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

Kiwon Ban, MSc; M. Hossein Noyan-Ashraf, PhD; Judith Hoefer, MD; Steffen-Sebastian Bolz, MD, PhD; Daniel J. Drucker, MD*; Mansoor Husain, MD*

***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, vasodilatation, 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;117:2348-2358.)**

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 preproglucagon, 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)

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The online Data Supplement, which contains a supplemental Methods section and figures, can be found with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.107.739938/DC1.

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functionally associated with adenylate cyclase through the stimulatory G_s.[17,18] 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.[19] Although GLP-1R is expressed in β cells and throughout the gut, lung, kidney, heart,[20,21] and central nervous system, including autonomic nuclei that control cardiovascular functions,[11,22] 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[23] 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.[11,19,24–28] In contrast to our earlier use of $Glp1r^{-/-}$ mice in the CD1 background and nonlittermate CD1 controls,[19] 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,[29] 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.[30]

Reverse-Transcription Polymerase Chain Reaction and Western Blot

GLP-1R–specific primers, protein extraction, and quantification were as described,[31–33] 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 exendin-4 and exendin(9-39) were produced by California Peptide (Napa, Calif). The DPP-4 inhibitor sitagliptin was obtained commercially, and L-phenylephrine, acetylcholine, N^G-nitro-L-arginine (L-NNA), and N^G-monomethyl-L-arginine (L-NAME) 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 ($U \cdot mL^{-1} \cdot min^{-1} \cdot g^{-1}$).

Glucose Uptake

Following a published methodology,[34] we collected the 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 (Analox Instruments, Lunenberg, Mass). Myocardial glucose uptake (mg/dL) was calculated as follows: $[G_{in} - G_{out}] \times$ coronary flow rate (mL/min)/heart weight (g), where G_{in} is glucose concentration (inflow) and G_{out} is glucose concentration (outflow).

Perfusion Myography

A video-dimension analyzer and pressure-controlled 3-N(Morpholino) propane sulfonfic acid–filled perfusion chamber (Living Systems Instrumentation, Burlington, Vt) were used as previously described.[35] 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 I 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.

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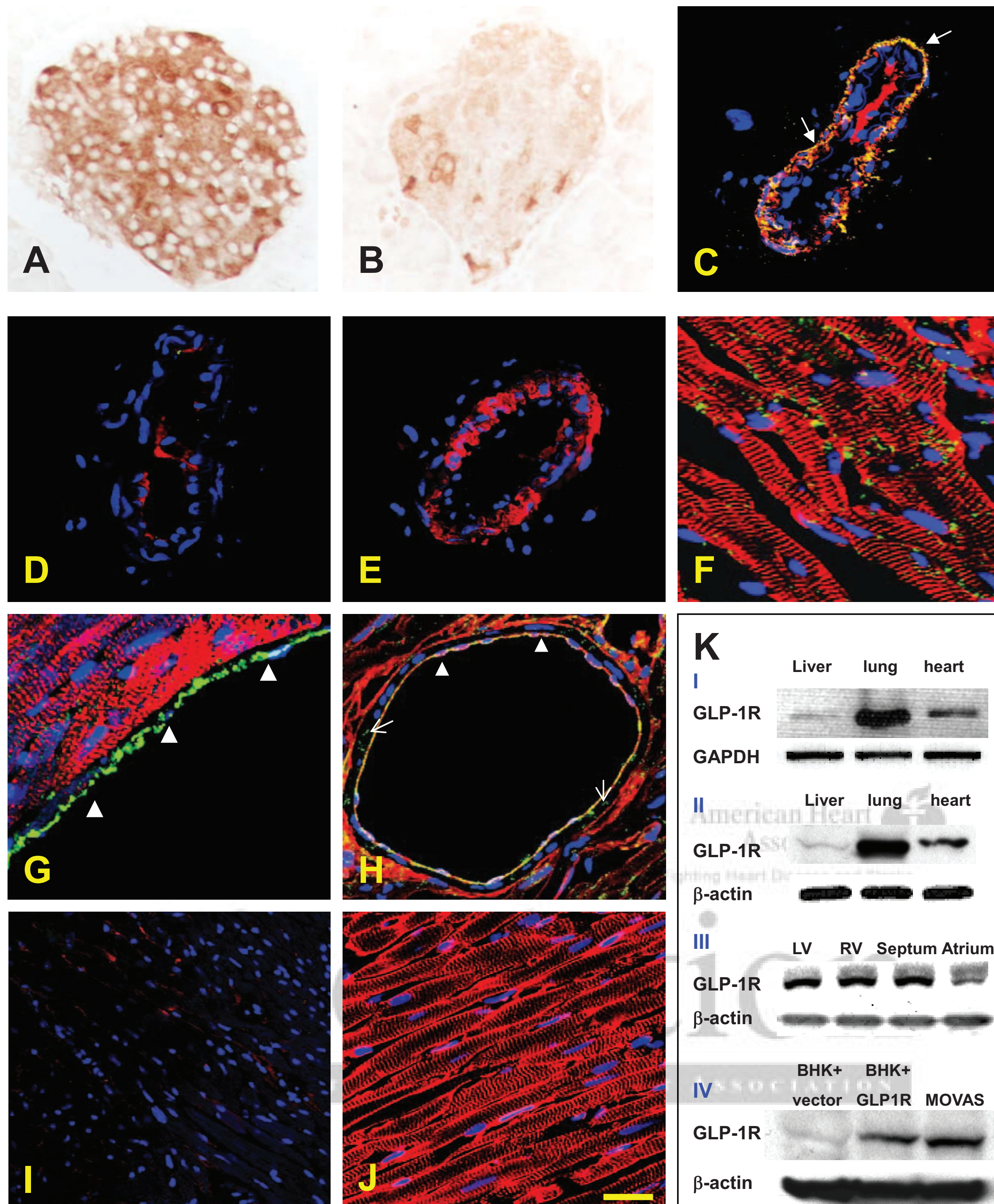

Figure 1. GLP-1R expression in mouse cardiac and vascular tissues. Staining of pancreatic islets by the polyclonal anti–GLP-1R antibody (A) was eliminated by preadsorption with a GLP-1R–specific peptide (B). Labeling of mesenteric arteries with anti–smooth muscle α-actin (red), anti–GLP-1R (green), and 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 (C 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-

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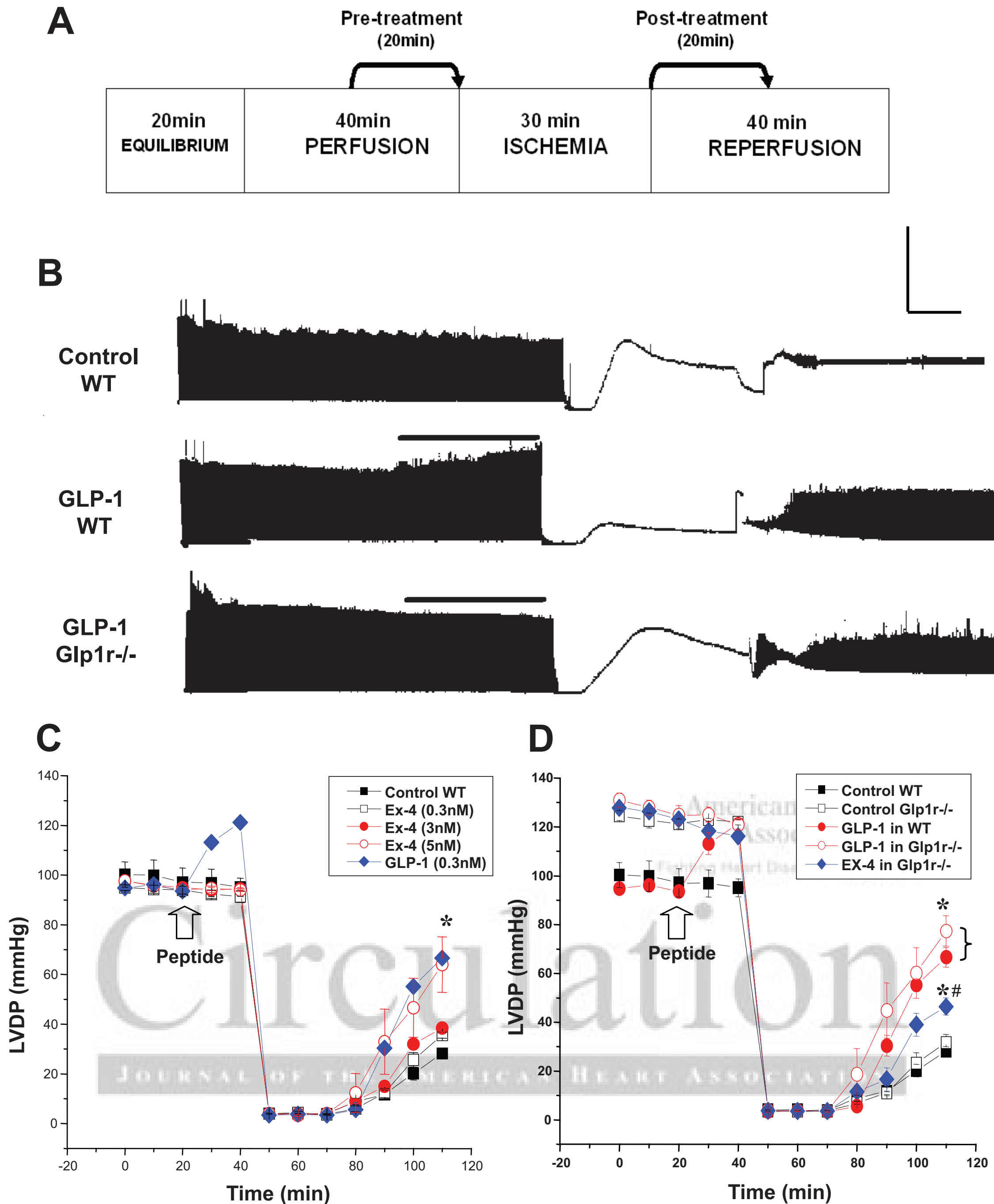

Figure 2. Functional recovery after I/R injury in WT and Glp1r$^{-/-}$ hearts pretreated with GLP-1 or exendin-4. A, Experimental protocol showing sequence and duration of peptide infusions, ischemia, and reperfusion. B, Representative LVDP recordings from isolated perfused hearts. Scale bars: *y* axis=100 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.

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, I).

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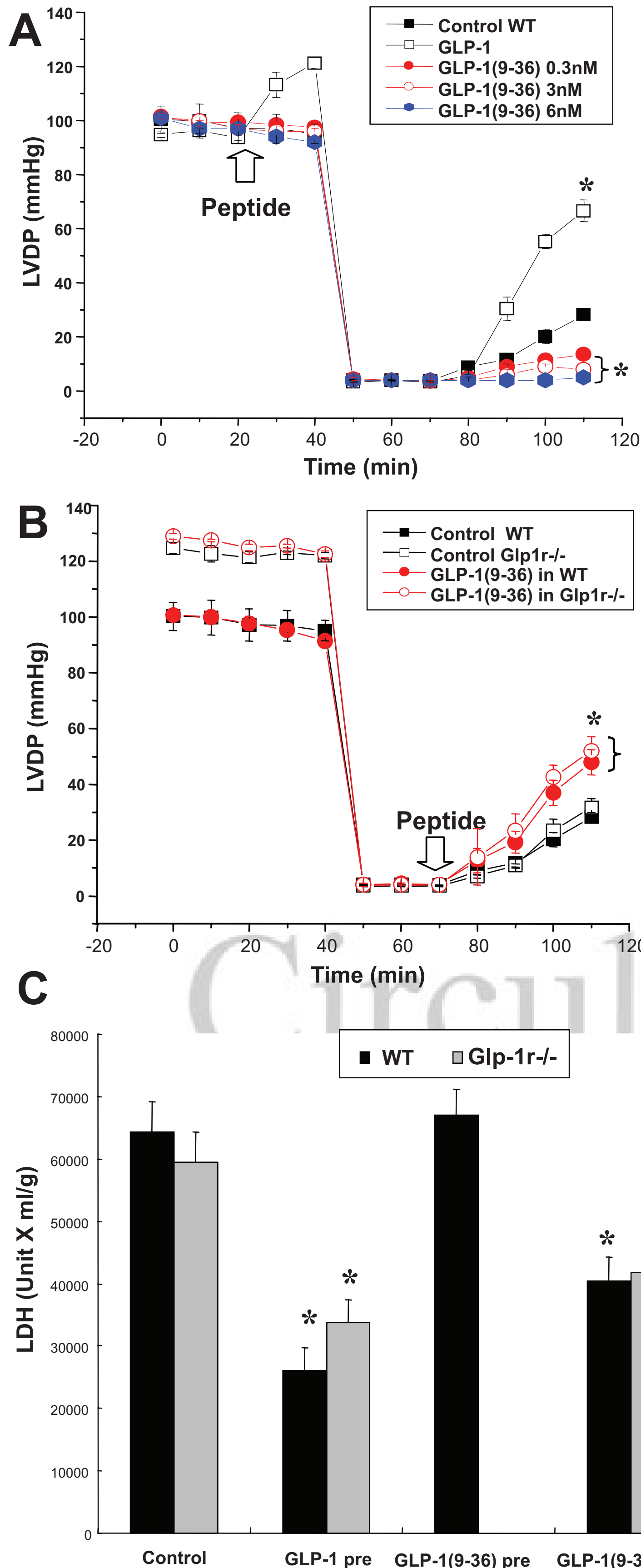

Figure 3. Functional recovery after I/R injury in WT and Glp1r$^{-/-}$ hearts treated with GLP-1(9-36). A, Pretreatment with GLP-1(9-36) (n=6 for each dose) impaired functional recovery compared with untreated (n=21) or GLP-1–pretreated (n=15) hearts from WT mice. B, Administration of GLP-1(9-36) during 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.

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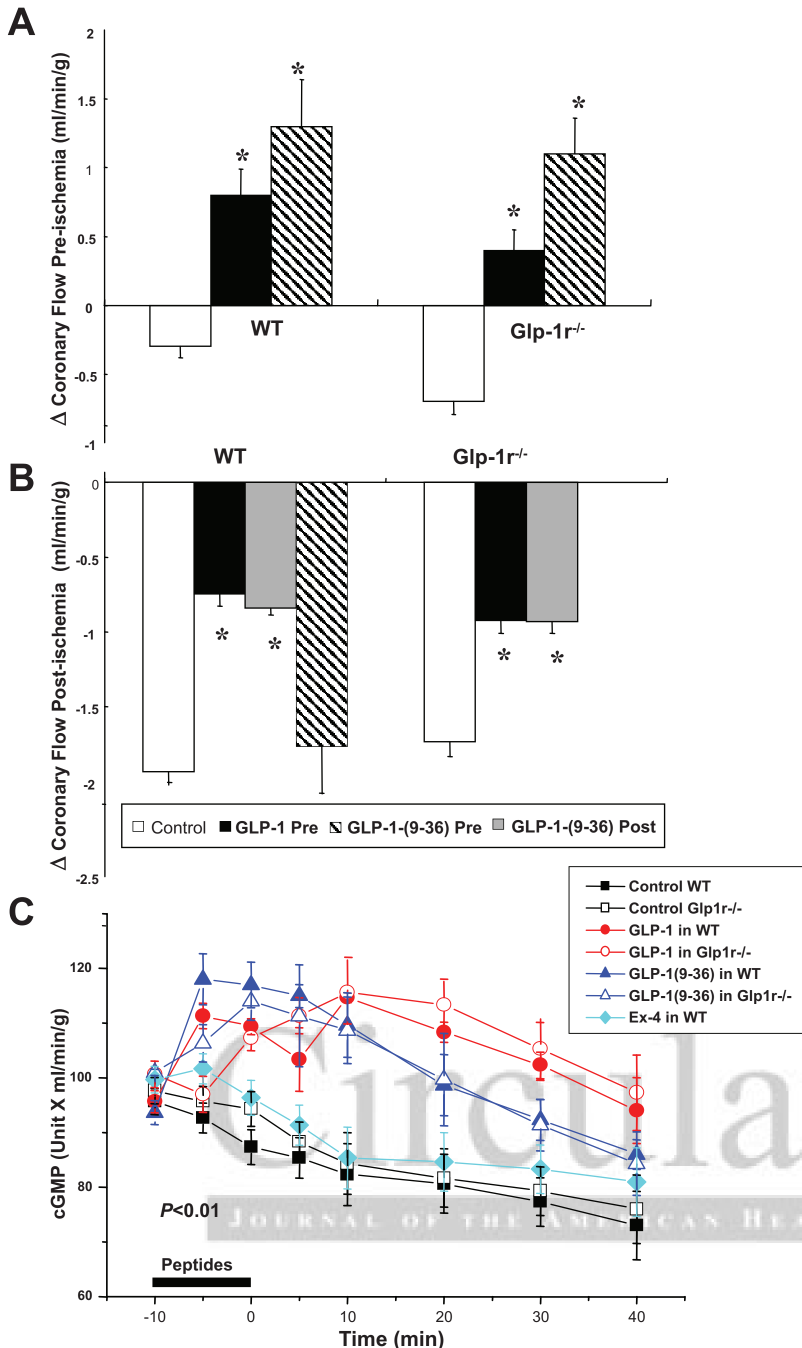

Figure 4. Effects of GLP-1 on coronary flow and cGMP release. Changes in coronary flow rate in the (A) preischemic and (B) postischemic periods were calculated as the mean change in recorded coronary flow rate between 40 minutes and (A) 60 minutes and (B) 100 minutes of the I/R protocol (see Figure 1A and Figures II and III of the Data Supplement). In WT and Glp1r hearts, pretreatment with GLP-1 increased coronary flow in the preischemic and postischemic periods, whereas pretreatment with GLP-1(9-36) only increased coronary flow in the preischemic period. Posttreatment with GLP-1(9-36) increased coronary flow in both phases. **P*<0.05 vs control untreated WT or Glp1r$^{-/-}$ by 1-way ANOVA. C, Levels of cGMP release in the coronary effluent were measured in normoxic perfused hearts (n=3 each). GLP-1 and GLP-1(9-36), but not exendin-4, increased cGMP release in WT and Glp1r$^{-/-}$ hearts. The effects of group, time, and group-by-time interaction were significant by repeated-measures ANOVA (*P*<0.01). All data shown are mean±SE.

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

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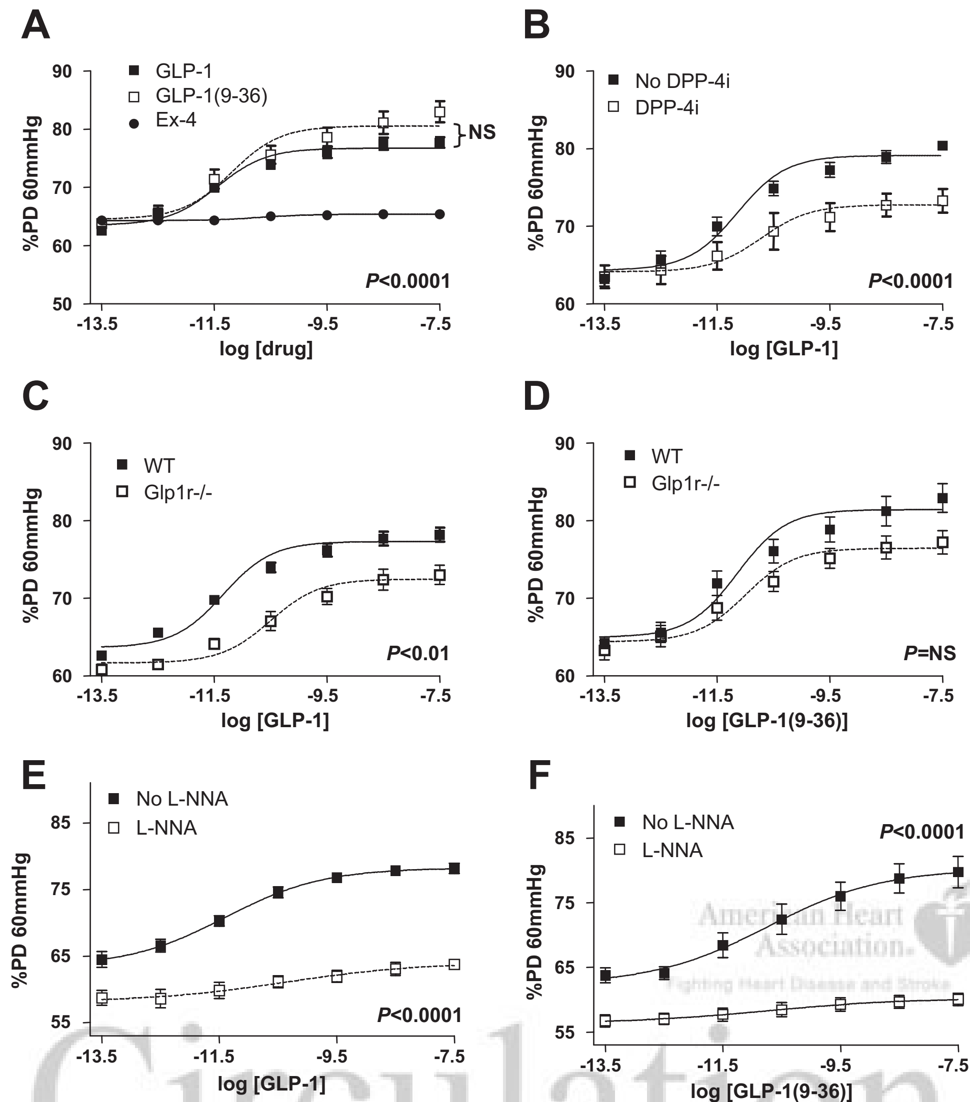

Figure 5. GLP-1 and GLP-1(9-36), but not exendin-4, have vasodilatory effects on mesenteric arteries from WT and Glp1r$^{-/-}$ mice. A, Vasodilatory effects of GLP-1(9-36) (n=6; EC_{50}, 10 ρmol/L) did not differ from those of GLP-1 (n=6; EC_{50}, 3.8 ρmol/L) in WT arteries, whereas the GLP-1R agonist 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; Ca^{2+}-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.

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 for each dose, 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

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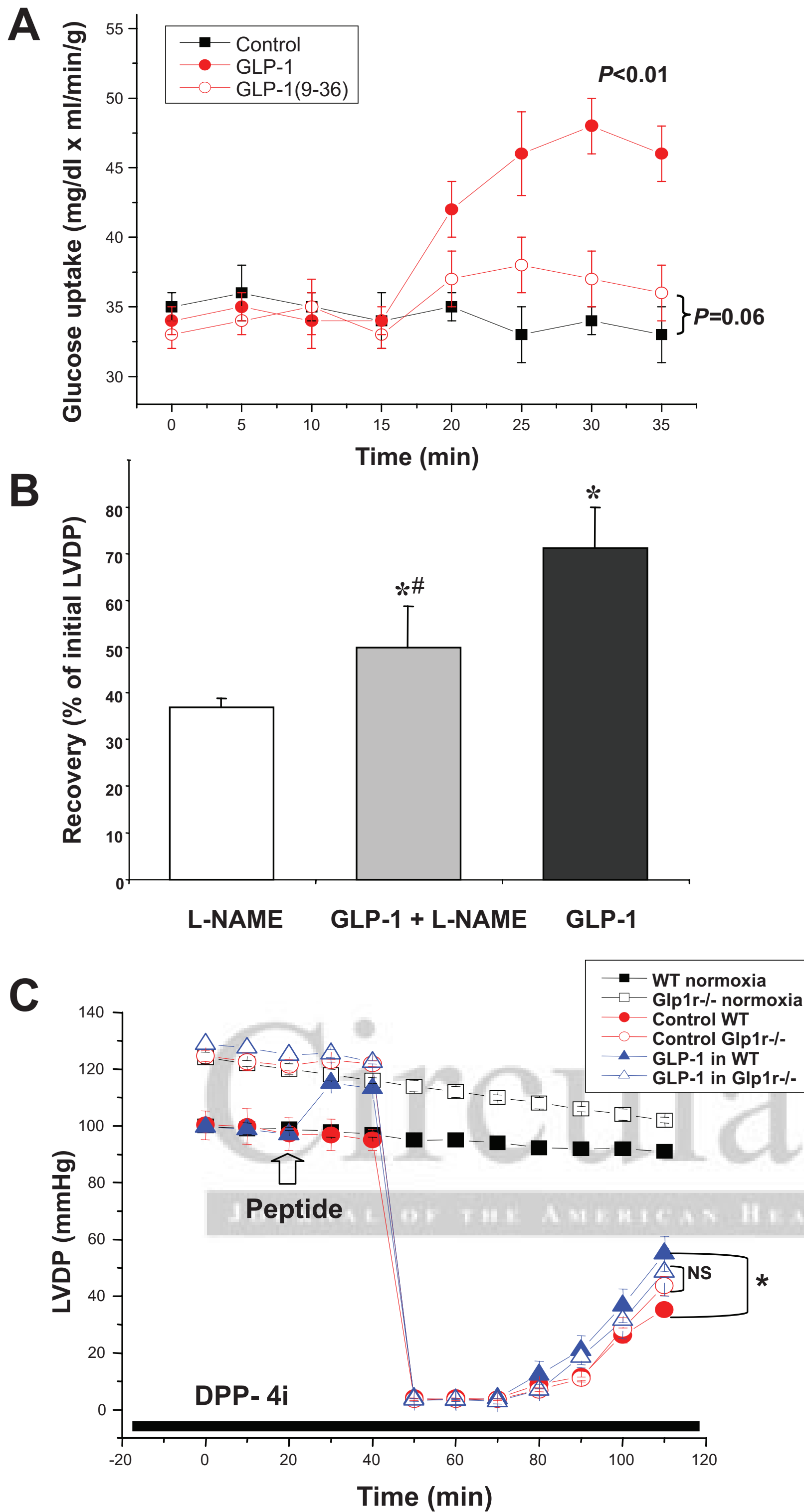

Figure 6. Cardioprotective effects of GLP-1 are partly mediated by GLP-1(9-36)–dependent NOS activity. A, Effects of GLP-1 and GLP-1(9-36) on myocardial glucose uptake were compared in normoxic perfused hearts. GLP-1 more than GLP-1(9-36) (n=3 each) increased levels of glucose uptake vs untreated controls. The effects of group, time, and group-by-time interaction were significant by repeated-measures ANOVA for GLP-1 ($P<0.01$) but not GLP-1(9-36) ($P=0.06$). B, Cardioprotective effects of GLP-1 were reduced after L-NAME (50 μmol/L) pretreatment (n=3) and (C) were lost in Glp1r$^{-/-}$ hearts (n=5) but not WT hearts (n=6) after DPP-4 inhibition (DPP-4i) with sitagliptin (5 μmol/L). Data shown are mean±SE. *$P<0.05$ vs control untreated WT or Glp1r$^{-/-}$; #$P<0.05$ vs GLP-1–treated WT by 1-way ANOVA at 110 minutes.

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

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LDH release in WT and Glp1r$^{-/-}$ hearts (WT: 40 401±3921 U · mL^{-1} · min^{-1} · g^{-1}; Glp1r$^{-/-}$: 41 831±3751 U · mL^{-1} · min^{-1} · g^{-1}; n=5 each; $P<0.05$ for both comparisons), although the effect of GLP-1(9-36) after ischemia was not as large as that of pretreatment with GLP-1. These data further suggest that the cytoprotective effects of GLP-1 are mediated, at least in part, by a GLP-1R–independent effect of GLP-1(9-36).

We next assessed the effects of the peptides on coronary blood flow. When GLP-1 or GLP-1(9-36) was administered after ischemia, coronary flow (and LVDP) was enhanced, just as when GLP-1 was administered before ischemia (see Figure 4A and 4B and Figures II and III in the Data Supplement). In contrast, when GLP-1(9-36) was administered before ischemia, it augmented immediate coronary flow (before ischemia; Figure 4A) but had no salutary effect on delayed (after ischemia; Figure 4B) coronary flow or on the recovery of LVDP after reperfusion (Figure 3A).

We also measured the release of the nitric oxide (NO) –driven signaling molecule cGMP in coronary venous effluent of normoxic hearts. In the absence of GLP-1 or GLP-1(9-36), cGMP release was comparable in WT and Glp1r$^{-/-}$ hearts. However, the addition of either GLP-1 or GLP-1(9-36), but not the DPP-4–resistant GLP-1R agonist exendin-4, significantly increased cGMP release from WT and Glp1r$^{-/-}$ hearts (Figure 4C).

To assess whether GLP-1 or GLP-1(9-36) exerted direct vasodilatory actions, we studied mesenteric arteries partially preconstricted with phenylephrine (3 μmol/L). Vasodilatory responses to GLP-1(9-36) (EC_{50}, 10 ρmol/L) did not differ from those elicited by GLP-1 (EC_{50}, 3.8 ρmol/L; P=NS; Figure 5A). Furthermore, the observed vasodilatory responses were not glucose dependent (data not shown). In contrast, the DPP-4–resistant GLP-1R agonist exendin-4 had no vasodilatory effects on mesenteric vessels (Figure 5A). To test whether the conversion of GLP-1 to GLP-1(9-36) was required for the vasodilatory action of GLP-1, we repeated the experiments in the presence of the DPP-4 inhibitor sitagliptin (5 μmol/L). Sitagliptin reduced but did not abolish the vasodilatory response to GLP-1 (Figure 5B), suggesting that both native GLP-1 and its metabolite GLP-1(9-36) have vasodilatory action. Consistent with this hypothesis, both GLP-1 and GLP-1(9-36) still induced vasodilation in arteries from Glp1r$^{-/-}$ mice (Figure 5C and 5D). Taken together, these data strongly support the existence of a vasodilatory signaling mechanism for GLP-1 and GLP-1(9-36) that is independent of the known functional GLP-1R.

To investigate participation of the L-arginine–NO pathway in the vasodilatory responses to GLP-1 and GLP-1(9-36), we repeated experiments on preconstricted mesenteric arteries (phenylephrine 3 μmol/L) before and after treatment with 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,[14,34] normoxic 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 implicate 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,[5,37] we have now localized GLP-1R expression to distinct heart chambers and cell types, including cardiomyocytes and endothelial and vascular SMCs, but not fibroblasts, of the normal adult mouse heart. Furthermore, both immunofluorescence 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,[12,14,15,38,39] the mechanisms 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 postischemic 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 effects in the setting of I/R injury.[40,41]

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,[42,43] 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-

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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 effects 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 12 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

1. Drucker DJ. The biology of incretin hormones. *Cell Metab*. 2006;3: 153–165.
2. Irwin DM. Molecular evolution of proglucagon. *Regul Pept*. 2001; 98:1–12.
3. Estall JL, Drucker DJ. Glucagon and glucagon-like peptide receptors as drug targets. *Curr Pharm Des*. 2006;12:1731–1750.
4. Mojsov S, Kopczynski MG, Habener JF. Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. *J Biol Chem*. 1990;265:8001–8008.
5. Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide, I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett*. 1995;358:219–224.
6. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab*. 1995;80:952–957.
7. Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology*. 1995; 136:3585–3596.
8. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology*. 2007;132:2131–2157.
9. Barragan JM, Rodriguez RE, Blazquez E. Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. *Am J Physiol*. 1994;266:E459–E466.
10. Golpon HA, Puechner A, Welte T, Wichert PV, Feddersen CO. Vasorelaxant effect of glucagon-like peptide-(7-36)amide and amylin on the pulmonary circulation of the rat. *Regul Pept*. 2001;102:81–86.
11. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest*. 2002;110:43–52.
12. Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J Card Fail*. 2006;12:694–699.
13. Nikolaidis LA, Doverspike A, Hentosz T, Zourelias L, Shen YT, Elahi D, Shannon RP. Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. *J Pharmacol Exp Ther*. 2005;312:303–308.
14. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelias L, Stolarski C, Shen YT, Shannon RP. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation*. 2004;110:955–961.
15. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation*. 2004;109:962–965.
16. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes*. 2005;54:146–151.

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17. Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, Drucker DJ. International Union of Pharmacology: XXXV: the glucagon receptor family. *Pharmacol Rev*. 2003;55:167–194.
18. Thorens B. Expression cloning of the pancreatic {beta} cell receptor for the gluco- incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci U S A*. 1992;89:8641–8645.
19. Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN, Parker TG, Huang Q, Drucker DJ, Husain M. Cardiac function in mice lacking the glucagon-like peptide-1 receptor. *Endocrinology*. 2003;144: 2242–2252.
20. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology*. 1996;137:2968–2978.
21. Wei Y, Mojsov S. Distribution of GLP-1 and PACAP receptors in human tissues. *Acta Physiol Scand*. 1996;157:355–357.
22. Nakagawa A, Satake H, Nakabayashi H, Nishizawa M, Furuya K, Nakano S, Kigoshi T, Nakayama K, Uchida K. Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton Neurosci*. 2004;110:36–43.
23. Nikolaidis LA, Elahi D, Shen YT, Shannon RP. Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2005;289:H2401–H2408.
24. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med*. 1996;2:1254–1258.
25. De Leon DD, Deng S, Madani R, Ahima RS, Drucker DJ, Stoffers DA. Role of endogenous glucagon-like peptide-1 in islet regeneration after partial pancreatectomy. *Diabetes*. 2003;52:365–371.
26. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes*. 2004;53:1326–1335.
27. Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A, Hansotia T, Drucker DJ, Wollheim C, Burcelin R, Thorens B. Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. *J Clin Invest*. 2004;113:635–645.
28. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med*. 2003;9:1173–1179.
29. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem*. 2003;278:471–478.
30. Afroze T, Yang LL, Wang C, Gros R, Kalair W, Hoque AN, Mungrue IN, Zhu Z, Husain M. Calcineurin-independent regulation of plasma membrane Ca^{2+} ATPase-4 in the vascular smooth muscle cell cycle. *Am J Physiol Cell Physiol*. 2003;285:C88–C95.
31. Flamez D, Van Breusegem A, Scrocchi LA, Quartier E, Pipeleers D, Drucker DJ, Schuit F. Mouse pancreatic beta-cells exhibit preserved glucose competence after disruption of the glucagon-like peptide-1 receptor gene. *Diabetes*. 1998;47:646–652.
32. Noyan-Ashraf MH, Wu L, Wang R, Juurlink BH. Dietary approaches to positively influence fetal determinants of adult health. *FASEB J*. 2006; 20:371–373.
33. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. *Anal Biochem*. 1985;150:76–85.
34. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP. Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. *J Pharmacol Exp Ther*. 2006;317:1106–1113.
35. Gros R, Van Wert R, You X, Thorin E, Husain M. Effects of age, gender, and blood pressure on myogenic responses of mesenteric arteries from C57BL/6 mice. *Am J Physiol Heart Circ Physiol*. 2002;282:H380–H388.
36. Vila Petroff MG, Egan JM, Wang X, Sollott SJ. Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. *Circ Res*. 2001;89:445–452.
37. Campos RV, Lee YC, Drucker DJ. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology*. 1994;134:2156–2164.
38. Herman GA, Bergman A, Stevens C, Kotey P, Yi B, Zhao P, Dietrich B, Golor G, Schrodter A, Keymeulen B, Lasseter KC, Kipnes MS, Snyder K, Hilliard D, Tanen M, Cilissen C, De Smet M, de Lepeleire I, Van Dyck K, Wang AQ, Zeng W, Davies MJ, Tanaka W, Holst JJ, Deacon CF, Gottesdiener KM, Wagner JA. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2006;91:4612–4619.
39. Bose AK, Mocanu MM, Carr RD, Yellon DM. Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. *Cardiovasc Drugs Ther*. 2005;19:9–11.
40. Brunner F, Maier R, Andrew P, Wolkart G, Zechner R, Mayer B. Attenuation of myocardial ischemia/reperfusion injury in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Cardiovasc Res*. 2003;57:55–62.
41. Kloner RA. Cardiovascular effects of the 3 phosphodiesterase-5 inhibitors approved for the treatment of erectile dysfunction. *Circulation*. 2004;110: 3149–3155.
42. Raufman JP. Bioactive peptides from lizard venoms. *Regul Pept*. 1996; 61:1–18.
43. Drucker DJ. Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nat Clin Pract Endocrinol Metab*. 2005; 1:22–31.
44. Daniel EE, Anvari M, Fox-Threlkeld JET, McDonald TJ. Local, exendin-(939)-insensitive, site of action of GLP-1 in canine ileum. *Am J Physiol Gastrointest Liver Physiol*. 2002;283:G595–G602.
45. Wang Y, Kole HK, Montrose-Rafizadeh C, Perfetti R, Bernier M, Egan JM. Regulation of glucose transporters and hexose uptake in 3T3-L1 adipocytes: glucagon-like peptide-1 and insulin interactions. *J Mol Endocrinol*. 1997;19:241–248.
46. Montrose-Rafizadeh C, Yang H, Rodgers BD, Beday A, Pritchette LA, Eng J. High potency antagonists of the pancreatic glucagon-like peptide-1 receptor. *J Biol Chem*. 1997;272:21201–21206.

CLINICAL PERSPECTIVE

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 structural differences relative to native GLP-1 and hence may not give rise to GLP-1(9-36) in vivo. Similarly, DPP-4 inhibitors, although enhancing the stability of cardioprotective molecules such as SDF-1, prevent the enzymatic cleavage of native GLP-1 to GLP-1(9-36). The data presented here extend previous findings on the biology of GLP-1(9-36) by demonstrating that this peptide metabolite exerts cardioprotective actions in the murine heart. Moreover, these actions are independent of the known GLP-1R, invoking a novel signaling mechanism for the DPP-4–generated GLP-1 metabolite. These findings suggest that GLP-1(9-36) itself may have therapeutic benefit in the setting of cardiovascular injury, a hypothesis that can be tested in future clinical studies. Moreover, the increasing complexity of the cardiovascular actions of GLP-1 and its cardioactive metabolite GLP-1(9-36) underscores the need for clinical studies examining the cardiovascular actions of GLP-1R agonists and DPP-4 inhibitors in subjects with type 2 diabetes.

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