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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.

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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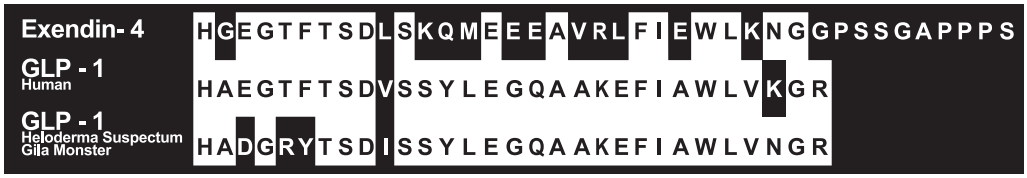

Fig. 1. Comparison of amino acid sequences for exendin-4, mammalian GLP-1, and Gila monster GLP-1.

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

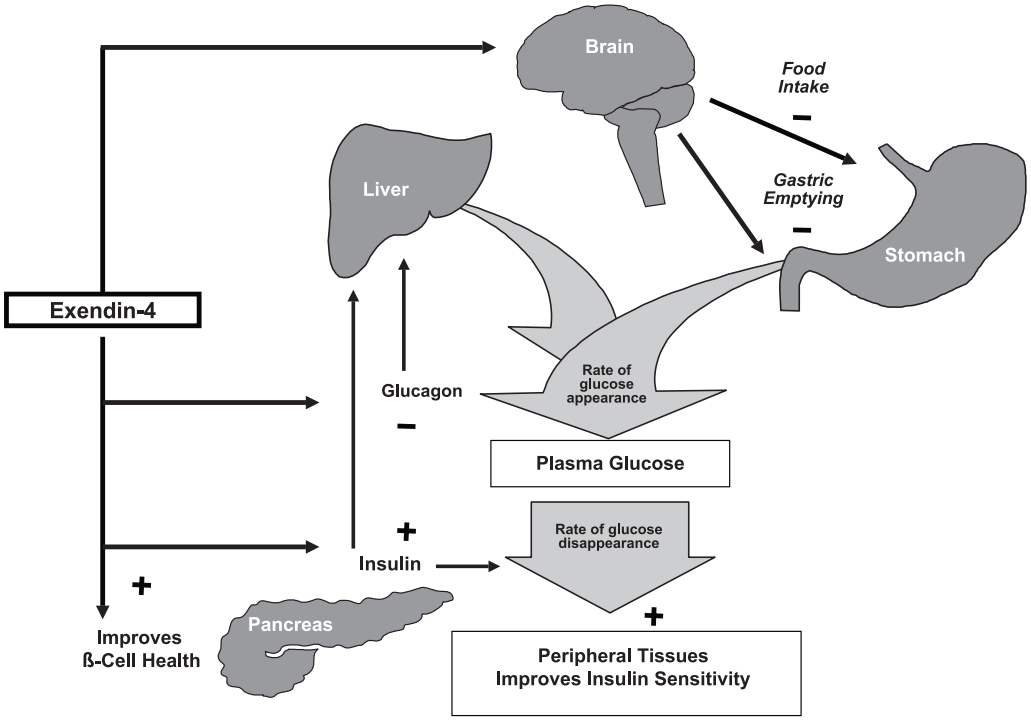

Fig. 2. Theoretical overview of the primary, anti-diabetic mechanisms of action for exendin-4 (or exenatide). Data suggest that exendin-4 enhances glycemic control by enhancing glucose-dependent insulin secretion, suppressing glucose-dependent glucagon secretion, slowing gastric emptying, reducing food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

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

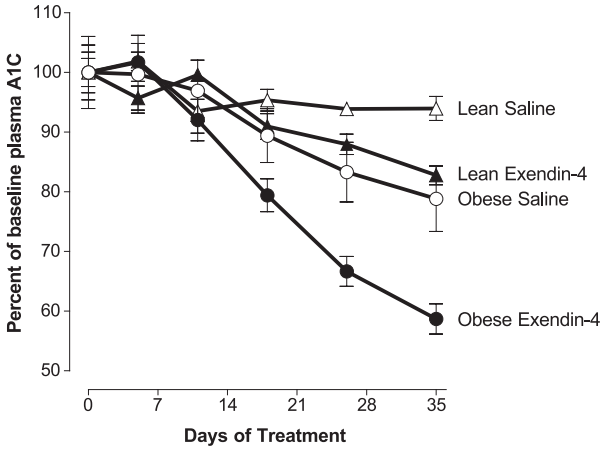

Fig. 3. Plasma A1C in diabetic and normoglycemic rats. Long-term administration of exendin-4 reduced plasma A1C in diabetic, obese ZDF rats and normoglycemic lean littermate control rats ($p < 0.01$ for obese rats and $p < 0.002$ for lean rats, respectively, compared with vehicle-treated lean rats). 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

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