Long-term metabolic benefits of exenatide in mice are mediated solely via the known glucagon-like peptide 1 receptor

Krystyna Tatarkiewicz, Emmanuel J. Sablan, Clara J. Polizzi, Christiane Villessac, and David G. Parkes


Tatarkiewicz K, Sablan EJ, Polizzi CJ, Villessac C, Parkes DG. Long-term metabolic benefits of exenatide in mice are mediated solely via the known glucagon-like peptide 1 receptor. Am J Physiol Regul Integr Comp Physiol 306: R490–R498, 2014. First published January 29, 2014; doi:10.1152/ajpregu.00495.2013.—Glucagon-like peptide 1 receptors (GLP-1R) are expressed in multiple tissues and activation results in metabolic benefits including enhanced insulin secretion, slowed gastric emptying, suppressed food intake, and improved hepatic steatosis. Limited and inconclusive knowledge exists regarding whether the effects of chronic exposure to a GLP-1R agonist are solely mediated via this receptor. Therefore, we examined 3-mo dosing of exenatide in mice lacking a functional GLP-1R (Glp1r−/−). Exenatide (30 nmol·kg−1·day−1) was infused subcutaneously for 12 wk in Glp1r−/− and wild-type (Glp1r+/+) control mice fed a high-fat diet. Glycated hemoglobin A1c (HbA1c), plasma glucose, insulin, amylase, lipase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), body weight, food intake, terminal hepatic lipid content (HLC), and plasma exenatide levels were measured. At the end of the study, oral glucose tolerance test (OGTT) and rate of gastric emptying were assessed. Exenatide produced no significant changes in Glp1r−/− mice at study end. In contrast, exenatide decreased body weight, food intake, and glucose in Glp1r+/+ mice. When compared with vehicle, exenatide reduced insulin, OGTT glucose AUC0–2h, alanine transaminase (ALT), and HLC in Glp1r+/+ mice. Exenatide had no effect on plasma amylase or lipase levels. Exenatide concentrations were approximately eightfold higher in Glp1r−/− versus Glp1r+/+ mice after 12 wk of infusion, whereas renal function was similar. These data support the concept that exenatide requires a functional GLP-1R to exert chronic metabolic effects in mice, and that novel “GLP-1” receptors may not substantially contribute to these changes. Differential exenatide plasma levels in Glp1r−/− versus Glp1r+/+ mice suggest that GLP-1R may play an important role in plasma clearance of exenatide and potentially other GLP-1-related peptides.

GLP-1 receptor knockout; mice; clearance

TWO FORMS OF INCRETIN-BASED THERAPIES have emerged within recent years as new treatments for Type 2 diabetes mellitus: glucagon-like peptide 1 (GLP-1) receptor agonists and dipeptidyl peptidase 4 (DPP-4) inhibitors, which prevent degradation of endogenous GLP-1. GLP-1 is a gut incretin hormone secreted in response to nutrient ingestion, and its pleiotropic physiological effects are mediated by the well-characterized GLP-1 receptor (GLP-1R), which is expressed in multiple peripheral tissues and in the central nervous system (see reviews in Refs. 7 and 19). Activation of the GLP-1R results in metabolic benefits including enhanced insulin secretion (20, 22, 31), preservation of pancreatic β-cells (5, 11, 56), slowing of gastric emptying (GE) (16, 51), glucagon suppression (9, 17), decreased food intake (28, 46), and improved hepatic steatosis (10). The direct and indirect mechanisms underlying these effects have not been fully elucidated, and there is still limited and controversial knowledge regarding GLP-1R distribution in different tissues and different species (33).

Several publications have implied that certain effects of full-length GLP-1 (7–36 amide, its truncated versions (metabolites) or synthetic GLP-1 analogs, may not be mediated exclusively by binding and activation of the known pancreatic GLP-1R. For instance, multiple cardioprotective effects of GLP-1 were demonstrated, including increased functional recovery and cardiomyocyte viability after ischemia-reperfusion injury of isolated hearts and arteries from normal wild-type mice. Unexpectedly, these actions of GLP-1 were preserved in Glp1r−/− mice, suggesting that cardioprotective effects of GLP-1 in ischemic mouse hearts were mediated through both GLP-1R-dependent and -independent pathways (3). In a second study, intraportal injection of GLP-1 significantly facilitated the afferent impulse discharge rate of the hepatic vagus nerve in rats; however, injections of the highly potent GLP-1R agonist exendin-4 did not facilitate these afferent impulses. The authors suggest that the neural receptor of GLP-1 involves a receptor mechanism distinct from that mediating its well-known insulinotropic action (32). Additionally, GLP-1 actions in isolated myocytes, reported as stimulation of glyco- gen synthesis, were not accompanied by significant induction of cellular cAMP, which is a well-documented signaling pathway for the pancreatic GLP-1R (27, 57). Similarly, in adipocytes, GLP-1 increased insulin-mediated glucose uptake and lipid synthesis along with a decrease in intracellular cAMP. A study in isolated rat hepatocytes suggested that GLP-1 activates glycogen synthase—a via receptor mechanisms that are distinct from insulinotropic actions mediated via GLP-1R in pancreatic β-cells (42). Moreover, exenatide (synthetic exen-4), but not native GLP-1, decreased ghrelin levels in fasted rats (40) and the central anorexic effects of exenatide differed from those of GLP-1 (4). Mechanisms contributing to these differential pharmacological actions potentially go beyond the improved pharmacokinetic properties of DPP-4-resistant GLP-1R agonists. Together, these observations suggest the existence of an additional novel receptor signaling pathway complementing the insulinotropic actions of GLP-1R agonists in β-cells, and the search for a second potential GLP-1R continues (52, 54).

The present study aimed to determine whether the long-term metabolic benefits of exenatide are exclusively mediated via activation of the known GLP-1R. To assess this hypothesis, exenatide was continuously infused at a supramaximal dose for 3 mo in mice lacking a functional GLP-1R. These mice exhibit normal basal glucose metabolism, feeding behavior, and body weight, together with relatively mild glucose intolerance and impaired insulin secretion (2, 41, 47). Hence, in the present...
study, mice were fed a high-fat diet to exacerbate their metabolic phenotype, allowing a greater dynamic range for observing exenatide-mediated pharmacological actions.

MATERIALS AND METHODS

Animals. All procedures were conducted in accordance with Animal Welfare Act guidelines and approved by the Institutional Animal Care and Use Committee at Amylin Pharmaceuticals. Male (7–13 wk old) GLP-1R knockout (Glp1r−/−) and wild-type control mice (Glp1r+/+) on the C57Bl/6 background were used (Dr. Daniel J. Drucker, University of Toronto). Animals were fed standard chow (7012 Teklad, Harlan Laboratories, Indianapolis, IN) and housed individually at 21–24°C with a 12-h light/dark cycle with ad libitum access to food and water. Six weeks before treatment with exenatide, the feed was replaced with a high-fat diet (58 kcal% from fat, D12331 Research Diet, New Brunswick, NJ). Glp1r−/− and Glp1r+/+ mice were age-matched littermates and both strain cohorts were randomized to treatment groups based on glycated hemoglobin A1c (HbA1c) values. At study end, animals were euthanized with isoflurane inhalation, livers collected, and blood was processed for plasma and frozen. Single-dose exenatide plasma exposure and assessment of glomerular filtration rate (GFR) was performed in a separate cohort of Glp1r−/− and Glp1r+/+ mice fed regular chow. Urine was collected using separate metabolic cages for each individual awake animal (Complete Metabolic Cage 2100, Lap Products, Seafood, DE).

Administration of exenatide. Exenatide was administered subcutaneously at 30 nmol·kg−1·day−1 (126 μg·kg−1·day−1) as a continuous infusion for 12 wk using osmotic minipumps (28-d model 2004, Direct, Cupertino, CA). Minipumps were replaced each month. Control groups received 50% DMSO in water with 0.1% bovine serum albumin as a vehicle. Body weight and food intake were measured at baseline and every other week. Nonfasting blood glucose and HbA1c were measured at baseline and after 10 wk of treatment when additional blood samples were collected and processed for plasma for determination of amylase, lipase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations.

Oral glucose tolerance and gastric emptying tests. An oral glucose tolerance test (OGTT) and a GE test were conducted after an overnight fast during the final week of the 12-wk study. Animals received an oral gavage with 2 g/kg glucose in sterile water and 132 mg/kg acetonelophosphate (Sigma Aldrich, St. Louis, MO) reconstituted in ORABLEND SF (Paddock Laboratories, Minneapolis, MN). Blood samples were collected at baseline and 15, 30, 60, and 120 min after the glucose-acetanomiphosphon load.

Exenatide exposure after a single-dose administration. Two additional acute studies to assess compound exposure in Glp1r−/− versus Glp1r+/+ mice at different ages were conducted. After a single subcutaneous dose of 30 nmol/kg exenatide, blood was collected at 60 min, and at 15 and 60 min from 3- and 10-mo-old mice, respectively.

Glomerular filtration rate. For the comparison of renal function of Glp1r−/− versus Glp1r+/+ mice, GFR was assessed in 6-mo-old conscious, untreated animals. GFR was calculated based on 24-h urine collection, using the following formula: [urine creatinine (mg/dl) × urine volume (ml)]/[plasma creatinine (mg/dl) × urine time collection (h)].

Biochemical analyses. Blood glucose during the chronic study was measured using a blood glucose meter (One Touch Ultra, LifeScan, Johnson & Johnson, Milpitas, CA). HbA1c, plasma amylase, lipase, AST, ALT, creatinine, and urine creatinine were measured using a clinical chemistry analyzer (AU680 Olympus America, Irving, TX) according to the manufacturer’s protocols. Concentrations of glucose and acetonemorphine in samples from the OGTT and GE test were measured simultaneously in each sample using a clinical chemistry analyzer (Olympus). Insulin concentration in terminal plasma was assessed using an insulin ELISA (Crystal Chem, Downers Grove, IL). Plasma exenatide was measured using a previously described and validated immunoenzymetric assay developed at Amylin Pharmaceuticals (12). Hepatic lipid content was measured as described previously (44).

Statistical analyses. Results are presented as means ± SE, graphed, and analyzed using GraphPad Prism 5 (GraphPad Software, San Diego, CA). Areas under the curve (AUC) were calculated using the trapezoid method. Statistical differences between treatment groups and respective vehicle controls for Glp1r−/− and Glp1r+/+ mice or between both strains were identified by analysis of variance (ANOVA; 1- or 2-way where appropriate) followed by Bonferroni post hoc testing (P < 0.05) for all metabolic changes. A t-test was used for analyzing differences between groups in plasma exenatide levels and renal parameters (P < 0.05).

RESULTS

Subchronic effects of exenatide on body weight, food intake, basal glucose, and HbA1c. Before initiation of the study, mean body weight of Glp1r−/− mice (30.9 ± 0.6 g) was slightly but significantly lower than that of control Glp1r+/+ mice (34.8 ± 0.6 g); however, from week 2 to week 10 this difference was not significant: mean body weight of vehicle-treated Glp1r−/− mice and Glp1r+/+ mice measured on week 10 of the study was 41.4 ± 0.4 g and 45.3 ± 1.7 g, respectively. Exenatide had no significant effect on vehicle-corrected body weight of Glp1r−/− mice (Fig. 1A), whereas it significantly reduced body weight of Glp1r+/+ mice (Fig. 1B). No statistically significant difference in cumulative food intake was noted in Glp1r−/− mice treated with exenatide (Fig. 1C) versus the respective vehicle-treated controls. Cumulative food intake was reduced by exenatide in Glp1r+/+ mice during the first 4 wk of administration, and the effect subsided to values comparable to vehicle controls thereafter (Fig. 1D). Overall, Glp1r−/− and Glp1r+/+ mice were normoglycemic and their mean baseline blood glucose was similar (9.9 ± 0.2 and 9.6 ± 0.3 mmol/l, respectively). A small but statistically significant difference was observed in the initial mean HbA1c value in Glp1r−/− mice (4.74 ± 0.05%) versus Glp1r+/+ mice (4.59 ± 0.04%). A modest but statistically significant change in nonfasting glucose from baseline was observed in Glp1r+/+ mice but not in Glp1r−/− mice administered with exenatide for 10 wk (Fig. 2A). No significant changes in HbA1c were seen in either strain following 10 wk of infusion of exenatide (Fig. 2B).

Effects of subchronic administration of exenatide on oral glucose tolerance and gastric emptying. Glucose excision profiles during the OGTT performed in Glp1r−/− and Glp1r+/+ mice are presented in Fig. 3, A and B, respectively. A slight suppression in the time-related glucose excursion was observed in Glp1r−/− mice administered with exenatide for 12 wk (P < 0.05 vs. vehicle, 2-way ANCOVA), and this effect, as measured by AUC0–t0.6 g), was significantly different from the OGTT profile seen in vehicle-treated Glp1r+/+ mice. A greater decrease in glucose excursion was observed with exenatide treatment (P < 0.05 vs. vehicle) in Glp1r−/− mice (Fig. 3B).

Subchronic administration of exenatide resulted in no measurable effect on GE in Glp1r−/− mice (Fig. 3C). A small suppression of GE with exenatide in Glp1r+/+ mice was observed at t = 15 min (P < 0.05) (Fig. 3D).

Subchronic effects of exenatide on plasma insulin, amylase, and lipase. Exenatide had no impact on terminal plasma insulin concentration in Glp1r−/− mice versus a significant reduction
in Glp1r<sup>+/+</sup> mice (Fig. 4A). To assess the potential effects of exenatide on pancreatic exocrine function, we measured plasma concentrations of pancreatic amylase and lipase after 10-wk continuous exposure to the drug. Concentrations of amylase (Fig. 4B) and lipase (Fig. 4C) were comparable in vehicle-treated groups from both strains and no effects of exenatide were observed.

**Subchronic effects of exenatide on hepatic enzymes in plasma and hepatic lipid content.** Hepatic end points were assessed based on previously reported beneficial effects of exenatide on liver steatosis (10) and to elucidate the existing controversy regarding whether these effects are solely mediated via signaling through the known GLP-1R. Mean plasma ALT concentrations were similar in Glp1r<sup>−/−</sup> and Glp1r<sup>+/+</sup> mice dosed with vehicle and, compared with respective vehicle controls, 10 wk of exenatide administration significantly reduced ALT in Glp1r<sup>+/+</sup> mice only (Fig. 5A). Mean plasma AST in both mouse strains was comparable and was not affected by exenatide treatment (Fig. 5B). Of note, Glp1r<sup>−/−</sup> mice were protected from lipid accumulation in the liver as exhibited by a significant increase in liver lipid content in vehicle-treated, high-fat fed Glp1r<sup>−/−</sup> mice (P < 0.05). Exenatide infusion reduced hepatic lipid content in Glp1r<sup>+/+</sup> mice to levels comparable to those in Glp1r<sup>−/−</sup> mice (Fig. 5C).

**Exenatide exposure in plasma.** After 12 wk of continuous infusion, plasma exenatide concentration was approximately eightfold higher in Glp1r<sup>−/−</sup> (450 ± 139 pg/ml) versus Glp1r<sup>+/+</sup> mice (55 ± 20 pg/ml) (Fig. 6A). To examine this observation further, we performed additional single-dose acute studies in both mouse strains at different ages. While elevated plasma concentrations of exenatide were noted in Glp1r<sup>−/−</sup> versus Glp1r<sup>+/+</sup> in 3- and 10-mo-old mice following a single injection of exenatide (Fig. 6, B and C), the difference was not as pronounced as that observed with 12 wk of infusion of exenatide.

**Glomerular filtration rate in untreated Glp1r<sup>−/−</sup> versus Glp1r<sup>+/+</sup>.** There was no noticeable difference between Glp1r<sup>−/−</sup> versus Glp1r<sup>+/+</sup> mice in plasma creatinine (Fig. 7A) or urine creatinine (Fig. 7B) concentrations, 24-h urine volume (Fig. 7C), or calculated GFR (Fig. 7D).

**DISCUSSION**

The present study is the first to compare the long-term effects of continuous administration of exenatide in high-fat fed Glp1r<sup>−/−</sup> versus Glp1r<sup>+/+</sup> mice. Overall, the findings support the hypothesis that the long-term metabolic benefits of exenatide administration in mice (36) require the known/extensively characterized GLP-1R to be present and fully...
we did observe similar findings to the Lamont study during a GE test where acetaminophen excursions were similar in Glp1r−/− mice regardless of whether the animals were treated with exenatide or vehicle. It is noteworthy that in our study, both the GE test and OGTT were performed simultaneously in each animal, and blood samples were collected and measurements of acetaminophen and glucose concentrations were also conducted simultaneously using a clinical analyzer allowing multianalyte analyses. Hence, this should minimize sample handling and assay variability contributing to the differences we observed between treated and untreated Glp1r−/− mice. Of note, the action of exenatide to slow GE after 3-mo dosing in Glp1r+/+ mice was smaller than might be expected. It is feasible that downregulation of this effect on GE is more pronounced with continuous-exposure GLP-1R agonists compared with shorter-acting, intermittently exposed agonists. Hence, our data supports the concept that there is no major slowing of GE with longer-term infusion of exenatide, at least in mice.

In our study, food intake and body weight in Glp1r−/− mice were not affected by subchronic administration of exenatide. We did not test responsiveness of these mice to other anorexigenic non-GLP-1R agonists; however, published data from the same mouse colony has shown that the food intake suppressive actions of leptin are robust in Glp1r−/− mice (48). Control Glp1r+/+ mice exhibited the expected response to exenatide, as reflected by significant body weight loss and transient food intake inhibition as shown previously (28). The greatest effect of continuous infusion of exenatide on food intake occurred during the first 2 wk of treatment in the present study and in previous studies in both mice and rats (28). We believe this is not suggestive of a loss of efficacy, but propose that once body weight resets to a lower level with continuous treatment, the drive to continually suppress food intake abates.

A visible trend for glucose lowering and complete lack of effect on GE in Glp1r−/− mice administered subchronically with exenatide led us to speculate about potential mechanisms of action for these observations. Baseline insulin levels in Glp1r−/− mice in our study were normal, and it has been shown that the insulin response to glucose is preserved in these animals (14). Total pancreatic β-cell volume and number are also not altered compared with Glp1r+/+ mice; however, the topography of β-cells is markedly different (24). Additionally, it has been hypothesized that other G protein-coupled receptors, members of structurally related receptors for the glucagon superfamily of peptide hormones, which are related to GLP-1, might convey response to GLP-1R ligands (52). Furthermore, it has been reported that the phenotype of the functional Glp1r−/− mice is likely diminished by compensatory upregulation of glucose-dependent insulinoctropic peptide (GIP) (13, 38). Therefore, we cannot exclude the possibility that the GIP receptor may play a role in our findings. The GIP receptor is expressed in β-cells (30); similarly to GLP-1R signaling, it mediates the profound postprandial insulinoctropic action of GIP (39), and an increase in GIP receptor (GIP-R) expression was observed during acute and chronic hyperglycemia (55). Moreover, it has been recently demonstrated in vitro that long-term incubation with exenatide slightly upregulated cell surface levels of the GIP-R in HEK293 cells transfected with cDNA encoding the GIP-R. In this same study, exenatide was shown to be a low potency GIP-R agonist (15).

As expected, and consistent with previous reports, Glp1r−/− mice in our study exhibited a phenotype with normal body weight, food intake, and basal glucose along with impaired glucose tolerance (2, 47). Intriguingly, long-term exposure to a supramaximal dose of exenatide seems to have produced a modest glucoregulatory effect in these Glp1r−/− mice, as we observed a trend for exenatide to suppress the glucose excursion accompanied by an OGTT in the GLP-1R-deficient animals. Of note, basal glucose and HbA1c were also lower than in control mice administered with vehicle. Indeed, a consistent trend for improvement in glucose excursions was observed during an OGTT at the end of the 12-wk study in both groups of mice treated with exenatide. These findings are somewhat in contrast to another recent report, where glucose excursions in exenatide-treated Glp1r−/− mice were superimposed with glucose excursions of vehicle controls; however, in that study exenatide was applied as a single relatively low dose (1 μg/animal) just before the glucose challenge (23). In contrast, functional. To this end, exenatide profoundly inhibited food intake and body weight gain in Glp1r+/+ mice, whereas these effects were abolished in mice lacking a functional GLP-1R. Basal and OGTT glucose concentrations were significantly reduced by exenatide in Glp1r+/+ mice, and to a lesser extent, in Glp1r−/− mice; however, no effect on HbA1c was noted in either mouse strain at the end of study. Glucoregulatory effects of exenatide in Glp1r+/+ mice were relatively modest despite the high dose used. While high-fat feeding was introduced 6 wk before study initiation, these mice were relatively normoglycemia. Since the antidiabetic activity of exenatide is known to be glucosel dependent (37), it would be unexpected for exenatide to reduce glucose levels beyond normoglycemia.

As expected, and consistent with previous reports, Glp1r−/− mice in our study exhibited a phenotype with normal body weight, food intake, and basal glucose tolerance (2, 47). Intriguingly, long-term exposure to a supramaximal dose of exenatide seems to have produced a modest glucoregulatory effect in these Glp1r−/− mice, as we observed a trend for exenatide to suppress the glucose excursion accompanied by an OGTT in the GLP-1R-deficient animals. Of note, basal glucose and HbA1c were also lower than in control mice administered with vehicle. Indeed, a consistent trend for improvement in glucose excursions was observed during an OGTT at the end of the 12-wk study in both groups of mice treated with exenatide. These findings are somewhat in contrast to another recent report, where glucose excursions in exenatide-treated Glp1r−/− mice were superimposed with glucose excursions of vehicle controls; however, in that study exenatide was applied as a single relatively low dose (1 μg/animal) just before the glucose challenge (23). In contrast,
also been reported that GIP does not inhibit GE in humans (29), potential compensatory increases in GIP-R activity would only influence glucoregulatory but not GE effects of GIP-R agonism. Of note, in a broad-based receptor screen (NovaScreen, Hanover, MD) exenatide did not bind to any of a panel of 67 receptors, ion channels, neurotransmitters, or transporter targets (51).

Another intriguing finding from our study is the observed eightfold higher plasma concentration of exenatide after 12 wk of continuous infusion in Glp1r<sup>−/−</sup> versus Glp1r<sup>+/+</sup> mice. We

Fig. 3. Oral glucose tolerance and gastric emptying based on a single dose of acetaminophen in Glp1r<sup>−/−</sup> (KO) and Glp1r<sup>+/+</sup> (WT) mice after 12-wk continuous subcutaneous infusion of 30 nmol·kg<sup>−1</sup>·day<sup>−1</sup> exenatide. Plasma glucose excursions are shown in Glp1r<sup>−/−</sup> (A) and Glp1r<sup>+/+</sup> (B), and plasma acetaminophen excursions are shown in Glp1r<sup>−/−</sup> (C) and Glp1r<sup>+/+</sup> (D) mice. Insets in A–D represent calculated AUC<sub>0–2h</sub> for specific excursion curves. Data are means ± SE. *P < 0.05 vs. vehicle controls (n = 10 KO, n = 7 WT).

Fig. 4. Plasma insulin (A), amylase (B), and lipase (C) in Glp1r<sup>−/−</sup> (KO) and Glp1r<sup>+/+</sup> (WT) mice after 10 wk of continuous subcutaneous infusion of 30 nmol·kg<sup>−1</sup>·day<sup>−1</sup> exenatide. Data are means ± SE. *P < 0.05 vs. vehicle WT control. (n = 10 KO, n = 7 WT).
performed additional acute studies with single-dose exenatide in separate cohorts of 3- and 10-mo-old mice to confirm these observations and to assess whether aging of mice, which might potentially lead to reduced renal function, differentially affects plasma clearance of exenatide between mouse strains. Regardless of mouse age, plasma exenatide concentrations in Glp1r^−/− (KO) mice were consistently higher than in Glp1r^+/+ (WT) mice; however, the difference was not as pronounced as that observed in the subchronic study. It is possible that the larger discrepancy in exenatide exposure seen in the subchronic infusion versus the acute single-dose study was due to accumulation of the drug over an extended period of continuous administration.

It is well established that exenatide is resistant to DPP-4 degradation, and the kidney is a primary route of clearance and enzymatic degradation of exenatide. It has been reported that exenatide, but not GLP-1, was cleared exclusively by glomerular filtration in anesthetized pigs (49). In renal-ligated rats, plasma exenatide concentrations were elevated and sustained versus intact animals (36), and in vitro the compound was almost completely degraded into peptide fragments by kidney membrane preparations from different species (8). Similarly, in patients with renal impairment exenatide clearance was reduced and exposure increased (25). In contrast, some studies indicate a component of nonrenal clearance, as in nephrectomized rats (35) or in patients on hemodialysis with end-stage renal disease (25), exenatide slowly disappeared from the circulation. It has also been shown that the GLP-1R is expressed in the rat kidney (6), in porcine renal proximal tubular cells (45), and possibly in renal vasculature and other cells. Additionally, exenatide treatment increased GLP-1R immuno-reactivity in glomeruli of diabetic db/db mice in a dose-dependent manner (34), increased GFR and suppressed proximal tubule reabsorption in the rat (50), and ameliorated renal dysfunction/sclerosis and hypertension in animal models (18, 26). Therefore, the findings from our study could be explained by the lack of a functional GLP-1R with subsequent impaired internalization of exenatide/GLP-1R complexes in renal proximal tubes. The lack of a functional GLP-1R may prevent enzymatic degradation of the peptide in the kidney, by impair-

![Fig. 5. Plasma alanine aminotransferase (ALT, A) and aspartate aminotransferase (AST, B) concentrations after 10 wk and hepatic lipid content (C) after 12 wk of continuous subcutaneous infusion of 30 nmol·kg⁻¹·day⁻¹ exenatide in Glp1r^−/− (KO) and Glp1r^+/+ (WT) mice. Data are means ± SE. *P < 0.05 vs. vehicle WT control. (n = 10 KO, n = 7 WT).](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00495.2013)

![Fig. 6. Plasma exenatide concentrations in Glp1r^−/− (KO) and Glp1r^+/+ (WT) mice after 12-wk continuous subcutaneous infusion (30 nmol·kg⁻¹·day⁻¹) (A) (n = 10 KO, n = 7 WT), 60-min post-single dose administration (30 nmol/kg) in 3-mo-old (B) (n = 5 KO, n = 6 WT) and 15- and 60-min post-single dose administration (30 nmol/kg) in 10-mo-old mice (C) (n = 7 KO, n = 9 WT). *P < 0.05 vs. vehicle WT control. (n = 10 KO, n = 7 WT).](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00495.2013)
ment of renal function induced by indirect mechanisms resulting from a lack of GLP-1R in other tissues/organs, or a combination of both mechanisms.

Based on measurements of GFR, urine volume, and plasma and urine creatinine, renal function of Glp1r<sup>-/-</sup> mice in our study appeared to be normal and comparable to function in Glp1r<sup>+/+</sup> mice. Similarly, the thorough characterization of Glp1r<sup>-/-</sup> and Glp1r<sup>+/+</sup> mice reported recently by Rieg et al. (43) revealed no substantial differences in renal physiology between both strains; however, GFR was modestly higher in Glp1r<sup>-/-</sup> versus Glp1r<sup>+/+</sup> mice. The absence of such a difference in our study may be due to methodology differences for assessing GFR, as a single-period (24-h) creatinine clearance-based method is less sensitive than the two-period (30-min), radio-labeled, inulin-based method used by Rieg et al. (43). Nevertheless, it seems unlikely that the differential exenatide exposure we observed in Glp1r<sup>-/-</sup> versus Glp1r<sup>+/+</sup> mice could be attributed primarily to impaired renal function. Our findings would suggest that this difference in renal and/or plasma clearance of exenatide is mainly attributed to the need for a functional GLP-1R.

In the present study, pancreatic and hepatic enzymes in plasma, and lipid content in the liver, were considered as secondary end points. The lack of effects of long-term administration of a supramaximal dose of exenatide on amylase and lipase in Glp1r<sup>-/-</sup> and Glp1r<sup>+/+</sup> mice is consistent with other studies performed previously (21). It was demonstrated previously that Glp1r<sup>-/-</sup> mice fed a high-fat diet, in contrast to Glp1r<sup>+/+</sup> controls, were protected from hepatic insulin resistance, which was associated with a decrease in hepatic triglycerides (1). In our study, total hepatic lipid content was measured and its decrease in Glp1r<sup>-/-</sup> mice is in agreement with these published observations. Additionally, our results demonstrated the expected beneficial effects of exenatide on certain hepatic end points in intact, wild-type mice.

**Perspectives and Significance**

Our data support the concept that exenatide requires a functional GLP-1R to exert its chronic metabolic effects in mice. The differential plasma levels of exenatide in Glp1r<sup>-/-</sup> versus Glp1r<sup>+/+</sup> mice and normal renal function in Glp1r<sup>-/-</sup> mice would suggest a significant and direct involvement of the GLP-1R in the clearance of exenatide from the blood. The apparent lack of pharmacological effect of chronic exenatide infusion in Glp1r<sup>-/-</sup> mice indicates that novel GLP-1R agonists, or indeed other pharmacological pathways, may not substantially contribute to the chronic metabolic actions of GLP-1R agonists. Further studies in mice with inducible and tissue-specific Glp1r gene deficiency will provide more comprehensive answers to the mechanistic questions arising from this study.

**ACKNOWLEDGMENTS**

We thank Pamela Smith (Amylin Pharmaceuticals) for assistance in preparation of the manuscript.

The majority of results from this study were presented in 2011 at the American Diabetes Association scientific sessions and at the European Association for the Study of Diabetes annual meeting.
DISCLOSURES

At the time when the work for this manuscript was performed, all authors were employed by Amylin Pharmaceuticals. All authors held stock in Amylin Pharmaceuticals.

AUTHOR CONTRIBUTIONS


REFERENCES


