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A novel GLP-1/xenin hybrid peptide improves glucose homeostasis, circulating lipids and restores GIP sensitivity in high fat fed mice

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ABSTRACT

Combined modulation of peptide hormone receptors including, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and xenin, have established benefits for the treatment of diabetes. The present study has assessed the biological actions and therapeutic efficacy of a novel exendin-4/xenin-8-Gln hybrid peptide, both alone and in combination with the GIP receptor agonist (DAla^2)GIP. Exendin-4/xenin-8-Gln was enzymatically stable and exhibited enhanced insulin secretory actions when compared to its parent peptides. Exendin-4/xenin-8-Gln also possessed ability to potentiate the *in vitro* actions of GIP. Acute administration of exendin-4/xenin-8-Gln in mice induced appetite suppressive effects, as well as significant and protracted glucose-lowering and insulin secretory actions. Twice daily administration of exendin-4/xenin-8-Gln, alone or in combination with (DAla^2)GIP, for 21-days significantly reduced non-fasting glucose and increased circulating insulin levels in high fat fed mice. In addition, all exendin-4/xenin-8-Gln treated mice displayed improved glucose tolerance, insulin sensitivity and metabolic responses to GIP. Combination therapy with (DAla^2)GIP did not result in any obvious further benefits. Metabolic improvements in all treatment groups were accompanied by reduced pancreatic beta-cell area and insulin content, suggesting reduced insulin demand. Interestingly, body weight, food intake, circulating glucagon, metabolic rate and amylase activity were unaltered by the treatment regimens. However, all treatment groups, barring (DAla^2)GIP alone, exhibited marked reductions in total- and LDL-cholesterol. Furthermore, exendin-4 therapy also reduced circulating triacylglycerol. This study highlights the positive antidiabetic effects of exendin-4/xenin-8-Gln, and suggests that combined modulation of GLP-1 and xenin related signalling pathways represents an exciting treatment option for type 2 diabetes.

1. Introduction

Gut-derived peptide hormones possess important physiological actions on specific target organs such as liver, muscle, adipose and pancreas to regulate metabolism [22,27,30]. In this regard, the well-characterised incretin hormone glucagon-like peptide-1 (GLP-1), secreted from intestinal L-cells in response to feeding, plays a fundamental role in glucose stimulated insulin secretion, as well as regulation of glucagon secretion, gastric emptying and satiety [3]. Xenin, another gastrointestinal hormone secreted from K-cells following a meal, is known to be involved in stimulating pancreatic insulin release [36,58] and inhibiting energy intake [8,51]. Thus, it is unsurprising that enzymatically stable, long-acting versions of both gut hormones have been actively pursued as potential drug candidates for the treatment of metabolic diseases [47,67]. Indeed, long-acting GLP-1 mimetics are now clinically approved for the treatment of both diabetes [57], and obesity [19].

Despite the obvious therapeutic indications for individual stabilised gut peptide forms, recent research highlights additive, or even synergistic, actions between gut hormones that can offer significantly enhanced metabolic benefits [16,23,27]. Thus, although effective, the antidiabetic actions of GLP-1 drugs in the clinic could be augmented [50]. In this regard, the impaired incretin effect in man, representing a fundamental pathophysiological characteristic of type 2 diabetes [44], consists of two main elements, decreased GLP-1 secretion and defective insulinotropic action of the sister incretin hormone to GLP-1, glucose-dependent insulinotropic polypeptide (GIP) [43]. Current GLP-1 mimetics only target issues relating to reduced GLP-1 secretion [62], with no impact upon compromised GIP bioactivity, which may help explain the lesser than expected benefits in man. However, xenin is known to potentiate the insulinotropic action of GIP [7,36,58,68], highlighting a clear therapeutic rationale for combination of GLP-1 and xenin therapies in diabetes. Moreover, numerous stable and long-acting GIP derivatives have been characterised [26], which could also be a useful addition within this combinatorial treatment approach. As such, modulation of different signalling pathways within the gut-pancreas axis by

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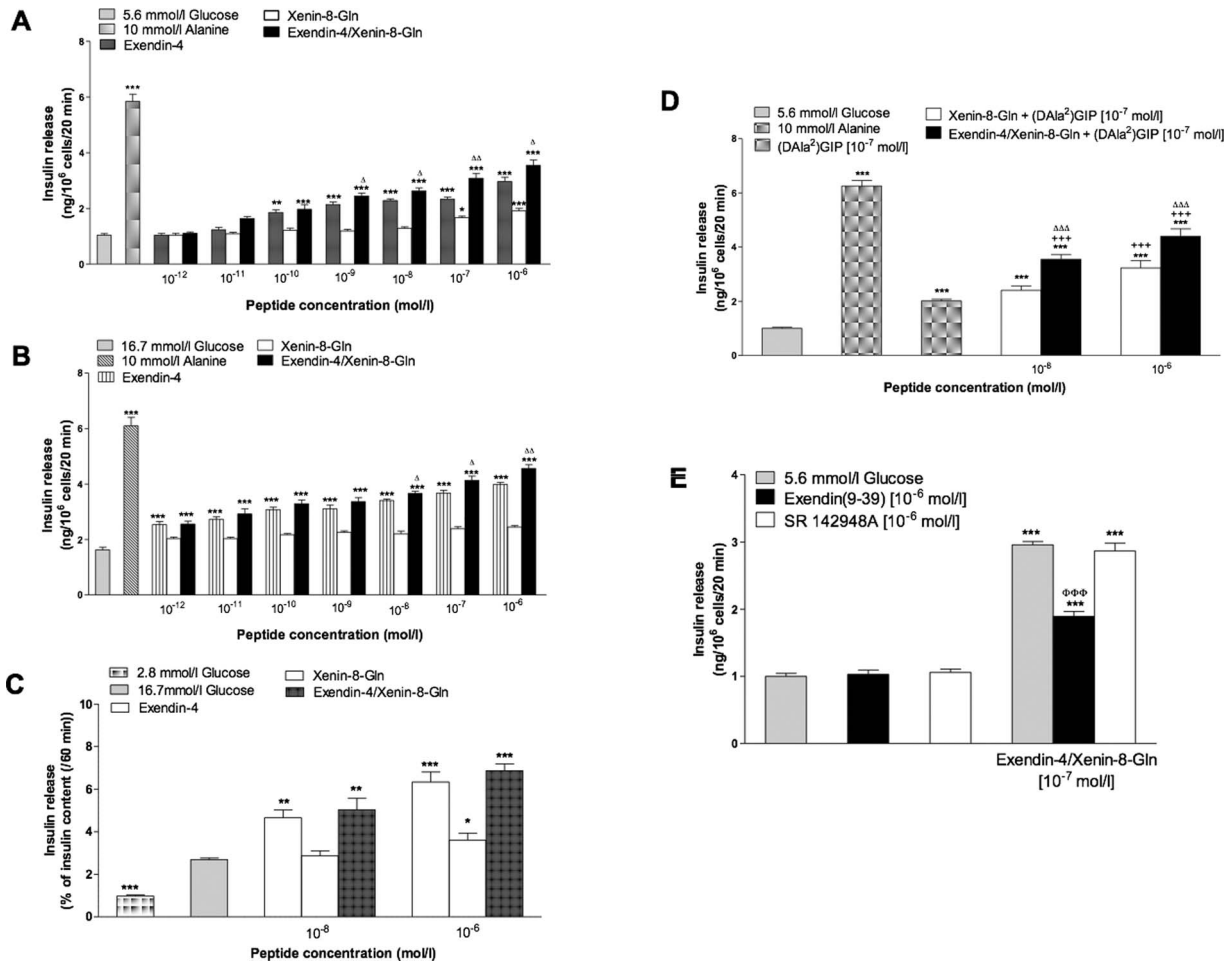

Fig. 1. Effects of exendin-4, xenin-8-Gln and exendin-4/xenin-8-Gln on insulin release from BRIN-BD11 cells and isolated mouse islets. (A,B) BRIN-BD11 cells were incubated (20 min) with exendin-4, xenin-8-Gln or exendin-4/xenin-8-Gln (10^{-12}–10^{-6} M) or alanine (10 mM) as positive control in the presence of (A) 5.6 mmol/l glucose or (B) 16.7 mmol/l glucose. (C) Isolated mouse islets were incubated (60 min) with exendin-4, xenin-8-Gln or exendin-4/xenin-8-Gln (10^{-8} and 10^{-6} M) at 16.7 mmol/l glucose. (D) Effects of xenin-8-Gln or exendin-4/xenin-8-Gln (10^{-8} and 10^{-6} M) on (DAla^2)GIP (10^{-7} M) mediated insulin release from BRIN BD11 cells. (E) Effects of the GLP-1 receptor and neurotensin receptor antagonists, exendin (9–39) and SR142948A, respectively (both at 10^{-6} M), on exendin-4/xenin-8-Gln (10^{-7} M) mediated insulin release from BRIN-BD11 cells. Values represent mean ± SEM (n = 8, A-D; n = 4, E). *P < 0.05, **P < 0.01 and ***P < 0.001 compared to respective glucose control. $^{\Delta}$P < 0.05, $^{\Delta\Delta}$P < 0.01 and $^{\Delta\Delta\Delta}$P < 0.001 compared to (A,B) exendin-4 or (D) xenin-8-Gln plus (DAla^2)GIP. $^{+++}$P < 0.001 compared to (DAla^2)GIP (10^{-7} mol/l) alone. $^{\Phi\Phi\Phi}$P < 0.001 compared to exendin-4/xenin-8-Gln alone.

GLP-1, xenin and GIP [35,45,58,63], along with beneficial centrally mediated effects on energy turnover [9,15], should lead to enhanced metabolic control and improved antidiabetic effects [1].

To increase therapeutic applicability of a combined treatment approach, and reduce polypharmacy, hybrid peptide technology is often employed [16,64]. This can be achieved through coupling the bioactive regions of the parent peptides together, in one molecule [31]. Therefore, in the current study, we have designed a novel GLP-1/xenin fusion hybrid that incorporates the key amino acid sequences of the stable and well-characterised GLP-1 and xenin mimetics, exendin-4 [59] and xenin-8-Gln [38] respectively, linked together via an 8-amino-3,6-dioxaoctanoic acid linker molecule [31]. Interestingly, previous work has shown that a shorter form of xenin, namely xenin-6, may also retain bioactivity [13], which could also be useful for creating xenin-based hybrid peptides. In addition, to also activate GIP receptor signalling pathways within our treatment paradigm, the stable and long-acting GIP analogue, (DAla^2)GIP [20], was employed. Initially we assessed plasma stability followed by *in vitro* and *in vivo* bioactivity of the peptides. In addition, the beneficial metabolic effects of twice daily administration of exendin-4/xenin-8-Gln and (DAla^2)GIP, alone or in combination, were evaluated in high fat fed mice. The results reveal that modulation of multiple gut hormone receptors requires further consideration as therapeutic option for obesity-diabetes.

2. Material and methods

2.1. Peptides synthesis and assessment of plasma enzymatic stability

All peptides were synthesised by standard solid-phase Fmoc protocols (> 95% purity) and purchased from American Peptide company (Sunnyvale, CA, USA). Supplementary Table 1 displays the amino acid sequence of the novel hybrid peptide, and related parent peptides. Before experimentation, all peptides were characterised in-house using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and Matrix-Assisted Laser Desorption Ionisation Time-of-Flight mass spectrometry (MALDI-ToF MS), as described previously [37]. Enzymatic stability of peptides was assessed *in vitro* using murine plasma, as outlined previously [55]. Briefly, test peptides (50 µg) were incubated at 37 °C in 50 mmol/l triethanolamine–HCl (pH 7.8) with mouse plasma (50 µl) for 0, 120, 240 and 480 min and degradation profiles followed by HPLC, with MALDI-ToF MS analyses of collected peaks.

2.2. Insulin secretory studies

Clonal pancreatic clonal BRIN-BD11 beta-cells [39] were used to assess *in vitro* insulin secretory activity of test peptides. For all studies, BRIN-BD11 cells were seeded into 24-well plates (150,000 cells per