Cardioprotective and Vasodilatory Actions of Glucagon-Like Peptide 1 Receptor Are Mediated Through Both Glucagon-Like Peptide 1 Receptor–Dependent and –Independent Pathways

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Background—The glucagon-like peptide 1 receptor (GLP-1R) is believed to mediate glucoregulatory and cardiovascular effects of the incretin hormone GLP-1(7-36) (GLP-1), which is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) to GLP-1(9-36), a truncated metabolite generally thought to be inactive. Novel drugs for the treatment of diabetes include analogues of GLP-1 and inhibitors of DPP-4; however, the cardiovascular effects of distinct GLP-1 peptides have received limited attention.

Methods and Results—Here, we show that endothelium and cardiac and vascular myocytes express a functional GLP-1R as GLP-1 administration increased glucose uptake, cAMP and cGMP release, left ventricular developed pressure, and coronary flow in isolated mouse hearts. GLP-1 also increased functional recovery and cardiomyocyte viability after ischemia-reperfusion injury of isolated hearts and dilated preconstricted arteries from wild-type mice. Unexpectedly, many of these actions of GLP-1 were preserved in Glp1r−/− mice. Furthermore, GLP-1(9-36) administration during reperfusion reduced ischemic damage after ischemia-reperfusion and increased cGMP release, vasodilation, and coronary flow in wild-type and Glp1r−/− mice, with modest effects on glucose uptake. Studies using a DPP-4–resistant GLP-1R agonist and inhibitors of DPP-4 and nitric oxide synthase showed that the effects of GLP-1(7-36) were partly mediated by GLP-1(9-36) through a nitric oxide synthase–requiring mechanism that is independent of the known GLP-1R.

Conclusions—These data describe cardioprotective actions of GLP-1(7-36) mediated through the known GLP-1R and novel cardiac and vascular actions of GLP-1(7-36) and its metabolite GLP-1(9-36) independent of the known GLP-1R. Our data suggest that the extent to which GLP-1 is metabolized to GLP-1(9-36) may have functional implications in the cardiovascular system. (Circulation. 2008;117:2340-2350.)

Key Words: diabetes mellitus ■ nitric oxide synthase ■ physiology ■ receptors ■ reperfusion

Glucagon-like peptide 1 (GLP-1) is a 30–amino acid gut hormone secreted in a nutrient-dependent manner that stimulates insulin secretion and inhibits glucagon secretion and gastric emptying, thereby reducing postprandial glycemia.1,2 GLP-1 is derived from posttranslational proteolysis of proglucagon, and its peptide sequence is identical in mouse, rat, and human.2,3 Active isoforms of GLP-1 include GLP-1(7-36) amide and glycine-extended GLP-1(7-37).4,5 After secretion from enteroendocrine L cells, GLP-1(7-36) amide is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) to its N-terminally truncated metabolite GLP-1(9-36), which does not interact with the known GLP-1 receptor.6,7

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The diverse actions of GLP-1 include the proliferation, differentiation, and protection from apoptosis of pancreatic β cells and the induction of satiety. GLP-1 also improves memory and learning, stimulates afferent sensory nerves, and has neuroprotective functions.1,8 Furthermore, GLP-1 receptor agonists have been reported to have cardiac and vascular actions in rodents and humans that include effects on contractility, blood pressure, cardiac output,9–12 and cardioprotection.13–16

GLP-1 is widely believed to exert its actions through a distinct heptahelical G protein–coupled receptor (GLP-1R)
functionally associated with adenylate cyclase through the stimulatory Gₐ. We previously showed that mice lacking a functional GLP-1R (Glp1r⁻/⁻) exhibit reduced resting heart rate, elevated left ventricular (LV) end-diastolic pressure, and increased LV thickness compared with wild-type (WT) CD-1 controls. Moreover, Glp1r⁻/⁻ mice exhibited impaired contractile responses to insulin and epinephrine. Although GLP-1R is expressed in β cells and throughout the gut, lung, kidney, heart, and central nervous system, including autonomic nuclei that control cardiovascular functions, the specific cellular localization, relative abundance, and functional importance of the GLP-1R in cardiovascular tissues have not been fully defined.

Here, we show in the C57Bl/6 strain that GLP-1R is expressed throughout the mouse cardiovascular system, with notable absence in cardiac fibroblasts and particular abundance in the endocardium. Remarkably, we demonstrate that some cardioprotective and vasodilatory actions of GLP-1(7-36) are independent of the known GLP-1R and are mediated, at least in part, by its metabolite GLP-1(9-36). These data extend previous findings regarding GLP-1(9-36) in dog and suggest the existence of an alternative signaling mechanism for GLP-1 and its metabolite in the mouse cardiovascular system. Importantly, our results imply that drugs targeting GLP-1R activation (GLP-1R agonists) versus GLP-1 degradation (DPP-4 inhibitors) for the treatment of diabetes may have different cardiovascular consequences.

Methods

Animals

Experiments conformed to protocols approved by Toronto General Hospital. C57Bl/6 mice were obtained from Charles River (Montreal, Quebec, Canada) and housed for at least 2 weeks before experimentation. The nature and origins of the Glp1r⁻/⁻ mice, now backcrossed for >6 generations in C57Bl/6, have been described. In contrast to our earlier use of Glp1r⁻/⁻ mice in the CD1 background and nonlittermate CD1 controls, all current studies were conducted in 10- to 12-week-old male WT and Glp1r⁻/⁻ littermates generated from heterozygous crosses.

Immunohistochemistry

Details on tissue preparation, staining, and microscopy shown in Figure 1 are provided in the online Data Supplement.

Cell Culture

BHK fibroblasts were grown in DMEM with 4.5 g/L glucose and 5% calf serum. Cells were transfected with a rat GLP-1R–encoding expression vector, and stably transfected cell populations were selected in G418 at 0.8 mg/mL for 2 weeks until confluent cultures were harvested for Western blot analysis. The mouse aortic smooth muscle cell (SMC) line (MOVAS) was grown as previously described.

Reverse-Transcription Polymerase Chain Reaction and Western Blot

GLP-1R–specific primers, protein extraction, and quantification were as described, with details provided in the Data Supplement.

Reagents

GLP-1 [GLP-1(7-36)] and GLP-1(9-36) were synthesized by Bachem (Torrance, Calif), and extendin-4 and extendin(9-39) were produced by California Peptide (Napa, Calif). The DPP-4 inhibitor sitagliptin was obtained commercially, and l-phenylephrine, acetylcholine, N⁶-nitro-l-arginine (L-NNA), and N⁶-monomethyl-l-arginine (L-NNAME) were from Sigma-Aldrich (St Louis, Mo). All other chemicals used were reagent grade (Sigma, Mississauga, Ontario, Canada).

Isolated Heart Preparations

Male 12-week-old mice were used in all experiments. Only isolated WT and Glp1r⁻/⁻ hearts exhibiting a heart rate >350 bpm (90% of all hearts available) were used in this study. Further details are provided in the Data Supplement.

Ischemia-Reperfusion

Hearts underwent a 20-minute equilibration phase followed by a 40-minute perfusion phase during which all hemodynamic parameters were continuously recorded. We generated 30 minutes of sustained global ischemia by clamping inflow to the heart; we then reperfused hearts for 40 minutes. For the pretreatment groups, agents were added to the perfusion buffer for only the final 20 minutes of the perfusion phase. For the posttreatment groups, agents were administered only for the first 20 minutes of the reperfusion phase (see Figure 2A). Recovery of LV developed pressure (LVDP) was measured at the end of reperfusion and was expressed as a percentage of the LVDP at the end of perfusion (ie, before ischemia).

Cell Viability

Release of the muscle enzyme lactate dehydrogenase (LDH) was used as a measure of cell viability as described in the Data Supplement.

cAMP and cGMP Assays

Isolated hearts were perfused first for 10 minutes with buffer containing the phosphodiesterase inhibitor IBMX (100 µmol/L; Sigma) to inhibit cAMP and cGMP degradation. Coronary effluent samples were then collected at timed intervals and used in either a cAMP radioimmunoassay kit (Amersham, Little Chalfont, UK) or a cGMP enzymatic assay kit (Amersham, Piscataway, NJ) and expressed as a function of coronary flow and heart weight (U/mL/g).

Glucose Uptake

Coronary effluent of isolated perfused normoxic hearts every 5 minutes during the infusion of GLP-1 or GLP-1(9-36) and measured glucose concentrations with an analyzer (Analogos Instruments, Lunenburg, Mass). Myocardial glucose uptake (mg/dL) was calculated as follows: [(Gₑ−Gₑ₀)−(Gₑ−Gₑ₀)]concentrations with an analyzer (Analox Instruments, Lunenberg, Mass). Myocardial glucose uptake (mg/dL) was calculated as follows: [(Gₑ−Gₑ₀)−(Gₑ−Gₑ₀)]coronary flow rate (mL/min)/heart weight (g), where Gₑ is glucose concentration (inflow) and Gₑ₀ is glucose concentration (outflow).

Perfusion Myography

A video-dimension analyzer and pressure-controlled 3-N(Morpholino) propane sulfonic acid–filled perfusion chamber (Living Systems Instrumentation, Burlington, Vt) were used as previously described. Additional details are provided in the Data Supplement.

Statistical Analyses

All data are presented as mean±SE. One-way ANOVA was used at specific time points in Figures 2C, 2D, 3, 4A, 4B, 6B, and 6C as described in the legends. If the ANOVA was significant, the Student-Newman-Keuls post hoc test was used to specify which groups were significantly different from each other. Repeated-measures ANOVA was used to evaluate the main effects of group, time (or dose), and the interaction of group by time (or dose) for data shown in Figures 4C, 5, and 6A and Figures 1 through in the Data Supplement. All analyses were performed with SPSS 13.0 (SPSS Inc, Chicago, Ill). Statistical significance was considered at values of P<0.05.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
To study the distribution of GLP-1R in mouse cardiovascular tissues, we first established the specificity of a GLP-1R antibody. Pancreatic islet cells showed abundant GLP-1R-specific labeling (Figure 1A), with complete loss of staining after preadsorption with a synthetic GLP-1R peptide (Figure 1B), or omission of the primary antibody and replacement with rabbit IgG (data not shown). Double labeling of mesenteric arteries with anti-smooth muscle α-actin (red), anti-GLP-1R (green), and Hoechst nuclear stain (blue) revealed GLP-1R expression on medial SMCs (arrows in C). Hearts labeled with anti–sarcomeric α-actinin (red), anti-GLP-1R (green), and Hoechst nuclear stain (blue) revealed GLP-1R expression on cardiomyocytes (F), endocardium (arrowheads in G), vascular endothelium, and SMCs (arrowheads and arrows, respectively, in H). Omitting primary antibody (D, I) or replacing it with rabbit IgG (E, J) resulted in complete loss of GLP-1R–specific signal. Reverse-transcription polymerase chain reaction demonstrated GLP-1R mRNA in liver, lung, and heart of WT mice (K-I), and Western blots demonstrated GLP-1R protein in these same organs (K-II) and in the LV, right ventricle (RV), interventricular septum, and atrium. Specificity of the antibody was further established in BHK cells transfected with vector only (left), cells transfected with GLP-1R (middle), and MOVAS (right). GAPDH and β-actin served as loading controls for polymerase chain reaction and Western blots, respectively. Scale bar=100 μm (A, B), 50 μm (G through E, H through J), and 30 μmol/L (F, G).

Results

To study the distribution of GLP-1R in mouse cardiovascular tissues, we first established the specificity of a GLP-1R antibody. Pancreatic islet cells showed abundant GLP-1R-specific labeling (Figure 1A), with complete loss of staining after preadsorption with a synthetic GLP-1R peptide (Figure 1B), or omission of the primary antibody and replacement with rabbit IgG (data not shown). Double labeling of mesen-
teric vessels revealed GLP-1R expression in SMCs of the media (Figure 1C through 1E). GLP-1R immunopositivity also localized to cardiomyocytes, endocardium, microvascular endothelium, and coronary SMCs (Figure 1F through 1J). In contrast, costaining with an anti-DDR2 antibody showed that cardiac fibroblasts did not express GLP-1R (data not shown). The intensity of anti–GLP-1R labeling was highest on the endocardium and more heterogeneous on vessels.

Reverse-transcription polymerase chain reaction demonstrated the presence of a GLP-1R–specific transcript, with levels of expression highest in lung, lower but easily detectable in heart, and much lower in liver (Figure 1K).

Figure 2. Functional recovery after I/R injury in WT and Glp1r−/− hearts pretreated with GLP-1 or exendin-4. A, Experimental protocol showing sequence and duration of peptide infusions, ischemia, and reperfusion. B, Representative LVDP recordings from isolated perfused hearts. Scale bars: y axis = 100 mm Hg; x axis = 10 minutes. C, Effect of no treatment (control; n = 21), GLP-1 (0.3 nmol/L; n = 15), and exendin-4 (n = 6 for each dose) pretreatments on isolated WT hearts undergoing I/R. D, Pretreatment with GLP-1 resulted in significant protection from I/R injury in both WT (n = 15) and Glp1r−/− (n = 10) hearts vs untreated controls (WT, n = 21; Glp1r−/−, n = 8). All data shown are mean ± SE. *P < 0.05 vs control untreated WT or Glp1r−/−; #P < 0.05 vs GLP-1–treated Glp1r−/− hearts by 1-way ANOVA at 110 minutes.
Figure 3. Functional recovery after I/R injury in WT and Glp1r⁻⁻⁻ hearts treated with GLP-1(9-36). A, Pretreatment with GLP-1(9-36) (n=6 for each dose) impaired functional recovery compared with untreated (n=21) or GLP-1–pretreated (n=15) hearts from WT mice. B, Administration of GLP-1(9-36) during reperfusion (posttreatment, n=6 to 8) improved functional recovery in WT and Glp1r⁻⁻⁻ hearts. *P<0.05 vs control untreated WT or Glp1r⁻⁻⁻ by 1-way ANOVA at 110 minutes. C, Area under the LDH release curve was integrated to determine total LDH release during the 40-minute reperfusion period (n=5 for each). Pretreatment with GLP-1, but not GLP-1(9-36), and posttreatment with GLP-1(9-36) reduced LDH release. Because no benefit of GLP-1(9-36) pretreatment was observed in WT hearts, its effect in Glp1r⁻⁻⁻ was not examined. All data shown are mean±SE. *P<0.05 by 1-way ANOVA.
Western blotting with the same antibody used for immunofluorescence confirmed GLP-1R protein in these organs (Figure 1K, II), including all chambers of the heart, with considerably lower expression noted in atria (Figure 1K, III). GLP-1R mRNA (not shown) and protein (Figure 1K, IV) were not detectable in BHK fibroblasts; however, GLP-1R protein was easily detected in BHK cells transfected with rat GLP-1R cDNA and in MOVAS cells (Figure 1K, IV).

To determine the functional importance of GLP-1R, we studied isolated mouse heart preparations subjected to ischemia-reperfusion (I/R) (Figure 2A). Representative tracings (Figure 2B) of LVDP and grouped data (Figures 2 and 3) showed that in WT mouse hearts, pretreatment with GLP-1 (0.3 nmol/L) significantly improved recovery of LVDP after I/R injury compared with untreated controls (71.1±4.2%, n=15, versus 29.6±1.9%, n=21; P<0.05; Figure 2B and 2C). Pretreatment with >10-fold-higher doses of the degradation-resistant GLP-1R agonist exendin-4 (5 nmol/L but not 0.3 or 3 nmol/L) resulted in a similar level of protection from I/R injury in WT mice (68.2±5.6%; n=6 for each dose; P<0.05; Figure 2C). Surprisingly, the cardioprotective effects of GLP-1 (0.3 nmol/L) remained evident in mice lacking a functional GLP-1R (64.1±5.9%, n=10, versus 25.9±5.3%, n=8; P<0.05; Figure 2B and 2D), whereas the salutary effects of exendin-4 (5 nmol/L) were reduced in
Glp1r−/− hearts (39.9 ± 3.1%; n = 5; P < 0.05; Figure 2C and 2D). These results suggest that some of the cardioprotective effects of native GLP-1 may be mediated through a mechanism independent of the known GLP-1R.

Of note, an increase in LVDP was observed during pretreatment with GLP-1 in WT but not Glp1r−/− hearts (26.7 ± 3.9 mm Hg for WT versus −2.6 ± 0.6 mm Hg for Glp1r−/−; P < 0.01), whereas pretreatment with GLP-1(9-36) had no such effect (Figure 3A and 3B). These data suggest that GLP-1, but not GLP-1(9-36), has direct inotropic action via the GLP-1R. Given previous reports of the ability of GLP-1 to increase cAMP levels in isolated rat cardiomyocytes,36 we next tested whether GLP-1 would increase cAMP production in perfused mouse hearts. Consistent with its observed inotropic action, GLP-1 (0.3 nmol/L) significantly increased cAMP release into coronary effluent (see Figure I in the Data Supplement).

We next examined whether GLP-1(9-36), generated from GLP-1(7-36) by DPP-4–mediated cleavage, might be responsible for some of the cardiovascular actions previously attributed to full-length GLP-1(7-36). Unexpectedly, pretreatment with GLP-1(9-36) worsened functional recovery after I/R compared with untreated WT controls (11.0 ± 1.0%, n = 6; versus 29.6 ± 1.9%, n = 21; P < 0.05; Figure 3A). In contrast, treatment with GLP-1(9-36) during the reperfusion phase significantly enhanced functional recovery in hearts from WT and Glp1r−/− mice (WT: 52.5 ± 8.0%, n = 8; Glp1r−/−: 44.8 ± 4.0%, n = 6; P < 0.05 versus untreated controls; Figure 3B). These results imply that the cardioprotective effects of GLP-1 are mediated, at least in part, by the

![Graphs](https://via.placeholder.com/150)

Figure 5. GLP-1 and GLP-1(9-36), but not exendin-4, have vasodilatory effects on mesenteric arteries from WT and Glp1r−/− mice. A, Vasodilatory effects of GLP-1(9-36) (n = 6; EC50, 10 μmol/L) did not differ from those of GLP-1 (n = 6; EC50, 3.8 μmol/L) in WT arteries, whereas the GLP-1R agonist exendin-4 (n = 3) did not produce vasodilation. B, Effect of DPP-4 inhibition (DPPi) using sitagliptin (5 μmol/L) to prevent generation of GLP-1(9-36) in WT arteries. C, Vasodilatory responses induced by GLP-1 in arteries from Glp1r−/− (n = 6) and WT (n = 6) arteries. D, Vasodilatory responses induced by GLP-1(9-36) in Glp1r−/− (n = 6) and WT arteries (n = 6). E, F, Vasodilatory responses from both GLP-1 and GLP-1(9-36) in WT arteries (n = 6) were completely abolished by pretreatment with L-NNA (10−4 mol/L). Data were normalized to passive diameter (PD; Ca2+-free buffer at 60 mm Hg) and are mean ± SE. The effects of group, dose, and group-by-dose interaction were evaluated by repeated-measures ANOVA, with levels of significance shown.
metabolite GLP-1(9-36) via mechanism(s) independent of the GLP-1R.

LDH release was measured in coronary effluent to assess the extent of cardiomyocyte damage. LDH levels during equilibrium and perfusion phases (Figure 2A) were similar in all groups, suggesting that the peptides themselves did not affect baseline cardiomyocyte viability (data not shown). However, after global ischemia, the amount of LDH released (area under the curve) during the reperfusion phase correlated with measures of functional recovery (Figure 3C). WT and Glp1r−/− mice pretreated with GLP-1 showed lower LDH release (WT: 26 018 ± 3720 U · mL⁻¹ · min⁻¹ · g⁻¹; Glp1r−/−: 33 800 ± 3590 U · mL⁻¹ · min⁻¹ · g⁻¹; n=5 each) compared with untreated controls (WT: 64 222 ± 4841 U · mL⁻¹ · min⁻¹ · g⁻¹; Glp1r−/−: 59 477 ± 4876 U · mL⁻¹ · min⁻¹ · g⁻¹; n=5 each; P<0.05). Posttreatment with GLP-1(9-36) also reduced
vasodilatory actions, we studied mesenteric arteries partially
L-NNA (10⁻⁴ mol/L), an inhibitor of NO synthase (NOS).
L-NNA significantly inhibited vasodilation in response to
GLP-1 and GLP-1(9-36) (P<0.0001; Figure 5E and 5F),suggesting that the vasodilatory effects of both peptides
require NOS.
To explore potential mechanisms underlying the cardioprotective effects of GLP-1(9-36), we examined its ability to improve myocardial glucose uptake. In contrast to the robust
increase in glucose uptake observed with GLP-1, nor-
moxic hearts perfused with GLP-1(9-36) demonstrated only a
trend toward a very modest increase in glucose uptake (Figure 6A).
We next examined the potential role of NOS in the cardioprotective effects of GLP-1. There was a substantial reduction in GLP-1–mediated functional recovery after I/R in hearts pretreated with the NOS inhibitor L-NAME (50 µmol/L for 20 minutes; Figure 6B). However, NOS inhibition did not completely abolish the protective effect of GLP-1 in WT hearts (L-NAME: 37.1±4.0%; L-NAME+GLP-1: 49.7±9.1%; n=3 each; versus GLP-1 alone: 71.1±4.2%; n=15; P<0.05; Figure 6B).
Finally, to investigate the importance of GLP-1(9-36) for the cardioprotective actions of full-length GLP-1, a DPP-4 inhibitor (sitagliptin) was added to all perfusion buffers. Although sitagliptin alone had no significant effect on the function of normoxic WT and Glp1r⁻/⁻ hearts (n=3 each; P=NS) or on the protective effects of GLP-1 on WT hearts, it abolished the beneficial effects of GLP-1 on Glp1r⁻/⁻ hearts undergoing I/R (n=5; Figure 6B). These data clearly impli-
cate GLP-1(9-36) as a critical component of the cardioprotective effects of native GLP-1.

Discussion
In the present study, we localized GLP-1R expression in cardiac tissues of the mouse. Although previous studies demonstrated the presence of GLP-1R mRNA transcripts in cardiac RNA, we have now localized GLP-1R expression to distinct heart chambers and cell types, including cardio-
myocytes and endothelial and vascular SMCs, but not fibro-
blasts, of the normal adult mouse heart. Furthermore, both immuno
centrone and Western blot analyses demonstrate expression of the translated GLP-1R in SMCs of mesenteric arteries and in an aortic SMC cell line.

Although evidence from multiple studies suggests that
GLP-1 has important cardiovascular actions, the mecha
nisms underlying these diverse effects have not been fully elucidated. Our results propose a novel 2-pathway schema for cardiovascular actions of GLP-1, one that depends
on the GLP-1R for inotropic action, glucose uptake, ischemic
preconditioning, and mild vasodilatory actions and the second that depends on rapid metabolism of GLP-1 to GLP-1(9-36), the latter having GLP-1R–independent effects on postische
mic recovery of cardiac function and vasodilation. Our results also suggest that GLP-1(9-36) is not an inotrope, has at best
modest effects on myocardial glucose uptake in vitro, and
causes vasodilatation through an NO/cGMP-dependent
mechanism, which also participates in cardioprotective ef
fets in the setting of I/R injury.

Pretreatment of isolated WT hearts with GLP-1 (0.3 nmol/L) significantly enhanced recovery of LVDP and reduced cellular damage as measured by LDH release after I/R injury. Somewhat surprisingly, it took a much larger dose of the GLP-1R agonist exendin-4 (5 nmol/L), a potent degradation-resistant GLP-1R agonist, to reproduce the protective effect of native GLP-1. These findings are consistent with the existence of both GLP-
1R–dependent and GLP-1R–independent pathways that mediate the cardioprotective effects of GLP-1. Our finding that pretreat-
ment with GLP-1 (0.3 nmol/L) afforded as much protection from I/R injury in Glp1r−/− hearts as in WT hearts provides direct genetic evidence for the existence of a GLP-1R–independent pathway coupled to cardioprotection. Furthermore, administration of GLP-1(9-36) after ischemia greatly augmented functional recovery and decreased LDH release in hearts from WT and Glp1r−/− mice. Mass spectrometry–based identification of GLP-1(9-36) in coronary effluents revealed that this metabolite is highly stable (data not shown), suggesting that its inability to effect cardioprotection as a pretreatment is not simply attributable to degradation. Together, these findings clearly suggest an important role for the DPP-4–generated GLP-1(9-36) metabolite acting through a GLP-1R–independent mechanism to produce protection from I/R injury.

We also observed that both GLP-1 and GLP-1(9-36) produce increased coronary flow in constant-pressure perfused isolated hearts and vasodilatation of resistance-level mesenteric arteries from WT and Glp1r−/− mice. Furthermore, this vasodilatory effect correlated with presumably NO-dependent cGMP release. Importantly, unlike GLP-1 or GLP-1(9-36), exendin-4 did not produce vasodilatation or cGMP release. Together, these results strongly suggest that the vascular effects of GLP-1 demonstrated here are mediated through GLP-1(9-36) and a GLP-1R–independent mechanism acting, at least in part, through NOS-dependent cGMP formation.

Several independent lines of evidence support multiple biological roles for GLP-1(9-36) likely acting through a structurally and functionally distinct receptor. Exendin(9-36), a classic GLP-1R antagonist, did not block the inhibitory actions of GLP-1 on gastrointestinal motility or gastric acid secretion.44 Furthermore, GLP-1 increased basal and acute insulin-stimulated glucose uptake and GLUT1 and GLUT4 protein levels in fully differentiated 3T3-L1 adipocytes, in which the existence of the known GLP-1R has not been reported.45,46 Finally, with specific reference to the cardiovascular system, infusions of GLP-1(9-36) in conscious dogs with dilated cardiomyopathy improved LV performance and increased myocardial glucose uptake in vivo, suggesting that GLP-1(9-36) was an independent, biologically active substance with its own functional receptor.23

Conclusions

The present data suggest a model wherein the rapidly produced metabolite of GLP-1, GLP-1(9-36), affords significant protection against I/R injury and induces vasodilatation via an NO/cGMP-associated mechanism that does not require a functional GLP-1R. With the rapidly expanding clinical use of GLP-1R agonists and DPP-4 inhibitors for the treatment of type II diabetes and obesity, it seems prudent to learn more about the cardiovascular effects of these agents that may be associated with substantial differences in circulating levels of GLP-1(9-36). Given the potentially deleterious long-term consequences of increased cAMP generation in the heart, our results suggest that additional studies examining the cardiovascular consequences of GLP-1R signaling in the cardiovascular system appear warranted.

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Disclosures

Dr Drucker has served as an advisor or consultant within the past 2 months to Amgen Inc, Amylin Pharmaceuticals, Arisaph Pharmaceuticals Inc, Chugai Inc, ConjuChem Inc, Eli Lilly Inc, Emisphere Technologies Inc, Glaxo Smith Kline, Glenmark Pharmaceuticals, Isis Pharmaceuticals Inc, Johnson & Johnson, Merck Research Laboratories, Novartis Pharmaceuticals, Novo Nordisk Inc, NPS Pharmaceuticals Inc, Phenomix Inc, Takeda, and Transition Pharmaceuticals Inc. Neither Dr Drucker nor his family members hold stock directly or indirectly in any of these companies. The other authors report no conflicts.

References

Two therapeutic strategies based on potentiation of glucagon-like peptide 1 action are now used to treat type 2 diabetes mellitus: glucagon-like peptide 1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. Most GLP-1R agonists under development for the treatment of diabetes are resistant to cleavage by the DPP-4 enzyme; many also exhibit glucose incompetence after disruption of the glucagon-like peptide 1 receptor gene.

These findings suggest that GLP-1(9-36) itself may have therapeutic benefit in the setting of cardiovascular injury, an independent of the known GLP-1R, invoking a novel signaling mechanism for the DPP-4–generated GLP-1 metabolite.

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CLINICAL PERSPECTIVE