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Review

Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes

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Abstract

Exenatide (synthetic exendin-4), glucagon-like peptide-1 (GLP-1), and GLP-1 analogues have actions with the potential to significantly improve glycemic control in patients with diabetes. Evidence suggests that these agents use a combination of mechanisms which may include glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, enhancement of β-cell mass, slowing of gastric emptying, inhibition of food intake, and modulation of glucose trafficking in peripheral tissues. The short in vivo half-life of GLP-1 has proven a significant barrier to continued clinical development, and the focus of current clinical studies has shifted to agents with longer and more potent in vivo activity. This review examines recent exendin-4 pharmacology in the context of several known mechanisms of action, and contrasts exendin-4 actions with those of GLP-1 and a GLP-1 analogue. One of the most provocative areas of recent research is the finding that exendin-4 enhances β-cell mass, thereby impeding or even reversing disease progression. Therefore, a major focus of this is article an examination of the data supporting the concept that exendin-4 and GLP-1 may increase β-cell mass via stimulation of β-cell neogenesis, stimulation of β-cell proliferation, and suppression of β-cell apoptosis.
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Keywords: Pharmacology; Exenatide; Type 2 diabetes

1. Introduction

Exendin-4, the naturally occurring form of exenatide (synthetic exendin-4; AC2993), was originally isolated from the salivary secretions of the lizard *Heloderma suspectum* (Gila monster; Fig. 1) [1]. In the Gila monster, exendin-4 circulates after the lizard bites down on its prey (ingestion of a meal) and thus represents the first example of an endocrine hormone secreted from salivary glands. [2]. It is unknown whether exendin-4 has a role in fuel homeostasis in the Gila monster [2]. Exendin-4 has a 53% amino acid sequence overlap with mammalian glucagon-like peptide-1 (GLP-1). In mammals, GLP-1 is processed from the proglucagon gene in L-cells in the small intestine [3]. Exendin-4 is transcribed from a distinct gene, not the Gila monster homologue of the mammalian proglucagon gene from which GLP-1 is expressed [4]. In mammals, exendin-4 is resistant to degradation by dipeptidyl peptidase-IV (DPP-IV) and has a much longer plasma half-life than GLP-1, which is degraded by DPP-IV with a half-life of less than 2 min [5,6].

Exendin-4 is not an analogue of GLP-1. In other words, the structure of the synthetic exendin-4 peptide (exenatide) was not created by sequential modification of the structure of GLP-1. However, exendin-4 and GLP-1 do share many glucoregulatory actions which may be mediated by the known pancreatic GLP-1 receptor [7]. Glucoregulatory actions of exendin-4 include glucose-dependent enhancement of insulin secretion [8–11], glucose-dependent suppression of inappropriately high glucagon secretion [10,12], slowing of gastric emptying [10,13] which may be paradoxically accelerated in people with diabetes [14], and reduction of food intake ([15,16]; Fig. 2). In addition, exendin-4 has been shown to promote β-cell proliferation and islet neogenesis from precursor cells in both in vitro and in vivo models [17–19]. These glucoregulatory actions of exendin-4, combined with enhanced pharmacokinetics, result in very high in vivo potency relative to native GLP-1 [11,20,21]. The putative mechanisms of action of exendin-4 are compared and contrasted with the actions of GLP-1 and a long-acting GLP-1 analogue in the following sections. One of the most provocative areas of recent research is

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Fig. 1. Comparison of amino acid sequences for exendin-4, mammalian GLP-1, and Gila monster GLP-1.

based on observations that exendin-4 may improve β-cell mass, thereby impeding or even reversing disease progression. Therefore, a major focus of this article will be to examine in detail the published reports supporting the concept that exendin-4 and GLP-1 may increase β-cell mass via stimulation of β-cell neogenesis, stimulation of β-cell proliferation, and suppression of β-cell apoptosis.

The onset of type 2 diabetes is characterized by the emergence of postprandial (post-meal) hyperglycemia and subsequently, fasting hyperglycemia [22]. In most individuals, hyperglycemia results from a failure of pancreatic β-cells to secrete adequate insulin to compensate for insulin-resistance in peripheral tissues [23,24]. The fraction of glycosylated hemoglobin (A1C) in circulating red blood cells provides an accurate indicator of average glucose concentrations in the blood for the previous 3 months. A1C levels in healthy humans typically comprises 5–6% of total hemoglobin, while A1C values in people with poorly controlled diabetes generally exceed 9% [25]. Results from the United Kingdom Prospective Diabetes Study (UKPDS) showed that a reduction in A1C was associated with a reduced risk of vascular complications, and also reaffirmed that type 2 diabetes is a progressive disease characterized by a continuous loss of β-cell function that current therapies cannot rectify. [26,27]. Exenatide is the USAN generic drug name for synthetic exendin-4, an investigational therapeutic being studied by Amylin Pharmaceuticals in partnership with Eli Lilly and Company, that may have a beneficial impact on the course of this disease.

2. GLP-1 receptor

The GLP-1 receptor (GLP-1R) is a seven-transmembrane domain, G-protein coupled receptor, initially described as the exendin receptor [28–30]. Distribution of the mammalian GLP-1R includes pancreatic periductal- and β-cells, kidney, heart, stomach, and brain [31]. The pancreatic

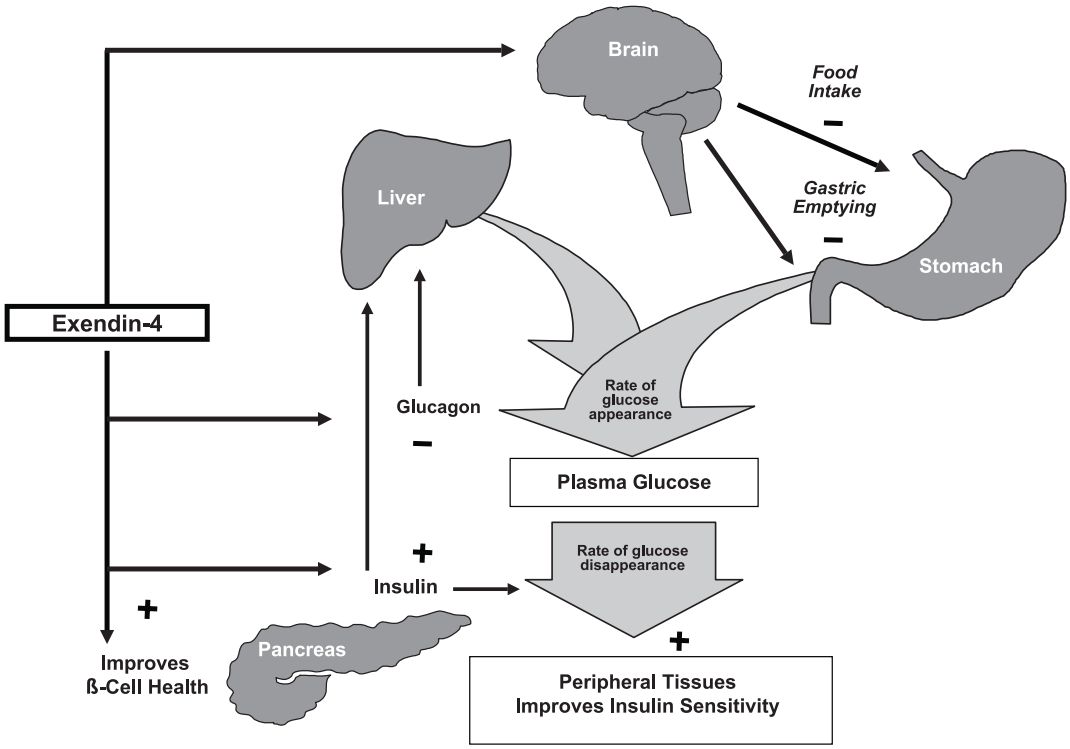

Fig. 2. Theoretical overview of the primary, anti-diabetic mechanisms of action for exendin-4 (or exenatide). Data suggest that exendin-4 enhances glycemic control by enhancing glucose-dependent insulin secretion, suppressing glucose-dependent glucagon secretion, slowing gastric emptying, reducing food intake, stimulating β-cell health, and increasing the insulin sensitivity of peripheral tissues.

SUN EXHIBIT 1037, IPR2024-00107, PAGE 2

GLP-1 receptor binds exendin-4 and GLP-1 with equal affinity in in vitro assays, and both peptides stimulate the receptor equipotently as demonstrated by the production of cyclic adenosine monophosphate (cAMP) in human and rat-based receptor systems [32–34]. Therefore, differences in receptor affinity and activation cannot explain the difference in in vivo potency between the two peptides. Under physiological conditions, the GLP-1R recognizes GLP-1 specifically, with no significant binding to secretin, vasoactive intestinal peptide (VIP), or other closely related, mammalian peptide hormones [28,31].

Based on studies using GLP-1R knockout (GLP-1R −/−) mice and in vitro receptor blockade in islets from these mice, a GLP-1 receptor appears to be involved in, and necessary for, the incretin/insulinotropic actions of exenatide and GLP-1, and the hepatic portal glucose sensor actions of GLP-1 [3,35–38]. GLP-1R −/− mice exhibit fasting hyperglycemia and abnormal blood glucose excursions in response to glucose challenge [39]. However, care must be taken when ascribing specific physiological functions to the GLP-1 receptor based on GLP-1R −/− mice, as these mice also exhibit abnormalities in the hypothalamic–pituitary–adrenal axis and alterations in the GIP (glucose-dependent insulinotropic polypeptide) hormone path- ways which may compensate for the absence of the GLP-1 signal transduction pathway [40,41]. Both GLP-1 and GIP stimulate insulin secretion under conditions of hyperglycemia (incretin actions) in GLP-1R −/− mice, resulting in lowered postprandial glucose excursions [3]. Truncated exendin-4(9–39)-NH_2 binds to and antagonizes mammalian pancreatic GLP-1 receptors [7] and is associated with an increased postprandial glucose excursion when administered to wild-type, male CD1 mice (5 μg/mouse IP 20 min before oral glucose challenge; [35]). In contrast, administration of truncated exendin-4(9–39)-NH_2 to GLP-1R −/− mice had no statistically significant effect on postprandial glucose excursions, although acute blood glucose levels (10–30-min post-challenge) trended lower after both oral and intraperitoneal glucose administration [35].

While exendin-4 and GLP-1 appear to share certain glucose-lowering actions, it is apparent that not all actions of exendin-4 are predictable based on the known pharmacology of GLP-1 [2]. For example, intraportal GLP-1 infusion triggers firing of the hepatic vagal afferent nerves, while exendin-4 does not [42]. Exendin-4 sensitized differentiated 3T3-L1 adipocytes to insulin-dependent glucose uptake, while GLP-1 had no effect in the same assay [43]. Exendin-4 may therefore exert at least some of its actions through a functionally different receptor, although this putative receptor has not yet been identified [38,42,43].

3. Glycemic control

Exendin-4, GLP-1, and GLP-1 analogues such as NN2211 have demonstrated abilities to control fasting and postprandial glucose excursions. The effects of GLP-1 on glycemic control in nonhuman models of diabetes have been reviewed elsewhere [44] and will not be extensively covered here, except for comparisons with exendin-4.

Exendin-4 had potent activity in reducing plasma glucose when administered as a single intraperitoneal dose of 0.001 to 10 μg to hyperglycemic *db*/*db* mice, a model of type 2 diabetes [11]. The maximal glucose-lowering effect of exendin-4 was sustained through the last time point measured (4 h). Comparable administration of GLP-1 in the range of 1–1000 μg also lowered glucose acutely; however, plasma glucose concentrations rapidly returned to their usual hyperglycemic levels. Exendin-4 lowered plasma glucose concentrations in a dose-dependent manner within 1 h after injection, with a maximum reduction of 37%. The half-maximal effective dose (ED_{50}) for exendin-4 averaged 0.06 μg/kg compared to 329 μg/kg for GLP-1. In the *ob*/*ob* mouse model of diabetes, exendin-4 had an average ED_{50} of 0.136 μg/kg compared to 744 μg/kg for GLP-1. Overall, exendin-4 was greater than 5000-fold more potent than GLP-1 in controlling hyperglycemia. In both strains of diabetic mice, the magnitude of the glucose lowering effect following a single dose of exendin-4 was related to the pre-existing plasma glucose concentration, i.e., exendin-4 activity was glucose-dependent.

The effects of prolonged 12- to 13-week treatment with once-daily intraperitoneal exendin-4 (24 nmol/kg; 100 μg/kg) has been studied in both diabetic *db*/*db* and nondiabetic mice [20]. After 1 week, fasting blood glucose concentrations in diabetic mice had declined from 232 to 90 mg/dl in the exendin-4-treated group, but remained at 238 mg/dl in the vehicle-control group. Fasting blood glucose concentrations were also lower in nondiabetic mice treated with exendin-4 (70 mg/dl) compared with vehicle-treated mice (135 mg/dl). At the completion of treatment, A1C values were 47% lower in exendin-4-treated diabetic mice than in vehicle-treated, averaging 4.7% and 8.8%, respectively. Nondiabetic mice also had a significant lowering of A1C values after exendin-4 treatment ($3.1 \pm 0.07\%$ vs. $3.5 \pm 0.08\%$. in vehicle-control mice). These A1C data affirmed the beneficial effects of exendin-4 on long-term glycemic control.

The effects of chronic intraperitoneal administration of exendin-4 on A1C and insulin sensitivity were further studied in male obese, diabetic Fatty Zucker (ZDF) rats [11]. These animals exhibit overt hyperglycemia starting at 8 to 10 weeks of age, are insulin-resistant, obese, dyslipidemic, and succumb to β-cell failure at 12 to 14 weeks of age. In a 5-week study using twice daily intraperitoneal injections of 100 μg exendin-4, initial pretreatment A1C values were approximately 73% greater in obese ZDF rats (6.0–6.9%) than in lean non-diabetic control rats (3.6–3.9%; Fig. 3). After 35 days, a significant decline in A1C of 41.3% was seen in the ZDF rats receiving exendin-4. This was 2.4-fold greater than the reduction observed in exendin-4-treated control lean rats. Measurements of glucose uptake

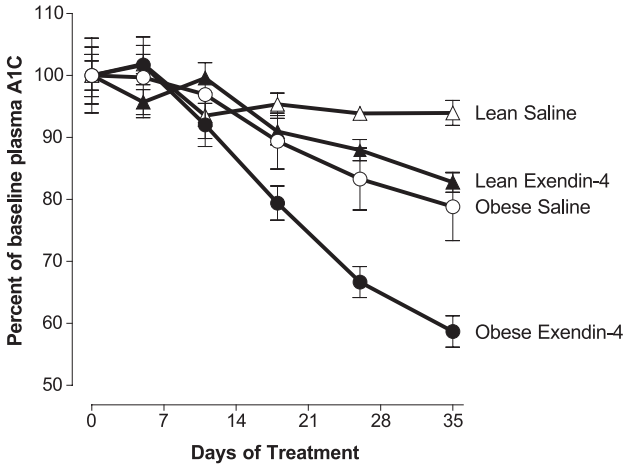

Fig. 3. Plasma A1C in diabetic and normoglycemic rats. Long-term administration of exendin-4 reduced plasma A1C in diabetic, obese ZDF rats and normoglycemic lean littermate control rats ($p < 0.01$ for obese rats and $p < 0.002$ for lean rats, respectively, compared with vehicle-treated lean rats). By day 35, A1C values had decreased 41% in obese ZDF rats treated with exendin-4. The relative reduction in A1C was 2.4-fold greater in obese than in lean exendin-4-treated rats, consistent with exendin-4 having a greater effect under hyperglycemic than normoglycemic conditions. Exendin-4 (100 μg) or vehicle was injected intraperitoneally, twice daily, for 5 weeks. Initial A1C values were $6.44 \pm 0.25\%$ in obese ZDF rats and $3.73 \pm 0.37\%$ in lean rats. Lean/Vehicle group, open triangles. Lean/Exendin-4 group, closed triangles. Obese/Vehicle group, open circles. Obese/Exendin-4 group, closed circles. $N = 6$ rats per group. (Adapted from Ref. [11]. Copyright ©1999 American Diabetes Association. Reprinted with permission from *The American Diabetes Association*).

into tissues under euglycemic and hyperinsulinemic conditions at the end of this study demonstrated an increase in insulin sensitivity (defined as plasma insulin concentration divided by the rate of glucose infusion) in exendin-4-treated obese ZDF rats (76%, $p < 0.02$) and lean, non-diabetic rats (51%, $p < 0.05$), compared with the saline-treated controls. In a second study, ZDF rats injected intraperitoneally once or twice daily with exendin-4 for 6 weeks had dose-dependent arrest or reversal of the trend for A1C to increase with increasing age/weight. Exendin-4 doses as low as 0.1 μg/rat twice daily were effective. Insulin sensitivity increased dose-dependently by up to 49% in the exendin-4-treated animals at the end of this study, as measured by glucose uptake under euglycemic hyperinsulinemic conditions. Whether this improvement in insulin sensitivity is a consequence of reduced glucose toxicity, or via direct actions of exendin-4 to improve glucose uptake into insulin-sensitive tissues, remains to be elucidated. Increases in total cholesterol levels were also significantly reduced in the exendin-4 treatment group over the 6-week period.

Analagous effects on glycemic control have also been reported in a larger mammalian model of human diabetes, using the GLP-1 analogue NN2211 to activate the GLP-1 receptor signal transduction pathway [45]. Chemical destruction of β-cells in male Göttingen minipigs caused the pigs to have impaired glucose tolerance or to be outright diabetic. Fasted β-cell-reduced pigs received intravenous doses of NN2211 (2 μg/kg; 0.6 nmol/kg) or vehicle under conditions of hyperglycemic glucose clamp. β-Cell-reduced pigs treated with the GLP-1 analogue required an approximately 200% increase in the glucose infusion rate compared to the vehicle-control group, and exhibited a temporally coincident increase in plasma insulin concentrations (AUC $72 \pm 28\%$ greater than vehicle) which was glucose-dependent. These data indicate an improvement in insulin-sensitivity after treatment with the GLP-1 analogue. NN2211 treatment also suppressed plasma glucagon concentrations during hyperglycemic clamp, but not during euglycemia following termination of the clamp, demonstrating the glucose-dependency of this anti-diabetic activity mediated through the GLP-1 receptor. Longer-term administration of subcutaneous NN2211 also had effects on glycemia in β-cell-reduced pigs. Once-daily NN2211 (3.3 μg/kg) was administered for 4 weeks. Treatment with the GLP-1 analogue reduced postprandial glucose excursions at both 2 and 4 weeks, and slowed gastric emptying. Overall, treatment with a GLP-1 analogue in a pig model of human diabetes confirmed several glucoregulatory mechanisms of action previously observed after administration of exendin-4 or GLP-1.

In fasted Rhesus monkeys with type 2 diabetes, a single subcutaneous injection of exendin-4 caused a reduction in plasma glucose that was dose-dependently accelerated, with a mean ED_{50} value of 0.25 μg/kg and a maximal glucose nadir of approximately 37% at a dose of 100 μg/kg [11]. Subsequently, plasma glucose concentrations tended to rise again towards control levels 2 to 3 h after exenatide injection.

The ability of GLP-1 to control glucose excursions in preclinical diabetes models led to a series of GLP-1 clinical trials in humans, which have been summarized [44]. However, it has become apparent that the short plasma half-life of GLP-1 was a significant barrier to clinical development. In particular, Zander et al. [46] demonstrated that subcutaneous infusion of GLP-1 efficiently lowers plasma glucose in patients with type 2 diabetes, but must be given continuously to be effective. The resistance of exendin-4 to degradation by dipeptidyl peptidase-IV and other putative mechanisms which together result in a better pharmacokinetic profile for exendin-4 offered one possible solution to this dilemma. A series of phase II trials of exenatide (synthetic exendin-4) have now been completed in greater than 300 subjects with type 2 diabetes (reviewed by Nielsen and Baron [47]). In general, a consistent pattern of safety and pharmacodynamics was observed. Dose-ranging studies identified an optimal exenatide glucose-lowering dose range of 0.05–0.2 μg/kg when injected subcutaneously. Fineman et al. [48] reported notable findings from a phase II study of exenatide in patients with type 2 diabetes not attaining A1C goals $\leq 8\%$ with oral sulfonylureas and/or metformin. Twenty-eight days of exenatide treatment reduced A1C by approximately 0.9% compared to baseline ($p \leq 0.006$). In addition, the proportion of patients achieving A1C $\leq 7\%$

was fourfold greater after exenatide treatment compared with placebo. Given that A1C only fully reflects a change in glycemia 3 months after a sustained change has occurred, this reduction in A1C and enhanced ability to achieve clinically relevant A1C target values after only one month of therapy is of great clinical interest. Glucose profiles during ingestion of a mixed meal demonstrated that the marked, acute ability of exenatide to reduce postprandial glycemia was sustained over the 28-day observation period. Another recent report [49] examined the impact of 1 month of SC exenatide dosing on blood glucose levels in poorly controlled, community dwelling, insulin-naive patients with type 2 diabetes. Although interpretation of these study results is limited by the lack of a placebo control, exenatide treatment resulted in reduced A1C compared with pretreatment values.

4. Insulin secretion

Glucose-dependent insulinotropism refers to the ability of agents such as exendin-4 and GLP-1 to stimulate insulin secretion during euglycemia or hyperglycemia, but not during hypoglycemia [2]. Glucose-dependent insulinotropism has also been defined as the amplification of β-cell insulin secretion when circulating glucose concentrations are above, but not below, the normal range [8,9]. In animal models of diabetes, a predominant acute action of exendin-4 is glucose-dependent insulinotropism, resulting in an amplification of glucose/insulin secretion coupling. This action of exendin-4 contrasts with the action of non-glucose-dependent insulin secretagogues or hypoglycemic agents, such as sulfonylureas, which predominantly increase insulin secretion independent of prevailing glucose concentrations [50] and thus have a greater potential to induce hypoglycemia [27].

Two in vitro models using pancreatic islets isolated from male Lewis rats have been used to examine the action of exendin-4 on insulin secretion [9]. In a static incubation model, islets were incubated in a medium containing either 3 mmol/l (basal) or 10 mmol/l (elevated) glucose. Raising the glucose concentration from 3 to 10 mmol/l increased insulin secretion 9.8-fold ($p < 0.05$). In the presence of elevated glucose (10 mmol/l) and a range of exendin-4 doses (1 nmol/l – 1 μmol/l), insulin secretion was increased over basal levels by up to 19.6-fold ($p < 0.01$). In a microphysiometer model, increasing the glucose concentration from 3 mmol/l (basal) to 7.5 mmol/l for 15 min increased insulin secretion up to 6.4-fold. The addition of exendin-4 (20 nmol/l) stimulated insulin secretion over and above the effect of 7.5 mmol/l glucose alone, to 13.5-fold over basal rate ($p < 0.01$). Insulin secretion returned to previous levels within 5 min of cessation of exendin-4 perfusion.

Thus, exendin-4 exerts direct effects on rat pancreatic islets in vitro to enhance glucose-stimulated insulin secretion. These effects do not appear to persist once exendin-4 is removed from the system, suggesting that the peptide may be binding and activating target receptors only while it is present in the perfusate. Furthermore, the insulinotropic action of exendin-4 in this system rapidly decreases when the ambient glucose is decreased back to 3 mmol/l, consistent with the glucose-dependence of insulinotropism being at least in part at the level of the pancreatic islet. Overall, the data support the interpretation that exendin-4 acts directly, but perhaps not exclusively, at the isolated pancreatic islet level to stimulate insulin secretion, and are consistent with published reports showing enhanced insulin secretion from islets exposed to GLP-1 [33,51–53].

Intravenous administration of exendin-4 (0.4 nmol/kg) acutely increased plasma insulin concentrations in fasted Wistar rats, eliciting approximately double the insulin peak elicited by the same dose of GLP-1. The ED_{50} value for this insulinotropic activity was 0.014 nmol/kg for exendin-4 compared with 0.19 nmol/kg for GLP-1 [20]. Similar results have been reported by Parkes et al. [9] examining the insulinotropic actions of exendin-4 during an intravenous glucose challenge (Fig. 4).

Insulinotropic actions have also been reported for exendin-4 in studies examining daily administration of the peptide (24 nmol/kg) to diabetic *db*/*db* mice for 12–13 weeks [20]. Fasting plasma insulin concentrations in diabetic mice treated with exendin-4 averaged 550% higher than vehicle-treated diabetic rats under conditions where the corresponding plasma glucose concentrations averaged 16 and 29 mmol/l, respectively, compared to 7–8 mmol/l blood glucose in nondiabetic (young) *db*/*db* mice.

In the human studies reported by Kolterman et al. [10], subcutaneous exenatide (synthetic exendin-4) rapidly lowered both fasting and postprandial plasma glucose in patients with type 2 diabetes. The glucose-dependent insulinotropism exhibited by exenatide was best illustrated by the data obtained in the fasting state, where there was a dose-dependent rise in serum insulin concentrations within the first 3 h after exenatide administration compared to placebo ($p < 0.001$). In sharp contrast, placebo treatment resulted in relatively stable insulin concentrations throughout the 8-h period of observation. The rise and peak of serum insulin concentrations following exenatide administration coincided with the rapid decline of fasting glucose concentrations. After 3- to 4-h postdose, and coincident with reaching glucose nadir, mean serum insulin returned to baseline with little difference among groups. Insulin $AUC_{(0-8\ h)}$ and C_{max} values for all exenatide treatments increased in an apparently dose-dependent manner compared to placebo ($p < 0.05$). Since exenatide concentrations remained elevated throughout the course of the assessment, the reduction in insulin beyond 3 h did not reflect a simple waning of exenatide effect due to lower circulating concentrations of exenatide.

Fineman et al. [48] used the homeostasis model assessment (HOMA) [54] methodology to assess β-cell function at baseline and at Days 14 and 28 in human subjects with

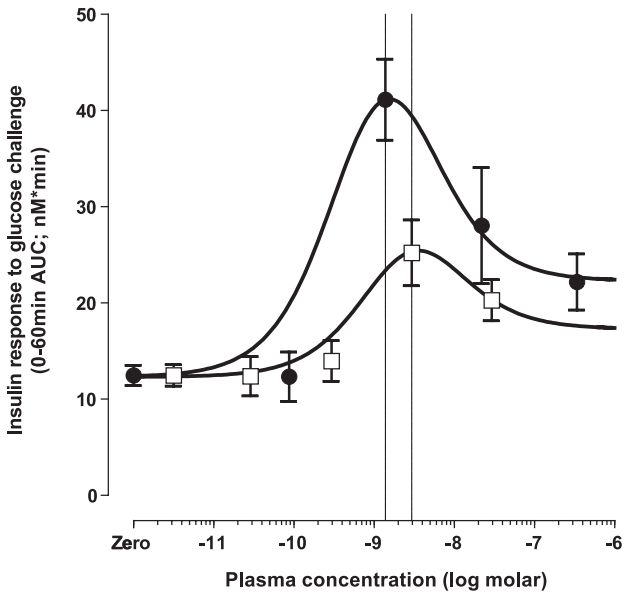

Fig. 4. Insulinotropic actions of exendin-4 and GLP-1: Concentration response. Male normoglycemic Harlan–Sprague–Dawley rats were anesthetized, then given a 2-h intravenous infusion of exendin-4 (3, 30, 300, or 3000 pmol/kg/min; $n = 4–8$ per dose), or GLP-1 (3, 30, 300, or 3000 pmol/kg/min; $n = 4–8$ per dose), or vehicle (1 ml/h, $n = 8$) at $t = -30$ min. At $t = 0$ min, D-glucose (5.7 mmol/kg) was infused at a rate of 0.5 ml/min over 1–2 min. This infusion regimen resulted in identical plasma glucose pharmacokinetic profiles in all treatment groups (see Ref. [9]). Plasma insulin was measured at −30, −15, 0, 5, 10, 15, 20, 30, 40, 50, 60, and 90 min. Peak insulinotropic effects of exendin-4 and GLP-1 occurred at a similar steady-state plasma concentration of approximately 1 to 2 nmol/l; thus, insulinotropic potencies were similar. However, the magnitude of the maximal effect was 63% greater with exendin-4 than with GLP-1 ($p < 0.03$). Exendin-4, black circles. GLP-1, white rectangles. (Reprinted from Ref. [9]).

type 2 diabetes receiving two or three daily subcutaneous injections of exenatide (0.08 μg/kg). HOMA analysis revealed improved β-cell secretory function following exenatide therapy. It is noteworthy that fasting values of plasma glucose and insulin, which were used to calculate HOMA, were obtained prior to the morning dose of exenatide when plasma concentrations of exenatide were negligible, suggesting a fundamental alteration in β-cell function following sustained exenatide exposure. Whether or not exenatide treatment increases pancreatic islet mass in humans awaits future studies, since β-cell function can improve independently of increased islet mass.

5. Glucagon secretion

Circulating glucagon was reduced after exenatide treatment in both the fasting and postprandial states in humans with type 2 diabetes [10]. The observation under fasting conditions supports the hypothesis that suppression of glucagon secretion is not merely a consequence of the slowing of nutrient presentation to the small intestine (gastric emptying). Given the well-documented elevations in fasting and postprandial glucagon levels in patients with type 2 diabetes [55] and the known activity of glucagon with respect to maintaining hepatic glucose output [56], it is reasonable to extrapolate that glucagon suppression by exenatide contributed to the overall effect of lowered plasma glucose in both the fasting and postprandial periods. Like insulin, glucagon concentrations also returned towards baseline beyond 3-h post-injection during the fasting state when the prevailing plasma glucose concentration reached its nadir. As the changes in concentrations of insulin and glucagon are coincident and reciprocal, the effects of exenatide on the enhancement of insulin secretion and the suppression of glucagon secretion may be considered glucose-dependent. It is noteworthy that currently available insulin secretagogues, as well as exogenous insulin, do not suppress the paradoxical postprandial glucagon rise observed in patients with diabetes [57]. This inappropriate elevation in postprandial plasma glucagon concentrations results in an inappropriately low insulin-to-glucagon ratio in the hepatic portal vein, contributing to sustained rates of excess hepatic glucose production [58]. Thus, by virtue of its ability to enhance endogenous insulin and lower glucagon secretion, exenatide would tend to re-establish a more physiologic and favorable portal vein ratio of insulin-to-glucagon compared to currently available agents.

6. Enhanced β-cell mass

One of the most provocative areas of recent research is based on observations that exendin-4 may improve β-cell health, thereby impeding or even reversing disease progression. A number of published reports support the concept that exendin-4 and GLP-1 increase β-cell mass via stimulation of β-cell neogenesis, stimulation of β-cell proliferation, and suppression of β-cell apoptosis (Table 1).

The development of the endocrine pancreas is under multifactorial control, and many of the key proteins or transcription factors involved in each developmental step have now been identified [59]. Expression of the transcription factor PDX-1/IDX-1 is essential for development and regeneration of the endocrine pancreas, and stimulation of PDX-1 may be one of the intracellular mechanisms by which exendin-4 and GLP-1 can enhance β-cell mass leading to long-term improvement in glucose control. Exendin-4 and GLP-1 have been proposed to produce differentiation of pancreatic non-β-cells including pancreatic ductal epithelial cells, acinar cells, and nestin-positive peri-ductal cells (potentially "functional" islet stem cells), into immunohistologically identifiable insulin-producing β-cell buds and subsequently, fully functional islets. In vitro studies in human and animal cell preparations support this hypothesis, and give insight into the molecular pathways mediating these changes.

Multipotential progenitor cells in the pancreatic islets and ducts, so-called nestin-positive islet-derived progenitor cells

Table 1
Effects on pancreatic β-cells

Model	Agent	Key findings	Reference
Nestin-positive islet-derived progenitor cells	Exendin-4, GLP-1	Stimulated differentiation of progenitor cells into insulin-producing cells.	[60]
AR42J pancreatic acinar cell line	Exendin-4, GLP-1	Stimulated differentiation into cells expressing insulin, pancreatic polypeptide, and glucagon.	[61]
Partially pancreatectomized rats	Exendin-4	Attenuated the development of diabetes; increased β-cell mass via differentiation (neogenesis) and replication.	[17]
Streptozotocin-treated Wistar rat	Exendin-4, GLP-1	Increased pancreatic insulin content, β-cell mass, and β-cell proliferation; decreased plasma glucose.	[18]
Goto–Kakizaki rat	Exendin-4, GLP-1	Increased pancreatic insulin content and β-cell mass; decreased plasma glucose.	[19]
Intrauterine growth-retarded neonatal Sprague–Dawley rats	Exendin-4	Increased β-cell mass and proliferation; increased Pdx-1 expression; decreased plasma glucose.	[66]
C57Bl/6 mouse, diabetic *db/db* mouse	Exendin-4, GLP-1	Increased β-cell mass, β-cell neogenesis, and Pdx-1 expression; increased insulin secretion; decreased plasma glucose; lower A1C; improved oral glucose tolerance.	[62]
Streptozotocin-treated C57Bl/6 mouse, BHK-GLP-1-R cells	Exendin-4	Increased β-cell mass; decreased β-cell apoptosis; increased plasma insulin; decreased plasma glucose; improved oral glucose tolerance; decreased cell apoptosis in vitro.	[63]
Diabetic *db/db* mouse	Exendin-4, tNN2211	Increased β-cell mass and proliferation; decreased plasma glucose.	[64]
Diabetic *db/db* mouse	Exendin-4	Increased pancreatic insulin content and β-cell mass; decreased β-cell apoptosis; improved hyperinsulinemia; enhanced glucose-stimulated insulin secretion; improved oral glucose tolerance; decreased plasma glucose.	[65]

(NIPs), have been previously identified by Habener et al. In a recent study, they now report the expression of functional GLP-1 receptors on these progenitor cells, and show that GLP-1 stimulates the differentiation of NIPs into insulin-producing cells [60]. Co-incubation of these cells with the GLP-1 receptor antagonist exendin-4(9–39) abolished the appearance of immunohistochemically detectable insulin, lending support to the idea of a specific GLP-1 receptor-mediated mechanism. Furthermore, confluent NIP cultures were shown to express the pro-glucagon gene and secrete GLP-1. Treatment of these cells with exendin-4 produced a two- to three-fold increase in insulin secretion. These findings suggest a model of islet development in which pancreatic progenitor cells express both GLP-1 receptors and pro-glucagon (subsequently processed into GLP-1). Locally produced GLP-1 may act as an autocrine/paracrine developmental morphogen on receptors on NIPs, resulting in the activation of PDX-1/IDX-1 and the expression of the proinsulin gene, conferring a β-cell phenotype. GLP-1 may thus be an important morphogen both for the embryonic development of the pancreas and for the neogenesis of β-cells in the islets of the adult pancreas.

In their basal state, AR42J cells, which are derived from a chemically induced pancreatic tumor, possess exocrine and neuroendocrine properties, but are negative for islet hormones and their mRNAs. Zhou [61] reported that both exendin-4 and GLP-1 can induce AR42J cells to differentiate into cells positive for insulin, pancreatic polypeptide, and glucagon. When these cells were exposed to GLP-1, intracellular cAMP levels and cell proliferation increased over the first 24 h, up to 50% of the cells became positive for islet hormones, and insulin was detectable in the cell culture medium in response to glucose. The mRNAs for GLUT 2 and glucokinase were also detected in GLP-1-treated cells. Exendin-4 caused the same cellular changes as GLP-1, albeit with a 10-fold higher potency. Hence, exendin-4 and GLP-1 can induce pancreatic precursor cells to differentiate into insulin and glucagon-secreting islet cells in vitro.

A number of studies have examined the abilities of exendin-4, GLP-1, or the GLP-1 analogue NN2211 to increase pancreatic β-cell mass in vivo [17–19,62–66]. In rats, mature β-cells have a lifespan of approximately 48–56 days. Expansion of β-cell mass can result from an increase in cell size without cell division (hypertrophy), an increase in cell number (hyperplasia due to mitosis of pre-existing β-cells and/or neogenesis from ductal stem cells), a reduction in β-cell apoptosis, or any combination of these mechanisms. In a partial pancreatectomy (90–95% Px) rat model of type 2 diabetes, the daily administration of exendin-4 at 1 nmol/kg IP for 10-day post-pancreatectomy attenuated the development of diabetes [17]. In exendin-4-treated Px rats, glucose levels were maintained at levels 40% lower than untreated rats for the entire 2.5-week observation period following the cessation of treatment, independent of changes in body weight. Notably, exendin-4 stimulated pancreatic regeneration, as assessed by a 40% expansion of β-cell mass in Px rats, and pancreatic cell proliferation, as assessed by a 40% expansion of β-cell mass in sham-operated rats with no increase in cell size.

The Goto–Kakizaki (GK) rat is a genetic model of type 2 diabetes in which a deficiency in neonatal β-cell mass is

considered the primary defect leading to nonfasted hyperglycemia, first detectable 3 weeks after birth. Tourrel et al. [19] investigated the effects of exendin-4 and GLP-1 on diabetes in GK female rats. Neonatal rats were treated with exendin-4 (3 μg/kg/day SC) or GLP-1 (400 μg/kg/day SC) during the pre-diabetic period on days 2 to 6 after birth. Exendin-4 and GLP-1 both enhanced pancreatic insulin content and total β-cell mass on day 7 as compared to untreated GK rats. In addition, at 2 months of age, rats treated with exendin-4 or GLP-1 still had significantly decreased basal plasma glucose levels as compared to untreated GK rats.

Neonatal Wistar rats injected with streptozotocin (STZ) at birth to induce β-cell destruction were used to examine whether early exposure to exendin-4 or GLP-1 could promote β-cell regeneration and consequently improve islet function in adults [18]. STZ-treated rats received daily IP injections of exendin-4 (3 μg/kg) or GLP-1 (400 μg/kg) from days 2 to 6 postnatal. On day 7, both agents decreased basal plasma glucose and increased pancreatic insulin content. In STZ/exendin-4 rats, β-cell mass was 71% of β-cell mass in healthy Wistar rats, while the comparable value in STZ/GLP-1 rats was 51%, versus only 21% in untreated STZ rats. At 2 months of age, STZ rats treated with either exendin-4 or GLP-1 had plasma glucose levels comparable to healthy Wistar rats, while untreated STZ rats had glucose concentrations 41% higher. Fasting pancreatic insulin content on a per gram basis was 39% and 33% higher after exendin-4 or GLP-1 treatments, respectively. The corresponding increases in β-cell mass on a per gram basis were 56% and 28%, respectively, and were attributable to cell proliferation, not hypertrophy. Exendin-4 or GLP-1 also enhanced plasma glucose clearance rates compared with untreated STZ rats. These findings in the neonatal-STZ model indicate that exendin-4 and GLP-1 can exert both short- and long-term beneficial effects on β-cells and glucose homeostasis.

In separate experiments using neonatal Sprague–Dawley rats, Stoffers et al. [66] induced intrauterine growth retardation (IUGR) to produce neonatal rats with deficient islet cell function. Groups of normal or IUGR rats were treated with daily doses of exendin-4 (1 nmol/kg) or vehicle on postnatal days 1–6. By 2 weeks postnatal, IUGR rats had impaired glucose tolerance which steadily worsened over time. In sharp contrast, the glucose tolerance results for 2-week-old IUGR rats treated with exendin-4 were no different from the test results for normal rats, and this effect persisted through at least 3 months postnatal. Fasting blood glucose concentrations in IUGF rats previously treated with exendin-4 averaged 65% lower than vehicle-treated IUGF rats at both 3 and 8 months of age, and were indistinguishable from fasting blood glucose concentrations in normal rats. The progressive deterioration in glycemic control in vehicle-treated IUGR rats was accompanied by a progressive decline in the mass of pancreatic β-cells. At 2 weeks postnatal, β-cell mass was normal in all groups, but by 3 months postnatal, the vehicle-treated IUGF rats had a pronounced reduction (approximately 80%) in β-cell mass. In contrast, IUGF rats treated with exendin-4 postnatally retained normal β-cell mass. This effect seemed to be due to decreased β-cell proliferation in vehicle-treated IUGF rats with no change in the β-cell apoptotic rate, and exendin-4 increased the rate of β-cell proliferation in IUGF rats back to normal. This was reflected in Pdx-1 expression rates near normal in exendin-4-treated IUGF rats compared to 60% lower levels of Pdx-1 mRNA in vehicle-treated IUGF rats at 2 weeks postnatal.

Three independent reports described experiments using the *db*/*db* mouse model of type 2 diabetes to assess β-cell mass. In Rolin et al. [64], the effects of exendin-4 and the GLP-1 analogue, NN2211, on blood glucose homeostasis and β-cell mass were examined. Female diabetic *db*/*db* mice were injected subcutaneously with exendin-4 (100 μg/kg), NN2211 (200 μg/kg), or vehicle, twice daily for 15 days. Exendin-4 and NN2211 each lowered blood glucose significantly when measured on days 1 and 15. By day 16, β-cell proliferation had significantly increased in both treatment groups compared to vehicle control with a corresponding increase in total β-cell mass of 30–60% as compared to vehicle-treated mice.

In Wang et al. [65] using the *db*/*db* mouse model, 6-week-old mice were treated for 2 weeks with daily intraperitoneal injections of vehicle or exendin-4 (1 nmol/kg). Fasting blood glucose (FBG) was normal at the initiation of treatment, but by 8 weeks of age vehicle-control mice were diabetic (FBG: 10.4 ± 0.5 mmol/l). In sharp contrast, 8-week-old mice previously treated with exendin-4 had significantly reduced FBG (6.1 ± 1.0 mmol/l; $p < 0.001$). Oral glucose tolerance tests demonstrated that exendin-4 significantly improved glucose tolerance, although insulin sensitivity tests showed no difference from vehicle-control mice. Fasting plasma insulin levels in vehicle-control mice were 81% higher than those of exendin-4-treated mice, consistent with hyperinsulinemia due to hyperglycemia. In addition, pancreatic insulin content was 38% lower in vehicle-control mice than in mice treated with exendin-4, and oral glucose tolerance tests demonstrated increased release of insulin in exendin-4 mice with a corresponding 1.4-fold increase in β-cell mass.

Stoffers et al. [62] investigated the activities of 2 weeks of once-daily intraperitoneal injections of exendin-4 (1 nmol/kg) on glycemic control in healthy C57Bl/6 and diabetic *db*/*db* mice. Exendin-4 lowered blood glucose, stimulated insulin secretion six-fold, and lowered A1C levels 20–45% versus both non-diabetic and diabetic animals. The investigators also studied the effects of 2 weeks of intraperitoneal injection of exendin-4 (100 pmol/kg) or continuous intraperitoneal infusion of GLP-1 (1.5 pmol/kg/min) on expression of the transcription factor IDX-1/PDX-1 in normoglycemic C57Bl/6 mice. Treatment with either exendin-4 or GLP-1 increased pancreatic expression of IDX-1/PDX-1, suggesting enhanced pancreatic β-cell

function. In addition, exendin-4 enhanced β-cell neogenesis as assessed by a 1.7-fold increase in islet cross-sectional area and increased immunohistochemical signal from insulin-producing cells as compared to vehicle-treated mice.

Li et al. [63] examined whether GLP-1R activation could inhibit β-cell apoptosis. Healthy adult C57Bl/6 mice were treated with low-dose STZ for 5 days to induce β-cell apoptosis. Daily intraperitoneal administration of exendin-4 (24 nmol/kg) commencing 2 days prior to STZ and continuing until 3 days after the end of STZ dosing lowered prevailing plasma glucose levels from days 9 to 29 compared to mice treated with STZ alone. When measured on day 30, the exendin-4-treated mice also had higher basal (fed) plasma insulin concentrations than mice treated with STZ alone, and these beneficial antidiabetic effects of exendin-4 treatment were still evident on day 55. In a separate experiment, C57Bl6 mice were treated with exendin-4 starting 7 days prior to STZ and continuing until day 28. On day 30, fasted STZ plus exendin-4 mice given an oral glucose tolerance test had enhanced glycemic control compared to STZ-mice. On day 55, these mice had comparable plasma insulin concentrations in the fed state to healthy mice administered a single dose of exendin-4. In healthy C57Bl/6 mice, some injected with exendin-4 and some not, pancreatic β-cells were rarely apoptotic. STZ-treatment dramatically increased the incidence of apoptotic β-cells, and this effect was greatly ameliorated by exendin-4 (approximately 76% decrease in apoptotic cells per islet). To further verify the anti-apoptotic activity of exendin-4, FACS-purified rat β-cells were incubated in vitro in the presence of pro-apoptotic cytokines with or without concomitant exendin-4. In this system, exendin-4 reduced the incidence of apoptosis by an average of 44%.

In summary, the published preclinical scientific literature examining both in vitro and in vivo actions of exogenously administered exendin-4 and GLP-1 provides strong evidence that these peptide can play an important role in the maintenance of β-cell mass and function through complementary actions to stimulate islet cell proliferation and neogenesis, and to inhibit islet cell apoptosis. Evidence suggests that this effect is mediated via the GLP-1 receptor, as recent studies in mice lacking a functional GLP-1 receptor demonstrate that these mice do not exhibit β-cell regeneration following pancreatectomy and exogenous exendin-4 treatment [67].

7. Gastric emptying

The importance of GLP-1 on gastric emptying and postprandial plasma glucose control was recognized when Dupre et al. [68] described the postprandial benefits of GLP-1 in insulin-deficient patients. The importance of gastric emptying on glycemic excursions has also been affirmed in insulin-replete subjects, and some authors propose that the gastric emptying effect predominates in post-prandial glucose control (reviewed in Refs. [2,69]). Delivery of nutrients from the stomach to the small intestine is a critical contributor to postprandial glucose excursions [70]. Indeed, non-diabetic patients who have undergone gastrectomy exhibit postprandial hyperglycemia in spite of apparently normal β-cell function and fasting euglycemia [71]. Whether gastric emptying rates are slow, normal, or accelerated in patients with diabetes without severe autonomic neuropathy is controversial [14]. One of the main confounders in understanding the pathophysiology of gastric emptying in diabetes is hyperglycemia itself, as elevated glucose concentrations in non-diabetics slow the gastric emptying rate [14]. Thus, a "normal" gastric emptying rate in the face of hyperglycemia may be considered pathophysiologic, as it is relatively accelerated compared to an expected normal slowing in the face of hyperglycemia. Meal entrained, endogenous entero-pancreatic hormones such as CCK, GLP-1, PYY, and amylin slow gastric emptying, attesting to the importance of this function for overall nutrient assimilation [72]. In addition, continuous subcutaneous infusion of GLP-1 for 6 weeks slowed gastric emptying in humans with type 2 diabetes [46], and a GLP-1 analogue slowed postprandial gastric emptying in β-cell-reduced pigs [45]. Exendin-4 also slowed gastric emptying in humans with type 2 diabetes [10].

8. Nutrient intake and body weight

Exendin-4 treatment has been associated with reduced food intake and weight loss in experiments in diabetic fatty Zucker (ZDF) rats. Young et al. [11] reported that administration of exendin-4 twice daily (0.1, 1, 10, or 100 μg) for 6 weeks resulted in a dose-dependent reduction in food intake ranging from 13% to 30%, with an exendin-4 ED_{50} value of 0.14 μg. The corresponding change in body weight exhibited an ED_{50} value of 0.42 μg exendin-4, with a maximum loss of 27 g (5.6% decrease) at the highest exendin-4 dose administered. In experiments reported by Szayna et al. [16], obese diabetic Zucker rats were given once-daily injections of exendin-4 (10 μg/kg) or vehicle over 14 days. Exendin-4 administration was associated with an acute reduction in food intake and body weight compared to vehicle-control rats over the first 5 days of treatment. Subsequently, the same Zucker rats were switched to a dosing regimen of twice daily injections of exendin-4 (10 μg/kg) or vehicle for an additional 42 days. In this long-term dosing phase, there was a sustained reduction in food intake and body weight compared to the vehicle-control group. This was accompanied by reductions in A1C, fasting blood glucose, and fasting blood insulin. Interestingly, during the first 4 weeks of exendin-4 treatment, reduced fat deposition in the subcutaneous abdominal regions was the primary finding. However, by the end of 8 weeks of exendin-4 treatment, both

subcutaneous abdominal and visceral fat deposition were reduced. Consistent with these observations in animal models, exendin-4 acutely reduced food intake in healthy human subjects [73]. In other experiments, Stoffers et al. [66] induced intrauterine growth retardation (IUGR) to produce neonatal rats with deficient islet cell function. Groups of normal or IUGR rats were treated with daily doses of exendin-4 (1 nmol/kg) or vehicle on postnatal days 1–6. At 2 weeks postnatal, body weights in both normal and IUGR rats treated with exendin-4 were 20% lower than the corresponding groups of vehicle-treated rats, and this weight difference was still statistically significant at 3 months postnatal (8% and 12% lower, respectively).

9. Insulin sensitivity

The effects of exendin-4, GLP-1, and GLP-1 analogues on insulin-sensitivity in peripheral tissues are currently under active investigation. However, published reports on the effects of GLP-1 on insulin sensitivity have been inconsistent until recently, perhaps due to the route of GLP-1 administration in these studies (peripheral or central vein) and the confounding effects of GLP-1 on insulin secretion. Based on evidence that GLP-1 may act within the hepatoportal region to regulate glucose uptake in peripheral tissues, Nishizawa et al. [74] infused GLP-1 directly into the hepatic portal vein of fasted, conscious dogs under conditions of hyperinsulinemic, hyperglycemic clamp (with somatostatin and basal glucagon infusions) to mimic the postprandial state when most GLP-1 release occurs. Hyperglycemia combined with hyperinsulinemia switched net hepatic glucose balance from output to uptake in all GLP-1 and vehicle-control treatment groups. Only GLP-1 (not vehicle) acutely stimulated whole body glucose disposal under conditions of hyperglycemia where pancreatic hormone concentrations were fixed, and this effect was dependent on GLP-1 blood concentration. Infusion of GLP-1 at a rate which resulted in physiological levels of GLP-1 in the circulatory system did not cause enhancement of glucose uptake by either the liver or non-hepatic tissues. In contrast, when frankly pharmacological levels of GLP-1 were achieved, both hepatic and non-hepatic glucose uptakes increased compared to vehicle-control. At intermediate GLP-1 levels, non-hepatic glucose uptake was enhanced with no concomitant change in hepatic glucose uptake. These data demonstrate that there is the potential for GLP-1 to acutely affect glucose uptake in both hepatic and non-hepatic tissues.

Vella et al. [75] reported the results of a phase I study in which healthy human volunteers were enrolled in a single-blind, crossover trial involving intravenous (IV) infusion of exenatide at 0.12 pmol/kg/min, GLP-1 at 1.2 pmol/kg/min, or saline (placebo) for 7 h. Glucose was infused to clamp blood glucose concentrations at 5.3 mmol/l and insulin was infused to progressively increase insulin concentrations to approximately 65, then 190, and finally 700 pmol/l. Endogenous insulin secretion was inhibited with somatostatin infusion at 120 ng/kg/min, while glucagon and growth hormone were maintained at basal levels by infusion of 0.65 and 3 ng/kg/min, respectively. There were no changes in circulating levels of glucose, insulin, C-peptide, glucagon, or growth hormone in non-diabetic humans. These data support the conclusion that neither exenatide nor GLP-1 acutely enhance insulin activity in non-diabetic humans. Another study in humans with type 2 diabetes has reported that a 6-week infusion of GLP-1 improves insulin resistance [46]. Whether this action is mediated by a direct effect on insulin responsive tissues to enhance glucose uptake, or is secondary to improvement in glycemic control, reduced glucose toxicity, and/or reduced body weight remains to be elucidated [2].

10. Summary

In conclusion, exenatide (synthetic exendin-4), GLP-1, and GLP-1 analogues have actions that significantly improve glycemic control in animal models of type 2 diabetes. Evidence suggests that these agents achieve improvements in glycemia through a combination of mechanisms which include glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, enhancement of pancreatic islet health, slowing of gastric emptying, reduction of food intake, and modulation of insulin-sensitivity in peripheral tissues. One of the most provocative areas of recent research is based on observations that exenatide may improve β-cell mass, thereby impeding or even reversing disease progression. Exenatide appears to increase β-cell mass through stimulation of β-cell neogenesis, stimulation of β-cell proliferation, and suppression of β-cell apoptosis. Ongoing phase 3 clinical trials will reveal whether or not exenatide is proven safe and effective for the treatment of patients with type 2 diabetes.

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References

[1] Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue from *Heloderma suspectum* venom. J Biol Chem 1992;267:7402–5.

[2] Young AA. Glucagon-like peptide-1, exendin and insulin sensitivity. In: Hansen B, Shafrir E, editors. Insulin Resistance and Insulin Resistance Syndrome. New York: Harwood Academic; 2002. p. 235–62.

SUN EXHIBIT 1037, IPR2024-00107, PAGE 10

[3] Drucker DJ. Glucagon-like peptides. Diabetes 1998;47:159–69.
[4] Chen YE, Drucker D. Tissue-specific expression of unique mRNAs that encode pro-glucagon-derived peptides or exendin-4 in the lizard. J Biol Chem 1997;272:4108–15.
[5] Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 1995;136:3585–96.
[6] Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes 1995;44: 1126–31.
[7] Göke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, et al. Exendin-4 is a high potency agonist and truncated exendin-(9–39)-amide an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor of insulin-secreting β-cells. J Biol Chem 1993;268:19650–5.
[8] Egan JM, Clocquet AR, Elahi D. The insulinotropic effect of acute exendin-4 administered to humans: comparison of nondiabetic state to type 2 diabetes. J Clin Endocrinol Metab 2002;87:1282–90.
[9] Parkes DG, Pittner R, Jodka C, Smith P, Young A. Insulinotropic actions of exendin-4 and glucagon- like peptide-1 in vivo and in vitro. Metabolism 2001;50:583–9.
[10] Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, et al. Synthetic exendin-4 (AC2993) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab 2003;88:3082–9.
[11] Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B, et al. Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (*ob*/*ob*, *db*/*db*) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (*Macaca mulatta*). Diabetes 1999;48:1026–34.
[12] Parkes D, Gedulin S, Smith P, Young A. Effect of exendin-4 on glucagon secretion in lean and obese Zucker (ZDF) rats. Diabetes 2001;50(Suppl. 2):A313.
[13] Jodka C, Gedulin B, Young A. Exendin-4 potently regulates gastric emptying in rats. Diabetes 1998;47:403A [Abs].
[14] Rayner CK, Samsom M, Jones KL, Horowitz M. Relationships of upper gastrointestinal motor and sensory function with glycemic control. Diabetes Care 2001;24:371–81.
[15] Bhavsar S, Watkins J, Young A. Comparison of central and peripheral effects of exendin-4 and GLP- 1 on food intake in rats. Program and Abstracts: 80th Annual Meeting of the Endocrine Society 1998;24: 433 [Abs].
[16] Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RGS, Greig NH, et al. Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. Endocrinology 2000;141:1936–41.
[17] Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. Diabetes 1999;48:2270–6.
[18] Tourrel C, Bailbé D, Meile M-J, Kergoat M, Portha B. Glucagon-like peptide-1 and exendin-4 stimulate β-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes 2001;50:1562–70.
[19] Tourrel C, Bailbé D, Lacorne M, Meile MJ, Kergoat M, Portha B. Persistent improvement of type 2 diabetes in the Goto–Kakizaki rat model by expansion of the β-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. Diabetes 2002;51: 1443–52.
[20] Greig NH, Holloway HW, De Ore KA, Jani D, Wang Y, Zhou J, et al. Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. Diabetologia 1999;42:45–50.
[21] Parkes D, Jodka C, Smith P, Nayak S, Rinehart L, Gingerich R, et al. Pharmacokinetic actions of exendin-4 in the rat: comparison with glucagon-like peptide-1. Drug Dev Res 2001;53:260–7.
[22] American Diabetes Association. Postprandial blood glucose. Diabetes Care 2001;24:775–8.
[23] Kahn SE, Porte Jr D . Pathophysiology of type II diabetes mellitus. In: Porte D, Sherwin RS, editors. Diabetes Mellitus. 5th ed. Stamford, CT: Appleton & Lange; 1997. p. 487–512.
[24] Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 1999;104:787–94.
[25] Harris MI, Flegal KM, Eastman RC, Eberhardt MS, Cowie CC. Racial and ethnic differences in glycemic control of adults with type 2 diabetes. Diabetes Care 1999;22:403–8.
[26] UK Prospective Diabetes Study Group. Overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes 1995;44:1249–58.
[27] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood–glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in subjects with type 2 diabetes. Lancet 1998;352:837–53.
[28] Thorens B. Expression cloning of the pancreatic β-cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A 1992;89:8641–5.
[29] Raufman JP, Singh L, Singh G, Eng J. Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. J Biol Chem 1992;267:21432–7.
[30] Raufman JP, Singh L, Eng J. Exendin-3, a novel peptide from *Heloderma horridum* venom, interacts with vasoactive intestinal peptide receptors and a newly described receptor on dispersed acini from guinea pig pancreas. Description of exendin-3(9–39), a specific exendin receptor antagonist. J Biol Chem 1991;266:2897–902.
[31] Brubaker PL, Drucker DJ. Structure–function of the glucagon receptor family of G protein-coupled receptors: the glucagon, GIP, GLP-1, and GLP-2 receptors. Receptors Channels 2002;8:179–88.
[32] Raufman JP. Bioactive peptides from lizard venoms. Regul Pept 1996;61:1–18.
[33] Fehmann HC, Bode HP, Ebert T, Karl A, Goke B. Interaction of GLP-1 and leptin at rat pancreatic β-cells: effects on insulin secretion and signal transduction. Horm Metab Res 1997;29:572–6.
[34] Thorens B, Porret A, Bühler L, Deng SP, Morel P, Widmann C. Cloning and functional expression of the human islet GLP-1 receptor. Diabetes 1993;42:1678–82.
[35] Baggio L, Adatia F, Bock T, Brubaker PL, Drucker DJ. Sustained expression of exendin-4 does not perturb glucose homeostasis, β-cell mass, or food intake in metallothionein–preproexendin transgenic mice. J Biol Chem 2000;275:34471–7.
[36] Scrocchi LA, Marshall BA, Cook SM, Brubaker PL, Drucker DJ. Identification of glucagon-like peptide 1 (GLP-1) actions essential for glucose homeostasis in mice with disruption of GLP-1 receptor signaling. Diabetes 1998;47:632–9.
[37] Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatei M, et al. Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9–39. Diabetes 1999;48:86–93.
[38] Burcelin R, Da Costa A, Drucker D, Thorens B. Glucose competence of the heptoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. Diabetes 2001;50:1720–8.
[39] Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide receptor gene. Nat Med 1996;2:1254–8.
[40] MacLusky N, Cook S, Scrocchi L, Shin J, Kim J, Vaccarino F, et al. Neuroendocrine function in mice with complete disruption of GLP-1 receptor signaling. Endocrinology 2000;141:752–62.
[41] Pederson RA, Satkunarajah M, McIntosh CH, Scrocchi LA, Flamez D, Schuit F, et al. Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinotropic action in glucagon-like peptide 1 receptor −/− mice. Diabetes 1998;47:1046–52.
[42] Nishizawa M, Nakabayashi H, Kawai K, Ito T, Kawakami S, Naka-

a RA, et al. The hepatic vagal reception of intraportal GLP-1 is via receptor different from the pancreatic GLP-1 receptor. J Auton Nerv Syst 2000;80:14–21.

[43] Idris I, Patiag D, Gray S, Donelly R. Exendin-4 increases insulin sensitivity via a PI-3-kinase-dependent mechanism: contrasting effects of GLP-1. Biochem Pharmacol 2002;63:993–6.

[44] Holst JJ. Therapy of type 2 diabetes mellitus based on the action of glucagon-like peptide-1. Diabetes Metab Res Rev 2002;18:430–41.

[45] Ribel U, Larsen MO, Rolin B, Carr RD, Wilken M, Sturis J, et al. NN2211: a long-acting glucagon-like peptide-1 derivative with anti-diabetic effects in glucose-intolerant pigs. Eur J Pharmacol 2002; 451:217–25.

[46] Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. Lancet 2002;359:824–30.

[47] Nielsen LL, Baron AD. Pharmacology of exenatide (synthetic exendin-4) for the treatment of type 2 diabetes. Curr Opin Invest Drugs 2003;4:401–5.

[48] Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, et al. Effect on glycemic control of synthetic exendin-4 (AC2993) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. Physiol Behav, Diabetes Care 2003;26:2370–7.

[49] Egan JM, Meneilly GS, Elahi D. Effects of one month bolus subcutaneous administration of exendin-4 in type 2 diabetes. Am J Physiol, Endocrinol Metab 2003;284:E1072–9.

[50] Henquin J-C. Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 2000;49:1751–60.

[51] Siegel EG, Schulze A, Schmidt WE, Creutzfeldt W. Comparison of the effect of GIP and GLP-1(7–36 amide) on insulin release from rat pancreatic islets. Eur J Clin Invest 1992;22:154–7.

[52] Zawalich WS, Zawalich KC, Rasmussen H. Influence of glucagon-like peptide-1 on beta cell responsiveness. Regul Pept 1993;44: 277–83.

[53] Heller RS, Apnte GW. Intra-islet regulation of hormone secretion by glucagon-like peptide-1(7–36)amide. Am J Physiol 1995;32: G852–60.

[54] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RL. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

[55] Reaven GM, Chen YD, Golay A, Swislocki AL, Jaspan JB. Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1987;64:106–10.

[56] Baron AD, Schaeffer L, Shragg P, Kolterman OG. Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. Diabetes 1987;36:274–83.

[57] Mahler RJ, Adler ML. Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. J Clin Endocrinol Metab 1999;84: 1165–71.

[58] Moore MC, Cherrington AD. Regulation of net hepatic glucose uptake: interaction of neural and pancreatic mechanisms. Reprod Nutr Dev 1996;36:399–406.

[59] Soria B. In-vitro differentiation of pancreatic β-cells. Differentiation 2001;68:205–19.

[60] Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF. Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. Endocrinology 2002;143:3152–61.

[61] Zhou J, Wang X, Pineyro MA, Egan JM. Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. Diabetes 1999;48:2358–66.

[62] Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, et al. Insulinotrophic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in the mouse pancreas. Diabetes 2000;49:741–8.

[63] Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. J Biol Chem 2003;278:471–8.

[64] Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, et al. The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increase beta-cell mass in diabetic mice. Am J Physiol, Endocrinol Metab 2002;283:E745–52.

[65] Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old *db*/*db* mice. Diabetologia 2002;45: 1263–73.

[66] Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. Diabetes 2003;52:734–40.

[67] De León DD, Deng S, Madani R, Ahima RS, Drucker DJ, Stoffers DA. Role of endogenous glucagon-like peptide-1 in islet regeneration after partial pancreatectomy. Diabetes 2003;52:365–71.

[68] Dupre J, Behme MT, Hramiak IM, McDonald TJ. Subcutaneous glucagon-like peptide-1 combined with insulin normalized postcibal glycemic excursions in IDDM. Diabetes Care 1997;20:381–4.

[69] Nauck MA, Niedereich-Holz U, Ettler R, Holst JJ, Orskov C, Ritzel R, et al. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 1997;273:E981–8.

[70] Green GM, Guan D, Schwartz JG, Phillips WT. Accelerated gastric emptying of glucose in Zucker type 2 diabetic rats: role in postprandial hyperglycaemia. Diabetologia 1997;40:136–42.

[71] Harju E. Nordback I: postprandial hyperglycemia after different carbohydrates in patients with total gastrectomy. Surg Gynecol Obstet 1987;165:41–5.

[72] Moyses C, Young A, Kolterman O. Modulation of gastric emptying as a therapeutic approach to glycaemic control. Diabetic Med 1996;13: S34–8.

[73] Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, et al. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am J Physiol, Endocrinol Metab 2001;281:E155–61.

[74] Nishizawa M, Moore MC, Shiota M, Gustavson SM, Snead WL, Neal DW, et al. Effect of intraportal glucagon-like peptide-1 on glucose metabolism in conscious dogs. Am J Physiol, Endocrinol Metab 2003;284:E1027–36.

[75] Vella A, Shah P, Reed AS, Adkins AS, Basu R, Rizza RA. Lack of effect of exendin-4 and glucagon-like peptide-1-(7,36)-amide on insulin action in non-diabetic humans. Diabetologia 2002;45:1410–5.