior to commencement of studies. Obesity and insulin resistance were clearly manifested compared to age matched mice maintained on normal laboratory chow (10% fat, 30% protein and 60% carbohydrate, Trouw Nutrition, Cheshire, UK) as verified by body weight and plasma insulin analyses. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

Study design

Mice received a once daily intraperitoneal (i.p.) injection (09:30 h) of either saline vehicle (0.9% w/v NaCl;), desHis^{1}Pro^{4}Glu^9-glucagon or desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon (both at 25 nmol/kg body weight) for 15 days. This dose was chosen based on our previous extensive *in vitro* and *in vivo* assessments with both peptides [26]. Food intake was measured daily, whilst body weight, blood glucose and plasma insulin concentrations were determined every 2–5 days.

Metabolic effects of desHis^{1}Pro^{4}Glu^9-glucagon and desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon in high fat fed mice

Oral (18 mmol/kg body weight) and i.p. (18 mmol/kg body weight) glucose tolerance tests were performed on day 15 in 18 h fasted mice. Insulin sensitivity (10 U/kg body weight; non-fasted) and glucagon (25 nmol/kg body weight; 18 h fasted) tolerance tests were also performed. The duration of the overnight fast is based on our extensive previous experience with this mouse model [16,24,26]. The oral glucose tolerance test was carried out by insertion of a gavage needle into the stomach of non-anesthetized mice. All metabolic tests ($n = 8$) were conducted 24 h after the daily i.p. injection of either saline, desHis^{1}Pro^{4}Glu^9-glucagon or desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon. At the end of the study, blood was collected for lipid profile analyses and pancreatic tissues were excised and insulin content measured following extraction with 5 ml/g of ice-cold acid ethanol (75% ethanol, 23.5% water, 1.5% concentrated HCl).

Biochemical analyses

Blood samples were taken from the tail vein of conscious mice at the times indicated in the figures. Blood glucose was measured directly using a hand-held Ascencia Contour blood glucose meter (Bayer Healthcare, Newbury, Berkshire, UK). For insulin analyses, blood samples were collected into chilled fluoride/heparin glucose microcentrifuge tubes (Sarstedt, Numbrecht, Germany) and centrifuged immediately using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 30 s at 13,000 × *g*. The resulting plasma was then aliquoted into fresh Eppendorf tubes and stored at −20 °C prior to insulin analysis. Insulin was measured using a modified dextran-coated charcoal radioimmunoassay, as described previously [8]. Blood triacylglycerol, HDL-, LDL- and total cholesterol levels were measured using a Hitachi Automated Analyzer 912 (Boehringer, Mannheim, Germany), as described previously [24].

Statistical analyses

Results are expressed as mean ± S.E.M. Data were compared using ANOVA, followed by a Student–Newman–Keuls *post hoc* test. Area under the curve (AUC) analyses were calculated using the trapezoidal rule with baseline subtraction. $P < 0.05$ was considered to be statistically significant.

Results

Effects of desHis^{1}Pro^{4}Glu^9-glucagon and desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon on body weight, food intake, blood glucose and plasma insulin

Once daily administration of desHis^{1}Pro^{4}Glu^9-glucagon or desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon had no effect on body weight (62.4 ± 1.9 and 62.1 ± 2.8 g; respectively) or daily food intake (4.4 ± 0.2 and 4.8 ± 0.3 g; respectively) when compared to saline treated controls (body weight 61.1 ± 3.2 g and daily food intake 4.9 ± 0.3 g). In addition, non-fasting blood glucose levels were not significantly different between groups (Fig. 1A). However, a significant decrease in plasma insulin concentrations ($P \leq 0.05$ to $P \leq 0.001$) was observed from day 6 onwards in both desHis^{1}Pro^{4}Glu^9-glucagon and desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon treated mice (Fig. 1B).

Effects of desHis^{1}Pro^{4}Glu^9-glucagon and desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon on glucose tolerance

During an oral glucose tolerance test, glucose levels were significantly ($P < 0.05$ to $P < 0.01$) reduced at 30 and 60 min post challenge in desHis^{1}Pro^{4}Glu^9-glucagon and desHis^{1}Pro^{4}Glu^9(Lys^{30}PAL)-glucagon treated high fat mice when compared with saline treated controls (Fig. 2A). This was corroborated by a significantly ($P < 0.01$) reduced overall glycemic excursion in both treatment groups (data not shown). Glucose-stimulated plasma insulin concentrations were also significantly ($P < 0.01$ to $P < 0.001$) reduced 15 and