Development and Characterization of a Glucagon-Like Peptide 1-Albumin Conjugate

The Ability to Activate the Glucagon-Like Peptide 1 Receptor In Vivo

Jung-Guk Kim,1 Laurie L. Baggio,1 Dominique P. Bridon,2 Jean-Paul Castaigne,2 Martin F. Robitaille,2 Lucie Jetté,2 Corinne Benquet,2 and Daniel J. Drucker1

The rapid degradation of native glucagon-like peptide 1 (GLP-1) by dipeptidyl peptidase-IV (DPP-IV) has fostered new approaches for generation of degradation-resistant GLP-1 analogues. We examined the biological activity of CJC-1131, a DPP-IV-resistant drug affinity complex (DAC) GLP-1 compound that conjugates to albumin in vivo. The CJC-1131 albumin conjugate bound to the GLP-1 receptor (GLP-1R) and activated cAMP formation in heterologous fibroblasts expressing a GLP-1R. CJC-1131 lowered glucose in wild-type mice, but not in GLP-1R−/− mice. Basal glucose and glycemic excursion following glucose challenge remained significantly reduced 10–12 h following a single injection of CJC-1131. Twice daily administration of CJC-1131 to db/db mice significantly reduced glycemic excursion following oral and IP glucose challenge (P < 0.01 to 0.05) but did not significantly lower body weight during the 4-week study period. Levels of random fed glucose were significantly lower in CJC-1131–treated +/+ and db/db mice and remained significantly lower even 1 week following discontinuation of CJC-1131 administration. CJC-1131 increased levels of pancreatic proinsulin mRNA transcripts, percent islet area, and the number of bromodeoxyuridine-positive islet cells. These findings demonstrate that an albumin-conjugated DAC:GLP-1 mimics the action of native GLP-1 and represents a new approach for prolonged activation of GLP-1R signaling.


Glucagon-like peptide 1 (GLP-1) is a proglucagon-derived peptide secreted from intestinal L-cells in response to nutrient ingestion (1,2). GLP-1 acts as an incretin to lower postprandial glycemic excursion via stimulation of insulin secretion and inhibition of glucagon secretion. GLP-1 also exerts actions independent of islet hormone secretion, including inhibition of both gastric emptying and food intake (3,4), and stimulation of β-cell proliferation (5,6).

Although the structurally related gut hormone glucose-dependent insulinotropic peptide (GIP) also potentiates glucose-dependent insulin secretion (7), unlike GLP-1, the insulinotropic actions of GIP are diminished in diabetic rodents or in human subjects with type 2 diabetes (8,9). In contrast, GLP-1 administration rapidly lowers glucose in both normal and diabetic subjects (9–11), and 6 weeks of continuous subcutaneous infusion of native GLP-1 significantly decreased blood glucose and HbA1c in human patients with type 2 diabetes (12). Hence there is considerable enthusiasm for the development of GLP-1–based pharmaceutical agents for the treatment of type 2 diabetes (2,13).

Although native GLP-1 effectively lowers blood glucose following acute peptide administration (14,15), both endogenous and exogenously administered GLP-1 exhibit a short t1/2 in vivo due primarily to NH₂-terminal cleavage and inactivation by the enzyme dipeptidyl peptidase (DPP-IV) (16,17). The physiological importance of DPP-IV for GLP-1 degradation and glucose homeostasis is exemplified by studies of mice or rats with inactivating mutations in the DPP-IV gene. These rodents exhibit enhanced glucose clearance following glucose challenge and increased circulating levels of intact GLP-1 in vivo (18,19). Similarly, administration of DPP-IV enzyme inhibitors is associated with reduced glycemic excursion, enhanced insulin secretion, and reduced degradation of GLP-1 in normal and diabetic rodents (20–22) and in human subjects (23).

Accordingly, there is considerable interest in complementary strategies for circumventing the rapid cleavage of GLP-1, including the development of GLP-1–based analogs with enhanced resistance to degradation and increased biological potency in vivo (13,24). The majority of these analogs exhibit one or more amino acid substitutions that reduce the affinity of the peptide for DPP-IV and subsequent cleavage both in vitro and in vivo. Similarly, the naturally occurring lizard peptide exendin-4 is a potent GLP-1R agonist that exhibits reduced DPP-IV mediated cleavage and a longer duration of action in both rodents and human subjects (25,26). We have recently initiated studies of GLP-1 derivatives that are resistant to DPP-IV and are synthesized with a short covalent reactive chemistries.
CJC-1131, A GLP-1 ALBUMIN GLP-1R AGONIST

RESEARCH DESIGN AND METHOD

Animals. All animal experiments were carried out in accordance with protocols approved by the Toronto General Hospital Animal Care Committee or the Comité Institutionnel de Protection des Animaux de l’UQAM. C57BL/6 db/db mice (The Jackson Laboratory, Bar Harbor, ME) and age- and sex-matched C57BL/6 wild-type mice from the same genetic background were used for chronic administration studies following a minimum 1-week acclimatization period in the animal facility. Wild-type CD-1 mice and GLP-1R mice (27) in the CD-1 background were used for acute peptide administration experiments. Mice were allowed ad libitum access to food and water, except where noted. Animals were on a 12-h light, 12-h dark cycle (lights on 0700 h). For dose-response experiments shown in Fig. 2B, female CD-1 mice (Charles River Canada, St-Constant, QC), 7- to 10-weeks old, were studied, whereas for glycemic measurements shown in Figs. 2C and D, experiments were carried out with female db/db mice aged 7–10 weeks. CJC-1131 is a synthetic modification of GLP-1 (Fig. 1A) consisting of a single amino acid substitution of α-Ala8 to β-Ala8 at position 2, enabling some additional protection from DPP-IV and the addition of a lysine (Lys37) to the COOH-terminal of the peptide. The other modification involves the selective attachment of a [2-[2-(maleimidopropionamido)(ethoxy)ethyl]amino]acetic acid-guanidinium isothiocyanate method. Total RNA (10 μg) was electrophoresed in a 1% (wt/vol) formaldehyde-agarose gel and transferred to a nylon membrane (Nytran Plus; Schleicher & Schuell, Keene, NH). For Northern blot analysis, the blot was hybridized to 32P-labeled random-primed complementary DNA probes corresponding to rat proglucagon, rat insulin, or 18S RNA.

RNA isolation and Northern blot analysis. Mice were anesthetized with CO2, and pancreata were removed immediately for RNA extraction by the acid-phenol:guanidinium isothiocyanate method. Total RNA (10 μg) was electrophoresed in a 1% (wt/vol) formaldehyde-agarose gel and transferred to a nylon membrane (Nytran Plus; Schleicher & Schuell, Keene, NH). For Northern blot analysis, the blot was hybridized to 32P-labeled random-primer complementary DNA probes corresponding to rat proglucagon, rat insulin, or 18S RNA.

Analysis of islet size and β-cell proliferation. Mice were injected with 100 μg/kg 5-bromo-2′-deoxyuridine (BrdU, Sigma-Aldrich, St. Louis, MO) intraperitoneally 6 h before being sacrificed. The pancreas was removed, fixed overnight in either 10% buffered formalin or 4% paraformaldehyde, and embedded in paraffin. Sections were obtained and stained with hematoxylin and eosin using standard protocols. Histological sections were immunostained for insulin using guinea-pig anti-insulin (Dako Diagnostics Canada, Mississauga, ON, Canada) as primary antibody (1:100 dilution) and rabbit anti-guinea-pig immunoglobulin (Dako Diagnostics Canada) as secondary antibody (1:50 dilution). Antibody binding was visualized by 3,3-diaminobenzidine, and sections were counterstained by Meyers hematoxylin. Islet histomorphometry was carried out according to the principles of Delesse (30). The sections were examined using a Leica (Leitz Labor Lux S; Leica Microsystems, Heerbrugg, Switzerland) microscope equipped with a video camera and connected to a computer with imaging software (Q500MC; Leica Microsystems). Estimates of islet area, islet number, number of BrdU immunopositive cells, and total pancreatic area were determined in a blinded manner as described (29,31).

Statistics. Results are expressed as mean ± SD or mean ± SE. Statistical significance was calculated by Student’s t test using SPSS windows version 10 (SPSS, Chicago, IL). P < 0.05 was considered to be statistically significant.

RESULTS

To circumvent the short biological t1/2 of native GLP-1 due principally to DPP-IV-mediated degradation, we designed a new DPP-IV-resistant GLP-1 derivative that would form
a covalent bond with albumin in vivo, thereby conferring to covalently linked GLP-1 the longer circulating $t_{1/2}$ of albumin (32). A d-alanine residue at position 2 was substituted for the native alanine, and the COOH-terminal end of the GLP-1 molecule was coupled to a reactive chemical linker capable of forming a 1:1 covalent bond to the Cys residue in serum albumin (Fig. 1A). The resultant GLP-1 derivative, designated CJC-1131, should retain the biological properties of native GLP-1 yet exhibit DPP-IV resistance and a prolonged $t_{1/2}$ consistent with the clearance of native serum albumin in vivo (33).

To assess the properties of a CJC-1131–human serum albumin (HSA) conjugate, we incubated HSA with CJC-1131 in vitro and utilized the purified CJC-1131:HSA conjugate for studies of GLP-1R binding using CHO cells transfected with human recombinant GLP-1R (Fig. 1B). The displacement of $^{125}$I-labeled GLP-1 by native GLP-1 versus the CJC-1131:HSA complex was highly similar over a range of CJC-1131 concentrations ($K_i/11005$ 5.16 nmol/l for native GLP-1 vs. 12 nmol/l for CJC-1131:HSA). To assess the bioactivity of CJC-1131, we measured cAMP accumulation using CHO cells transfected with human recombinant GLP-1R. These experiments demonstrated virtually identical dose-response relations for cAMP accumulation, with native GLP-1 exhibiting an EC$_{50}$ of 13 nmol/l, whereas the EC$_{50}$ for CJC-1131–HSA was 11–13 nmol/l (Fig. 1C).

We next assessed whether the route of CJC-1131 administration was an important determinant of bioactivity in mice or subcutaneous administration of saline or CJC-1131 (25 µg); $n = 5$ mice/group. Values are expressed as means ± SE. B: Blood glucose levels in 7- to 10-week-old female CD-1 mice during an oral glucose tolerance test performed 5 min after an intravenous administration of saline ( ), or 100 nmol/kg CJC-1131 ( ); $n = 5$ mice/group. Values are expressed as means ± SE; $^P < 0.05$, **$P < 0.02$, ***$P < 0.001$ for differences in area under the curve (AUC) glucose for mice treated with saline vs. 1, 10, and 100 nmol/kg CJC-1131, respectively. C: Basal glycemia in 6- to 7-week-old female diabetic db/db mice after a single subcutaneous injection of either vehicle ( ) or 100 nmol/kg CJC-1131 ( ); $n = 5$ mice/group. Values are expressed as means ± SE; $^P < 0.02$ for AUC glucose in saline versus CJC-1131–treated mice. D: Blood glucose levels in 7- to 10-week-old female db/db mice during an oral glucose tolerance test performed 12 h after intravenous administration of saline ( ), 100 nmol/kg of native GLP-1 ( ), or 100 nmol/kg of CJC-1131 ( ); $n = 5$ mice/group. Values are expressed as means ± SE; $^P < 0.05$ for AUC glucose in saline versus CJC-1131–treated mice. E: Blood glucose levels in wild-type CD-1+/+ ( ) and GLP-1R−/− ( ) mice during an oral glucose tolerance test performed 60 min after intraperitoneal administration of CJC-1131 (25 µg); $n = 9–10$ mice/group. Values are expressed as mean ± SE; $^P < 0.05$, **$P < 0.01$, #$P < 0.001$ for AUC glucose in GLP-1R+/+ vs. GLP-1R−/− mice.
C57BL/6 mice following glucose loading. CJC-1131 markedly reduced glycemic excursion following intraperitoneal or subcutaneous administration (Fig. 2A). Similarly, intravenous CJC-1131 administration produced a dose-dependent reduction in glycemic excursion following glucose loading (Fig. 2B). To ascertain whether a single injection of CJC-1131 would lower blood glucose in diabetic mice, we administered CJC-1131 by subcutaneous injection to db/db mice. Remarkably, basal random glycemia decreased rapidly and remained lower in db/db mice for up to 10 h following a single subcutaneous injection of CJC-1131 (Fig. 2C). Furthermore, the glucose-lowering effect of CJC-1131 was still evident during an oral glucose tolerance test performed 12 h after a single intravenous CJC-1131 injection (Fig. 2D). Although CJC-1131 consistently lowered glucose in wild-type mice, no effect was observed in GLP-1R−/− mice (Fig. 2E), demonstrating the critical importance of an intact GLP-1R for the biological activity of CJC-1131 in vivo.

These findings demonstrated that single injections of CJC-1131 exert glucose-lowering effects in normal and diabetic mice. We next assessed whether more prolonged repeated administration of CJC-1131 would lower glucose in mice with severe diabetes. Wild-type control C57BL/6 (C57+/+) or db/db mice were treated with saline or CJC-1131 twice daily for 4 weeks. Before initiation of CJC-1131, mean fasting glucose was 4.3 ± 0.9 mmol/l in C57+/+ vs. 17.9 ± 6.7 mmol/l in db/db mice (Fig. 3A). After 2 weeks of twice daily saline or CJC-1131 administration, fasting blood glucose was significantly lower in both control C57+/+ and db/db mice treated with CJC-1131 (4.5 ± 0.7 vs. 3.3 ± 0.5 mmol/l and 19.2 ± 5.8 vs. 12.5 ± 3.7 mmol/l, saline vs. CJC-1131 in C57+/+ vs. db/db mice, respectively; P < 0.01, Fig. 3A). Fasting glucose remained significantly lower in C57+/+ mice but not in db/db mice treated with CJC-1131 at the end of the 4-week treatment period (Fig. 3A). In contrast, fed blood glucose was significantly lower in CJC-1131–treated C57+/+ and db/db mice throughout the 4-week experiment (Fig. 3B; P < 0.01, saline versus CJC-1131). Furthermore, fed blood glucose remained significantly lower in db/db mice 1 week following the last injection of CJC-1131 (Fig. 3B, P < 0.05). Although CJC-1131 produced a small but significant decrease in body weight in C57+/+ mice, no differences in body weight were detected in db/db mice treated with saline versus CJC-1131 over the 4-week experiment (Fig. 3C).

To determine whether repeated administration of CJC-1131 was associated with improvement in glucose tolerance, we performed oral and intraperitoneal glucose tolerance testing in C57+/+ and db/db mice treated with saline or CJC-1131 for 2 weeks. Blood glucose excursion was significantly lower in CJC-1131–treated control C57+/+ mice following either oral or intraperitoneal glucose loading (Fig. 4A and B). Similarly, both fasting glucose and the glycemic excursion following glucose loading were modestly but significantly reduced in CJC-1131–treated db/db mice at multiple time points (Fig. 4A and B). Although plasma insulin levels were significantly greater in db/db mice compared with C57+/+ mice both in the fasting state and after glucose loading, plasma insulin levels were not further increased in db/db mice treated with CJC-1131 (data not shown).

GLP-1R agonists have been shown to increase proinsulin gene expression and promote islet neogenesis and β-cell proliferation (5,6,34). To determine whether a larger CJC-1131:albumin conjugate exhibits comparable actions on the diabetic pancreas in vivo, we carried out Northern blot analysis using pancreatic RNA from C57+/+ and db/db mice. Levels of pancreatic insulin mRNA transcripts were comparable in normoglycemic wild-type C57+/+
mice treated with either saline or CJC-1131. In contrast, 4 weeks of CJC-1131 administration significantly increased the levels of proinsulin mRNA transcripts in db/db mice (Fig. 5A). Pancreatic insulin content was reduced in db/db mice treated with CJC-1131 compared with control C57+/+ mice, but was not significantly different in saline– vs. CJC-1131–treated C57+/+ or db/db mice (Fig. 5B).

To determine whether CJC-1131 administration was associated with changes in the number or size of pancreatic islets, we carried out morphometric analysis of pancreata from C57+/+ and db/db mice following 4 weeks of treatment with either saline or CJC-1131 (Fig. 6). Total percent islet area was increased in C57+/+ mice and significantly increased in db/db mice following CJC-1131 administration (P < 0.01; Fig. 6B). We next assessed the proportion of small, medium, and large islets, as previously described (35). CJC-1131 treatment significantly increased the percent large islet area in db/db mice (Fig. 6C; P < 0.01). The percentage of large islets was also increased in control mice treated with CJC-1131, but this difference did not achieve statistical significance (Fig. 6C). In contrast, the number of small islets was significantly increased in both C57+/+ (212.2%; P < 0.05) and db/db mice (94.6%; P < 0.05) treated with CJC-1131 (Fig. 6D). Furthermore, CJC-1131 significantly increased the total number of islets in both C57+/+ and db/db mice (Fig. 6E).

To determine whether the observed differences in percent islet area and number of islets were attributable in part to increased rates of islet cell replication, we assessed the numbers of BrdU+ islet cells in saline– or CJC-1131–treated mice (Fig. 6F and G). CJC-1131 administration significantly increased the number of BrdU+ islet cells in db/db mice (413.1%, P < 0.01; Fig. 6G).

**DISCUSSION**

The observation that GLP-1 administration effectively reduces blood glucose in human subjects with type 2 diabetes has stimulated considerable effort toward the development of GLP-1–based agonists for pharmaceutical administration. Although infusion of native GLP-1 is highly effective in lowering blood glucose in diabetic patients (12,36), the native peptide is an excellent substrate for DPP-IV (16,17), and exhibits a very short half-life in vivo.

**FIG. 4.** Blood glucose levels following oral (A) or intraperitoneal (B) glucose challenge in C57+/+ (○) and db/db mice (△), n = 20 mice per group, after 2 weeks of twice daily administration of either saline (●) or 25 μg CJC-1131 (γ, Δ). AUC for oral glucose tolerance testing in C57+/+ mice (saline treated 825.4 ± 36.3 and CJC-1131–treated 550.2 ± 29.0; P < 0.001) and db/db mice (saline treated 3,620.6 ± 55.0 and CJC-1131–treated 3,081.4 ± 161.0; P < 0.01). AUC for intraperitoneal glucose tolerance testing in C57+/+ mice (saline treated 958.1 ± 65.6 and CJC-1131–treated 434.2 ± 28.0; P < 0.001) and db/db mice (saline–treated 3,298.1 ± 204.8 and CJC-1131–treated 2,824.6 ± 218.6; P < 0.05). Values are expressed as mean ± SE; *P < 0.05, **P < 0.01.

**FIG. 5.** **A:** Pancreatic insulin mRNA; the lower panel depicts relative ratios of insulin/18s RNA from multiple analyses. **B:** Insulin content in C57+/+ (n = 6) and db/db (n = 6) mice after 4 weeks of saline or CJC-1131 (25 μg twice daily) administration.

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FIG. 6. Histological and morphometric analysis of pancreatic islets from C57+/+ and db/db mice after 4 weeks of saline (□) or twice daily CJC-1131 (25 µg) treatment (■). A: Immunohistochemical staining for insulin. B: Percent total islet area/total pancreatic area. C: Percent large islet area/total pancreatic area. D: Number of small islets/pancreatic area (1 × 10^6 µm^2). E: Total islet number/pancreatic area. F: Histological representation of BrdU immunopositive islet cells. G: quantitation of BrdU positive cells per pancreatic area (1 × 10^7 µm^2) in C57+/+ (n = 6) and db/db (n = 6) mice. Values are expressed as the means ± SE; *P < 0.05, **P < 0.01.
of action suitable for subcutaneous administration in vivo (13).

Several approaches have been undertaken to develop stable long-acting GLP-1 analogs, with DPP-IV–resistant molecules such as NN2211 currently being tested in clinical trials for the treatment of type 2 diabetes (37). Exendin-4, a naturally occurring lizard salivary gland peptide (38) that exhibits potent GLP-1–like activity and exerts its actions through the GLP-1R, is also undergoing clinical evaluation for the treatment of type 2 diabetes (28,39). The data presented here describe the results of introducing two specific modifications of the native GLP-1 molecule, one to engineer DPP-IV resistance, the second to achieve chemical coupling of the GLP-1 analog (CJC-1131) to serum albumin. Albumin is known to exhibit a longer $t_{1/2}$ in vivo, ~19 days in humans (40), which is much greater than the $t_{1/2}$ of short-lived regulatory peptides such as GLP-1. Accordingly, peptide binding to albumin has been used to improve the pharmacokinetic properties of several smaller proteins, including Fab antibody fragments (33), coagulation factor VIIa inhibitor 1a (41), and insulin (42). The present results indicate that a much larger albumin–GLP-1 covalently linked conjugate retains the biological properties of native GLP-1, including binding to and activation of the GLP-1R in vitro. Furthermore, the glucose-lowering effects of CJC-1131 were abolished in GLP-1R−/− mice, providing important complementary evidence that the actions of CJC-1131 in vivo are mediated by the known GLP-1R.

Several experimental results provide indirect evidence for a sustained duration of CJC-1131 action on glucose lowering in vivo. First, oral glucose tolerance remained significantly improved in mice even 12 h after a single CJC-1131 injection. Furthermore, fed glucose remained significantly lower in db/db mice treated with CJC-1131, even 1 week after the last CJC-1131 injection. These findings are consistent with data demonstrating sustained improvements in insulin secretion in Zucker diabetic fatty rats detectable even 1 week following a transient GLP-1 infusion (43). Similarly, blood glucose remained lower in exendin-4–treated partially pancreactectomized rats several weeks following discontinuation of exendin-4 administration (5). Although fed glucose and glucose tolerance were clearly improved in db/db mice treated with CJC-1131, significant hyperglycemia persisted even during repeated CJC-1131 administration. In contrast, daily administration of exendin-4 to younger, less severely diabetic db/db mice (starting fasting hyperglycemia of 5.1 mmol/l) prevented the progression to more severe hyperglycemia over the 2-week treatment period (44). However, a 2-week treatment of older, more severely diabetic db/db mice with twice daily administration of 200 $\mu$g/kg NN2211 or exendin-4 (100 $\mu$g/kg) did not prevent ongoing deterioration in glucose tolerance, with mice remaining very hyperglycemic even after the 2-week treatment period (45). Hence the effects of chronic GLP-1 agonist administration in the db/db mouse appear dependent, in part, on the clinical severity of the diabetes and age of the mice at the onset of treatment.

Although treatment of rodents and human subjects with GLP-1R agonists has been associated with prevention of weight gain or modest weight loss (12,25,46), we did not observe a significant reduction in body weight in db/db mice treated with CJC-1131 over the 4-week treatment period. Similarly, body weight was not significantly reduced in db/db mice treated with NN2211 or exendin-4 over comparable 2- to 4-week treatment periods (45,47). At present, it remains unclear whether restricted transport of an albumin–GLP-1 conjugate across the blood-brain barrier might compromise the ability of molecules such as albumin–CJC-1131 to reduce food intake and weight gain in vivo. Nevertheless, significant weight loss was observed in control C57+/+ mice treated with CJC-1131 over the same time period. Furthermore, intravenous infusion of CJC-1131 rapidly induced c-fos immunoreactivity in several regions of the rat central nervous system (H. Yamamoto, J. Elmquist, and D.J.D., unpublished observations), providing complementary evidence suggesting that an albumin–GLP-1 conjugate is capable of activating central nervous system functions in vivo.

Despite the failure to observe normalization of glycemia in the older db/db mouse, CJC-1131 administration increased islet area, with a significant increase in both the number and the relative percentage of small and large islets detected following 4 weeks of CJC-1131 treatment. Furthermore, the number of BrdU+ islet cells was significantly increased in CJC-1131–treated mice, consistent with previous studies demonstrating that activation of the GLP-1R is coupled to expansion of islet mass in the db/db mouse (6,44,45). Hence, although fenestration of islet capillaries does restrict access and reduce permeability toward larger molecules in the islet microcirculation (48), the comparatively larger CJC-1131–albumin conjugate apparently retains the ability to promote islet growth and enhance insulin gene expression in the mouse in vivo.

Despite the increase in islet area and proinsulin mRNA transcripts in CJC-1131–treated mice, we did not observe a significant increase in insulin content in the db/db mice treated with CJC-1131. Indeed, pancreatic insulin content was modestly decreased in control and CJC-1131–treated db/db mice relative to C57+/+ mice consistent with the functional exhaustion of $\beta$-cell insulin stores in db/db mice with blood glucose levels approaching 20 mmol/l.

Given the increasing interest in strategies based on enhancement of incretin action for the treatment of diabetes, there remains an unmet need for developing approaches that circumvent the requirement for daily or continuous administration of GLP-1 analogs. Accordingly, long-acting or slow-release forms of GLP-1 analogs that exhibit desirable therapeutic properties are awaited with interest. The administration of albumin-based GLP-1 derivatives should confer a longer circulating $t_{1/2}$ to the newly derived albumin-peptide conjugate, compared with the native GLP-1 peptide alone (32,33). The data presented here demonstrate that a prototype DPP-IV–resistant GLP-1 analog that covalently couples to albumin, CJC-1131, retains the biological properties generally ascribed to GLP-1R agonists. The findings that CJC-1131 binds to and activates the GLP-1R, lowers blood glucose, increases proinsulin gene expression, and stimulates islet cell proliferation suggest that further assessment of the efficacy and mechanisms of action of albumin-based GLP-1 derivatives appears warranted.
REFERENCES

13. Drucker DJ: Development of glucagon-like peptide-1-based pharmaceuti-
16. Kieffer TJ, McIntosh CH, Pederson RA: To present this data in abstract form at the 2002 Annual Meeting of the American Diabetes Association. D.J.D. is a Senior Scientist of the Canadian Institutes of Health Research.

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