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Short communication

Exendin-4 increases insulin sensitivity via a PI-3-kinase-dependent mechanism: contrasting effects of GLP-1

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Abstract

The insulinotropic agent, exendin-4, is a long-acting analogue of glucagon-like peptide-1 (GLP-1) which improves glucose tolerance in humans and animals with diabetes, but the underlying mechanisms and the effects of exendin-4 on peripheral (muscle/fat) insulin action are unclear. Previous *in vivo* and clinical studies have been difficult to interpret because of complex, simultaneous changes in insulin and glucagon levels and possible effects on hepatic metabolism. Thus, the comparative effects of exendin-4 and GLP-1 on insulin-stimulated 2-[^{3}H]deoxyglucose (2-DOG) uptake were measured in fully differentiated L6 myotubes and 3T3-adipocytes, including co-incubation with inhibitors of the PI-3-kinase (wortmannin) and mitogen-activated protein (MAP) kinase (PD098059) pathways. In L6 myotubes, there was a concentration-dependent and PI-3-kinase-dependent increase in insulin-stimulated 2-DOG uptake with exendin-4 and GLP-1, e.g. for exendin-4 the $C_{I\text{-}200}$ value (concentration of insulin required to increase 2-DOG uptake 2-fold) decreased from $1.3 \pm 1.4 \times 10^{-7}$ M (insulin alone, $n = 16$) to $5.9 \pm 1.3 \times 10^{-8}$ M (insulin + exendin-4 0.1 nM, $n = 18$, $P < 0.03$). A similar insulin-sensitizing effect was observed with exendin-4 in 3T3-adipocytes, but GLP-1 had no effect on adipocyte insulin sensitivity. In conclusion, this is the first direct evidence showing that exendin-4 increases insulin-stimulated glucose uptake in muscle and fat derived cells *via* a pathway that involves PI-3-kinase activation. Furthermore, the contrasting responses of exendin and GLP-1 in 3T3-adipocytes suggest that the peripheral insulin-sensitizing effect of exendin-4 (in contrast to the insulinotropic effect) does not involve the GLP-1 receptor pathway.

Keywords: Exendin-4; GLP-1; Insulin sensitivity; L6 myotubes; 3T3-adipocytes

1. Introduction

GLP-1 and gastric inhibitory polypeptide are the hormones secreted from gastrointestinal endocrine cells in response to food intake which account for the incretin effect, i.e. the enhanced insulin secretion after oral vs. intravenous glucose administration [1]. It has recently been shown that postprandial GLP-1 concentrations are reduced in patients with type 2 diabetes [2], while administration of exogenous GLP-1 has transient glucose-lowering effects *via* stimulation of glucose-dependent insulin secretion [3], inhibition of pancreatic glucagon release [4] and delayed gastric emptying [5]. GLP-1 is unsuitable for therapeutic use because it is rapidly cleared from the circulation (half-life 1.5 min) by the ubiquitous enzyme dipeptidyl peptidase-IV (DPP-IV).

Exendin-4 is an analogue of GLP-1 (53% structural homology) which was first isolated from the salivary secretions of a South American lizard known as the Gila monster (*Heloderma suspectum venum*) [6]; it is a distinct protein derived from a separate set of genes [7] and a potent agonist at the pancreatic GLP-1 receptor [8]. Exendin-4 is resistant to DPP-IV cleavage, and therefore has a long-acting antidiabetic profile that is potentially suitable for therapeutic use in humans [9,10]. Synthetic exendin-4, AC2993, is undergoing phase III clinical trials, and other GLP-1 analogues resistant to DPP-IV, e.g. LY307161, show promising antidiabetic effects in animal models of diabetes.

The extent to which peripheral actions contribute to the antidiabetic activity of GLP-1 and exendin-4 remains

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Abbreviations: GLP-1, glucagon-like peptide-1 (7-36); PI-3, phosphatidylinositol-3′; 2-DOG, 2-[^{3}H]deoxyglucose; DPP-IV, dipeptidyl peptidase-IV; MAP, mitogen-activated protein; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; $C_{I\text{-}200}$, concentration of insulin required to increase glucose uptake 2-fold.

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unclear. Previous work from our group and others has shown that GLP-1 augments insulin-stimulated glucose uptake and metabolism in isolated cells [11–13], and in Zucker diabetic rats 'whole-body' insulin sensitivity increased after chronic administration of exendin-4 [10]. The nature and explanation for this insulin-sensitizing effect is still unclear [10], mainly because the interpretation of *in vivo* studies in humans and animals is complicated by simultaneous changes in insulin and glucagon levels, as well as possible effects on hepatic metabolism [14], and the longer-term changes in insulin sensitivity may be secondary to improved glycaemic control [5,10,15]. Thus, the purpose of this *in vitro* study was to investigate the direct effects of exendin-4 on insulin-stimulated glucose uptake in skeletal muscle derived (L6 myotubes) and fat derived cells (3T3-L1 adipocytes), and to compare exendin and GLP-1 responses.

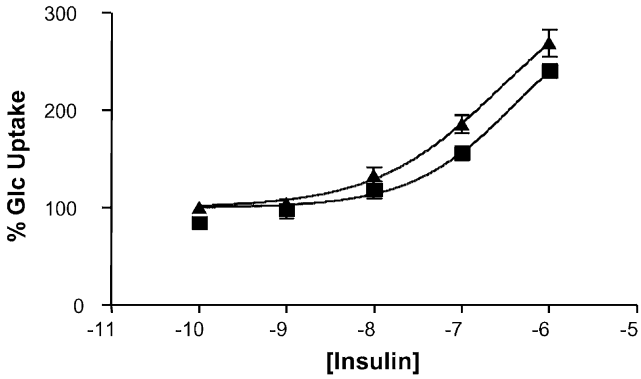

Fig. 1. Dose–response curves for insulin-stimulated 2-DOG uptake in L6 myotubes in the absence (▲) and presence (■) of exendin-4 0.1 nM. Derived $C_{I\text{-}200}$ values were significantly lower with exendin-4 ($P < 0.02$).

2. Materials and methods

L6 myoblasts, a rat skeletal muscle derived cell line (ECACC), were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% FCS in 5% CO_2, at 37°. After the cells became confluent, the medium was changed to one containing 2% FCS for 5 days in order to transform the cells from L6 myoblasts to mature myotubes. 3T3-L1 fibroblasts were grown in DMEM containing 10% fetal bovine serum and maintained in 5% CO_2 at 37°. The cells were incubated in the presence of DMEM containing 0.5 mM isobutylmethylxanthine, 250 nM dexamethasone, 400 nM insulin and 10% FCS for 3 days to differentiate them into insulin-responsive adipocytes. Thereafter, the cells were maintained in DMEM containing 10% FCS. All experiments began 8–10 days after the start of differentiation.

2.1. Measurement of insulin stimulated 2-DOG uptake

As described previously [11], L6 myotubes and 3T3-adipocytes were incubated in serum-free DMEM for 24 hr prior to performing the glucose uptake assay. Cells were incubated in the absence and presence of insulin, and in the presence of insulin + varying concentrations of exendin-4 or GLP-1 100 nM. Co-incubations were undertaken with wortmannin 100 nM, a specific phosphatidylinositol-3′ (PI-3) kinase inhibitor [16], or PD098059 20 μM, an inhibitor of MAP kinase [17]. Following this incubation period, cells were washed in Krebs buffer and incubated with 1 mL 'hot' Krebs solution containing [^{3}H]-2-DOG (0.2 μCi, specific activity 10 Ci/mmol) and 1 μM 2-DOG for 10 min. After washing, the cells were solubilized in

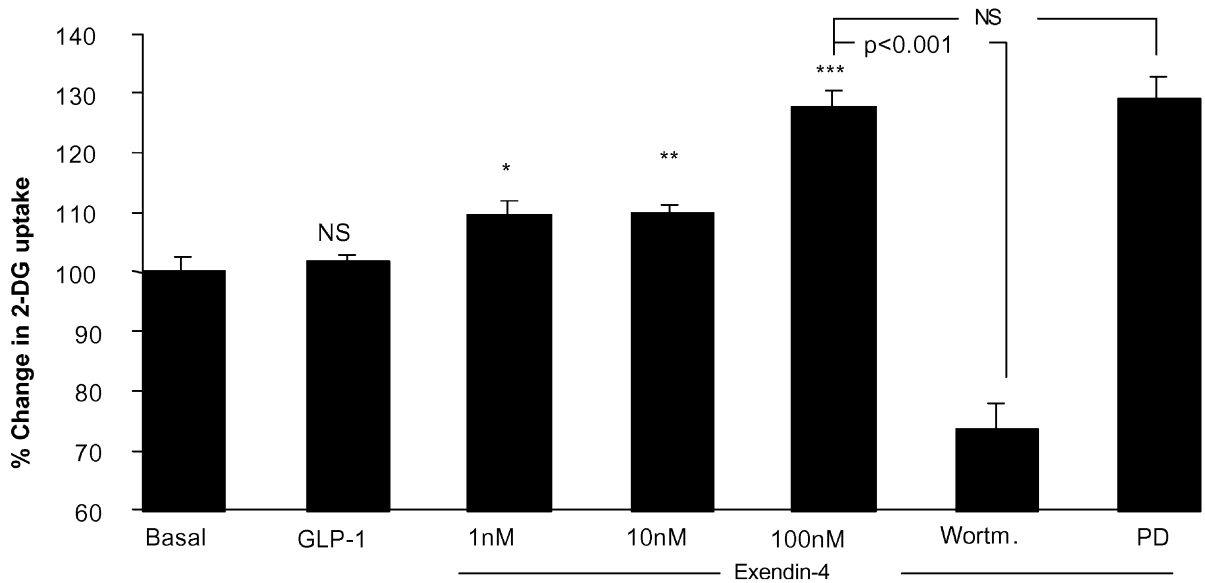

Fig. 2. Percentage change in 2-DOG uptake (relative to basal conditions, 100%) in 3T3-adipocytes exposed to insulin 100 nM + GLP-1 100 nM or exendin-4 (1–100 nM); and insulin + exendin-4 100 nM + wortmannin (or PD098059).

500 μL of 1 M NaOH prior to measurement of the incorporated radioactivity.

Concentration–response curves were derived for insulin-stimulated 2-DOG uptake in the presence or absence of exendin-4 and the curves fitted to a quadratic function to derive $C_{I\text{-}200}$ values (concentration of insulin required to increase 2-DOG uptake 2-fold relative to baseline control, 100%). Statistical analysis was performed using a Student's two-tailed *t*-test with unequal variance; Mann–Whitney *U*-test was used for non-parametric variables. Results are expressed as mean ± SEM for the percentage increase in 2-DOG uptake relative to control (100%). Individual values represent means of 4–6 measurements, each derived from experiments performed in triplicate.

3. Results

Exendin-4 had no effect on basal (non-insulin-mediated) glucose uptake, but significantly augmented insulin-stimulated glucose transport in L6 myotubes (Fig. 1). For example, the $C_{I\text{-}200}$ value decreased from $1.3 \pm 1.4 \times 10^{-7}$ M (insulin alone, $n = 16$) to $5.9 \pm 1.3 \times 10^{-8}$ M (insulin + exendin-4 0.1 nM, $n = 18$) ($p < 0.03$).

A similar, concentration-dependent increase in insulin sensitivity was observed with exendin-4 in 3T3-adipocytes, an effect that was attenuated by wortmannin but not PD098059 (Fig. 2). Although GLP-1 also increased 2-DOG uptake in L6 cells, there was no effect of GLP-1 on insulin sensitivity in 3T3-adipocytes (Fig. 2).

4. Discussion

Exendin-4 is a potent agonist at GLP-1 receptors on pancreatic islets [8,18], and the insulinotropic and glucagonostatic effects of exendin are similar to those of GLP-1. The main difference is in the time-course of the pancreatic responses, reflecting the much longer plasma half-life of exendin-4. *In vivo* studies have indicated that additional (extra-pancreatic) mechanisms may contribute to the anti-diabetic activity of exendin-4, e.g. increased 'whole-body' insulin sensitivity was reported after chronic administration to diabetic rats and monkeys [10]. Whether exendin-4 affects peripheral (i.e. muscle/fat) insulin sensitivity—the pathway primarily abnormal in type 2 diabetes—is still unclear, mainly because the interpretation of *in vivo* studies is complicated when the test drug might be simultaneously changing insulin action and both insulin and glucagon production [15].

This is the first study to directly investigate the effects of exendin-4 on insulin action *in vitro*. In isolated muscle and fat cells, exendin-4 augmented insulin-stimulated glucose uptake *via* an intracellular signalling pathway that requires PI-3-kinase activation. PI-3-kinase is important in the transduction of insulin-induced glycogen synthesis and the translocation of GLUT transporters to the plasma membrane, and exendin-4 has previously been shown to increase hepatic glycogen synthesis [14]. In contrast, blocking the MAP-kinase pathway had no effect on the insulin-sensitizing effect of exendin-4.

The contrasting positive (exendin-4) and neutral (GLP-1) effects on insulin sensitivity in 3T3-adipocytes strongly suggests the existence of different mechanisms underlying the extra-pancreatic effects of these proteins. In previous studies, the effects of GLP-1 on adipocyte glucose uptake were much smaller [13] than the 35% increase in insulin sensitivity observed in L6 myotubes [11], but in muscle the effects of GLP-1 are mediated by a receptor that is different from the pancreatic GLP-1 receptor [12]. Whether the GLP-1 receptor is expressed in 3T3-adipocytes is controversial [13,19], but if so it may be structurally and functionally different [20]. Thus, the insulin-sensitizing effect of exendin-4 in adipose cells may not involve the GLP-1 receptor pathway. In summary, this is the first study to directly address the effects of exendin-4 on peripheral insulin action. Whereas GLP-1 and exendin-4 have similar modes of action on islet cells, their extra-pancreatic effects on insulin sensitivity are quantitatively and qualitatively different, especially in adipose-derived cells, perhaps suggesting the involvement of distinct GLP-1 and exendin-4 receptor pathways.

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