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Biological activity of EXf, a peptide analogue of exendin-4

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ABSTRACT

Exendin-4 is an incretin mimetic that has been developed for the treatment of patients with type 2 diabetes. EXf is an available carboxy-terminal truncated fragment of exendin-4 with two amino acid substitutions. The purpose of these studies was to evaluate the biological activity of EXf. After a single subcutaneous injection, EXf significantly decreased plasma glucose concentration and glucose excursion following the administration of an oral glucose challenge both in non-diabetic (ICR), monosodium L-glutamate induced insulin resistance (MSG-IR) and diabetic KK-ay mice. Meanwhile, EXf resulted in an increase of first-phase insulin secretion in normal mice and KK-ay mice following the glucose challenge. EXf was also shown to inhibit small intestinal transit in rodent models. EXf activated the cAMP response element (CRE) of the rat insulin I gene promoter (RIP1) GFP-construct in a dose-dependent manner in the cultured mouse insulinoma cell line, termed NIT-1, and this agonist activity was blocked by the glucagon-like peptide 1 (GLP-1) receptor antagonist exendin(9–39). In summary, EXf, an analogue of exendin-4, has agonist activity to GLP-1 receptor in vitro and glucoregulatory activities in vivo, thus it can be considered as a new candidate for the treatment of type 2 diabetes.

1. Introduction

Exendin-4, a 39-amino acid peptide amide originally isolated from the salivary secretions of *Heloderma suspectum* (Gila monster) (Eng et al., 1992), is an incretin mimetic that has been developed for the treatment of patients with type 2 diabetes. In vitro, exendin-4 binds to and activates the known glucagon-like peptide 1 (GLP-1) receptor (Göke et al., 1993; Thorens et al., 1993). Exendin-4 shares many of the glucoregulatory actions observed with GLP-1 (Buse et al., 2004; DeFronzo et al., 2005; Holst, 2002; Kendall et al., 2005; Nauck, 2004; Nielsen, 2005). Clinical and nonclinical studies have shown that exendin-4 has several beneficial antidiabetic (glucose-lowering) actions that include glucose-dependent enhancement of insulin secretion, glucose dependent suppression of inappropriately high glucagon secretion, slowing of gastric emptying, reducing of food intake and body weight (Degn et al., 2004; Edwards et al., 2001; Egan et al., 2002). Relative to the endogenous mammalian incretin hormone, GLP-1, exendin-4 is resistant to cleavage by dipeptidylpeptidase IV resulting in a longer half-life.

EXf is a novel carboxy-terminal truncated fragment of exendin-4 with two amino acid substitutions, tyrosine for phenylalanine at position 6, which favors the binding of ^{125}I in the pharmacokinetic research, and the carboxy-terminal is proline, which is more resistant to carboxypeptidase's cleavage (Fig. 1). Shortening the peptide facilitates its synthesis. The purpose of the present study was to characterize the biological activity of EXf, which may be potentially the substitute for exendin-4, both in vitro and in vivo.

2. Materials and methods

2.1. Materials

EXf (patent application number: 200510040823.8) was obtained from Changzhou pharmaceutical factory Co., Ltd (Changzhou, China). Exendin(9–39) was purchased from Sigma (America). EXf and exendin(9–39) were dissolved in phosphate-buffered saline.

2.2. In vivo studies

2.2.1. Animals

ICR mice, pregnant ICR mice and KK-ay diabetic mice were purchased from the Experimental Animal Center, Chinese Academy of Medical Science, Beijing, China. Neonatal mice underwent a subcutaneous injection of monosodium L-glutamate (MSG, dissolved in sterile water; purchased from Huaboyuan Technologic Development Center, Beijing, China) in dose of 4 mg/g body weight for 7 days after birth. Control group received equal volume of saline isosmotic to the MSG

Abbreviations: CRE, cAMP response element; RIP1, rat insulin I gene promoter; GLP-1, glucagon-like peptide 1; MSG, monosodium L-glutamate; GFP, green fluorescent protein; MSG-IR mice, monosodium L-glutamate induced insulin resistance mice; AUC_{0-135}, area under curve (0–135 min).

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EXf
HGEGTYTSDLSKQMEEEAVKLFIEWLKNGP-NH2

Exendin-4
HGEGTFTSDLSKQMEEEAVKLFIEWLKNGGPSSGAPPPS-NH2

Fig. 1. Amino acid sequences of EXf and exendin-4.

solution. After weaning, pups were separated by sex and allowed to grow to the age of 16–18 weeks before experiments were initiated. The mice with MSG treatment neonatally are accompanied by obesity, insulin resistance, and a number of neuroendocrine dysfunctions. Male weight-matched MSG mice were randomly divided into four groups. All protocols for animal use and killing were in accordance with National Institutes of Health guidelines.

2.2.2. Acute time-course test of EXf in ICR, MSG induced insulin resistance (MSG-IR) and KK-ay diabetic mice

After fasting overnight (ICR mice) or for 4 h (MSG-IR and KK-ay diabetic mice), blood samples were collected via tail tip. Mice ($n=10$) were then injected subcutaneously with vehicle (5 ml/kg) or EXf at doses of 0.125, 0.5, or 2.0 μg/kg. Between 30 min and 3 h after the injections, 10 μl of blood from tail tip was collected and assayed for glucose. The whole blood glucose concentration was analyzed by the immobilized glucose oxidase method (Trinder, 1969).

2.2.3. Acute effects of EXf on glucose tolerance in ICR and MSG-IR mice

Before the oral glucose tolerance test was performed, animals were fasted overnight (ICR mice) or for 4 h (MSG-IR mice). Mice ($n=10$) were injected subcutaneously with vehicle (5 ml/kg) or EXf dosed as described above. Glucose (2 g /kg) was orally given to the animal 15 min after peptide administration. Blood samples were collected via tail tip prior to peptide injection and for up to 2 h after glucose challenge. Blood glucose concentration was measured as described above.

2.2.4. Effect of EXf on first-phase insulin secretion in ICR mice and KK-ay diabetic mice

To examine the effect of EXf on first-phase insulin release during hyperglycemia, insulin levels were determined after an oral glucose challenge (2 g/kg) in ICR and KK-ay diabetic mice. Six groups of ICR mice ($n=10$) fasted overnight were studied: four glucose-loading groups, and two control groups. In the glucose-loading groups, 15 min before the animals were given an oral glucose challenge, each animal received a subcutaneous injection of vehicle (5 ml/kg) or EXf dosed as described above. In the control groups, distilled water was administered orally (10 ml/kg) 15 min after subcutaneous injection of saline or EXf (2.0 μg/kg). Animals were decapitated 5 min after glucose challenge, and trunk blood samples were collected immediately. Plasma glucose was measured, and plasma insulin concentration was determined using commercially available radioimmunoassay kit (Beijing north institute of biological technology, China) with Gamma counter (Wallac1470 WINZARDγ counter).

KK-ay diabetic mice were subjected to a 4 h fasting before an oral glucose challenge. Mice were administered vehicle (5 ml/kg) or EXf at doses of 1.25, 2.5, or 5.0 μg/kg. Glucose was administered by oral gavage (2 g /kg) 15 min after peptide administration. Blood samples were collected from the retro orbital sinus 5 min after glucose challenge. Plasma glucose and plasma insulin concentration were measured as described above.

2.2.5. Effect of EXf on gastric emptying and small intestinal transit in ICR mice

2.2.5.1. Semi-solid test meal preparation. Two grams methylcellulose was added to 50 ml distilled water, heated to 90–95 °C and the mixture was stirred for 5–10 min to allow thorough wetting of the methylcellulose. Milk powder (3.2 g), powdered sugar (1.6 g), starch (1.6 g) and active carbon (1.0 g) were added in turn, with 2 min blending between each addition. The resulting meal was refrigerated for at least 36 h to allow for the escape of air trapped as a result of blending. Then it was kept at 4 °C, and prior to administration the test meal was warmed at 37 °C in a water bath and vigorously mixed.

2.2.5.2. Procedures. Four groups of ICR mice ($n=10$) were food deprived for 24 h with free access to water, and then received a subcutaneous injection of vehicle (5 ml/kg) or EXf as described above. At 15 min after the final injection, test meal (0.5 ml/mouse) was administered by oral gavage, and then mice were euthanized by cervical dislocation 15 min later. The abdominal cavity was opened,

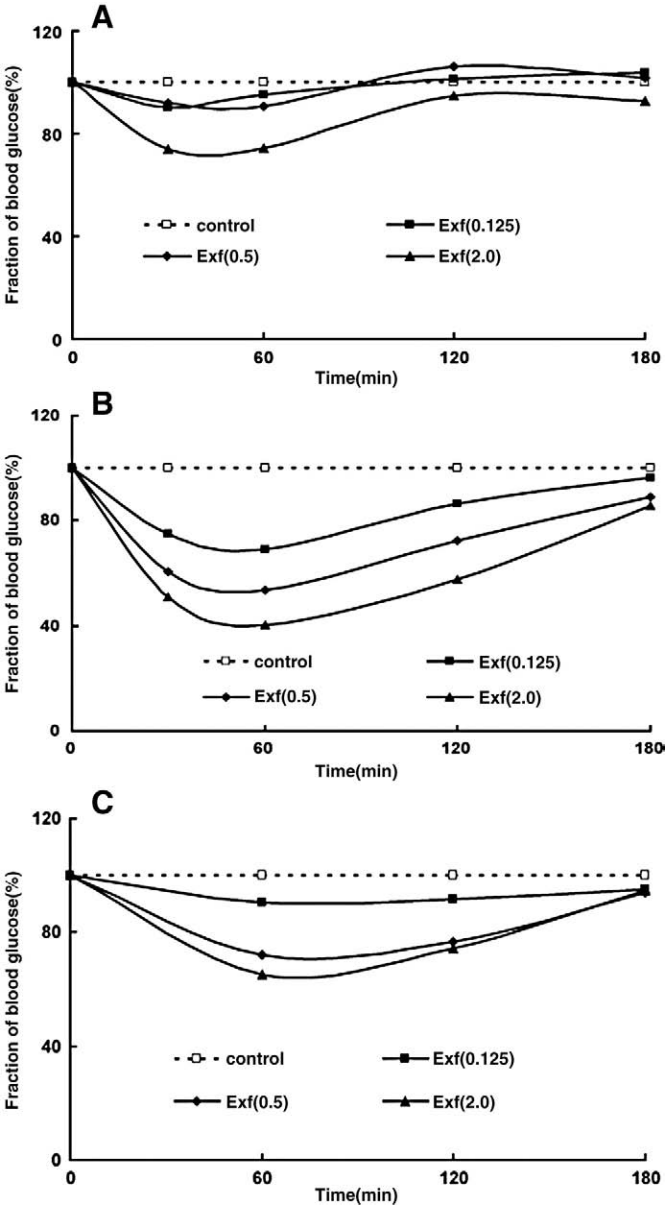

Fig. 2. Effect of EXf on blood glucose concentrations in ICR mice (A), MSG-IR mice (B) and KK-ay diabetic mice (C). After fasting overnight (ICR mice) or for 4 h (MSG-IR and KK-ay diabetic mice), baseline blood samples were collected via tail tip. Mice were then injected subcutaneously with vehicle (5 ml/kg) or EXf at doses of 0.125, 0.5, or 2.0 μg/kg. Between 30 min and 3 h after drug injections, 10 μl of blood from tail tip was collected and assayed for glucose. Time course of change of blood glucose is shown using the glucose concentration of control group at each time point as the baseline.