Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy

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GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is an incretin hormone that acts as a potent insulin secretagogue in response to nutrient ingestion and stimulates glucose disposition (8, 10, 15, 16, 19). GLP-1(1-37) is first cleaved from proglucagon with the first six NH2-terminal amino acids cleaved subsequently to form the active peptide GLP-1(7-36) that is then amidated to form GLP-1(7-36) amide (10, 15, 19). Although an attractive therapeutic agent, GLP-1(7-36) amide is unstable, being rapidly metabolized by dipeptidyl peptide IV (DPP IV) to generate a truncated metabolite GLP-1(9-36) amide (1, 11). In vitro studies (25) have suggested that GLP-1(9-36) amide antagonizes the insulinotropic effects of GLP-1(7-36) amide and the effects to slow gastric emptying. More recent studies in humans (25) and pigs (12) have suggested that that GLP-1(9-36) amide does not attenuate the insulinotropic effects of the 7-36 peptide. Notably, in pigs, GLP-1(9-36) amide stimulated glucose disposition independent of insulin stimulation, whereas similar effects were not seen in healthy humans (25). As such, whether and to what extent the effects of the parent peptide GLP-1(7-36) amide are attributable to the active metabolite remain controversial (2).

Increasing interest has focused of late on the potential cardiovascular effects of GLP-1, given its G protein-coupled receptor has been found in the heart (9, 13, 26), as well as in areas of the central nervous system that govern autonomic control (20, 27). GLP-1(7-36) amide (4, 5), GLP-1(9-36) amide (6), and the DPP IV-resistant receptor agonist exendin-4 (3, 6, 27) have been reported to increase both blood pressure and heart rate in rodents. Furthermore, mice deficient in the GLP-1 receptor (GLP-1(−/−)) have lower heart rates and blood pressures and increased cardiac mass (14). GLP-1(7-36) has been shown to stimulate glycolysis in anesthetized pigs undergoing coronary artery occlusion (18) and to mitigate ischemia-reperfusion injury in isolated isovolumic heart preparations (7, 17).

Our laboratory has recently demonstrated that continuous intravenous infusion of GLP-1(7-36) amide is associated with salutary hemodynamic effects in humans with LV systolic dysfunction after acute myocardial infarction (23) and in dogs with myocardial stunning (21). In conscious dogs with dilated cardiomyopathy (DCM) and myocardial insulin resistance (24), GLP-1(7-36) administration was associated with marked hemodynamic improvements and significant increases in basal and insulin-stimulated myocardial glucose uptake in the absence of increases in plasma insulin (22). These findings raise the possibility that the myocardial effects of GLP-1 are mediated by its metabolite, which is the predominant form of the peptide in the plasma.

Accordingly, the purpose of the present study was to determine whether a continuous intravenous infusion of GLP-1(9-36) amide mimicked the cardiovascular actions of the parent peptide in conscious dogs with myocardial dysfunction.
peptide GLP-1-(7–36) amide in conscious dogs with pacing-induced DCM. A second goal was to determine whether GLP-1-(9–36) amide stimulated myocardial glucose uptake and whether the metabolic effects were insulinotropic or insulinominetic.

**METHODS**

**Instrumentation.** Twenty-eight mongrel dogs of either sex weighing 15–20 kg were instrumented as described previously from our laboratory (22, 24). Briefly, instrumentation included a LV pressure transducer, aortic and coronary sinus catheters, transonic flow probes in the ascending aorta and proximal left circumflex coronary artery for continuous measurement of cardiac output (CO), and coronary blood flow (CBF) and sonomicrometry crystals for quantification of LV dimensions and volumes. All animals received analgesics as needed for the first 72 h after surgery, and cephalixin (1 g) was administered daily for 7 days. The dogs were allowed to recover from the surgical procedure for 2 wk, during which time they were trained to lie quietly on the experimental table in a conscious, unrestrained state. All catheters were flushed with saline, and all instruments were appropriately calibrated daily. Heparin was avoided due to its known lipolytic effects.

Hemodynamic measurements were made with the dogs fully awake, lying quietly on their right side. Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86–23, Revised 1996) and the guidelines of the Institutional Animal Care and Use Committee at Allegheny General Hospital.

**Hemodynamic measures.** Baseline experiments in normal conscious dogs consisted of systemic and coronary hemodynamic recordings to determine LV contractility (LV dp/dt), stroke volume (SV), CO, and CBF. SV was determined as the quotient of the CO determined by the aortic flow probe and the heart rate. The LV ejection fraction was determined as the quotient of the SV divided by the LV end-diastolic volume. The LV end-diastolic volume was determined from the ultrasonic dimension crystals (24). Arterial and coronary sinus blood samples were obtained to calculate myocardial oxygen consumption (MV\(_{O_2}\)) as the product of the left circumflex coronary artery blood flow and the myocardial arterio-venous O\(_2\) content difference:

\[
MV_{O_2} = [CBF \times (a - v)O_2\text{ content}]
\]

where \(a\) and \(v\) refer to arterial and coronary sinus blood samples, respectively.

DCM was induced by rapid right ventricular pacing (240 min\(^{-1}\)) as described previously (22, 24). A 30-min stabilization period after deactivation of the pacemaker preceded all experiments and all measurements in DCM.

After 28 days of rapid pacing, when the animals had clinical and hemodynamic signs and symptoms of advanced symptomatic DCM, dogs were randomized to a 48-h intravenous infusion of GLP-1-(7–36) amide (1.5 pmol·kg\(^{-1}\)·min\(^{-1}\), \(n = 9\) dogs), GLP-1-(9–36) amide (1.5 pmol·kg\(^{-1}\)·min\(^{-1}\), \(n = 7\) dogs), or an equivalent volume intravenous infusion of saline (control, 3 ml saline/24 h; \(n = 7\) dogs), administered via MiniMed pump (model 407C, Medtronic) through the right atrial catheter. Rapid pacing was suspended during the 48 h of infusion. In all groups, hemodynamics and metabolic parameters were measured before and at 1, 24, and 48 h during GLP-1 or saline administration, respectively. In a subset of five dogs in each group, measurements of LV and systemic hemodynamics and myocardial glucose uptake were made at 6 and 24 h after cessation of the respective GLP-1 or saline infusions.

Both forms of GLP-1 were synthesized in the protein/peptide core facility of the Endocrine Unit of the Massachusetts General Hospital. The peptide contents of each were at least 83%; each peptide was 99% pure and gave a single peak on high-performance liquid chromatography. The peptides were lyophilized in vials under sterile conditions for single use and were certified to be both pyrogen free and sterile. Net peptide content was used for all calculations. Each form of the peptide used in this protocol was from a single lot. The respective forms of GLP-1 were dissolved in 3 ml of normal saline that contained 0.2 ml of fresh plasma from each dog.

**Measurement of plasma levels of GLP-1-(7–36) and GLP-1-(9–36).** Intravenous infusions of the respective forms of GLP-1 were administered chronically through a right atrial catheter, and plasma samples were obtained from the aorta. The plasma concentrations of GLP-1-(7–36) and (9–36) were determined in plasma collected in ice-cooled tubes containing EDTA and a DPP-IV inhibitor using an antibody directed against the COOH-terminal portion of the peptides (Linco Research, St. Charles, MO). The COOH-terminal assay measured total GLP-1. The lower level of sensitivity of the assay is 3 pM with an ED\(_{50}\) of 82 ± 10 pM. The assay is 100% specific for the two forms of GLP-1. To measure the GLP-1-(7–36) alone, a second antibody (Linco Research), specific for the NH\(_2\) terminal of GLP-1-(7–36) peptide, was employed with comparable sensitivity, specificity, and ED\(_{50}\), as well as intra- and interassay coefficients of variation.

The NH\(_2\) terminal assay measured active GLP-1.

To establish the specificity of the GLP-1 antibodies, five doses of each form of GLP-1 (1.5, 2.5, 5, 10, 20 pmol·kg\(^{-1}\)·min\(^{-1}\)) were infused in an additional five normal dogs, instrumented as described in **Instrumentation.** Each dose was infused for 10 min into the right atrium (RA), and samples were obtained from the aorta.

During the 48-h continuous infusion of the respective peptides in DCM, plasma samples were taken daily from the aorta in all dogs. Right atrial samples were obtained from a right atrial catheter implanted through the internal jugular vein in four dogs receiving GLP-1-(7–36) and in four dogs receiving GLP-1-(9–36) and were used to measure peptide concentrations before systemic circulation.

**Metabolic determinations.** All dogs were fed a standard diet with fixed carbohydrate and fat content. Body weights were monitored weekly. Metabolic parameters were measured at 8:00 AM after an overnight fast. Arterial and coronary sinus blood samples were obtained in all dogs at baseline (preparing) and 28 days after the initiation of rapid pacing. Transmyocardial substrate balance was calculated as the difference between arterial and coronary sinus content. Myocardial substrate uptake was calculated as the product of myocardial substrate balance and CBF.

The measurements of insulin and glucagon levels in the plasma were carried out using specific RIA kits from Linco Research. The measurements of nonesterified (or free) fatty acids (NEFA) in the plasma were carried out using the NEFA C test kit purchased from Wako Diagnostics (Richmond, VA). The plasma glucose levels were measured by using a Yellow Springs Instrument (2300) Stat Plus Analyzer (Giangarlo Scientific, Pittsburgh, PA).

Myocardial insulin sensitivity was assessed at baseline, after the development of DCM, and after a 48-h infusion of GLP-1 using the hyperinsulinemic-euglycemic clamp technique (22, 24). In the fasting state, a primed constant infusion of insulin (480 pmol·m\(^{-2}\)·min\(^{-1}\)) was administered for 120 min to create a steady state concentration of plasma insulin (~1,000 pmol/l). Arterial glucose concentrations were measured every 5 min, and glucose was infused to maintain plasma glucose concentrations at 5 mmol/l ± 10%. Myocardial glucose balance and CBF were sampled every 15 min to determine myocardial glucose uptake.

**Statistical analysis.** Data are means ± SE. Differences in hemodynamic and metabolic responses over time between the groups were determined by repeated-measures ANOVA. Post hoc analysis employed the use of Student-Newman-Keuls test. A level of \(P < 0.05\) was considered statistically significant.
RESULTS

Plasma levels of GLP-1 during respective infusions. To determine the dominant circulating form of GLP-1, we performed acute intravenous infusion of escalating doses (1.25, 2.5, 5, 10, and 20 pmol·kg⁻¹·min⁻¹) of GLP-1-(7–36) and GLP-1-(9–36) in five normal conscious dogs. There was a steady and comparable increase in the total COOH-terminal peptide from 51 ± 2 to 207 ± 12 pmol/l during acute GLP-1-(7–36) infusion and a comparable increase from 53 ± 2 to 193 ± 11 pmol/l during acute GLP-1-(9–36) infusion. In contrast, there were negligible concentrations of the active NH₂-terminal peptide, even after maximal doses of GLP-1-(7–36) infusion, indicating that the two NH₂-terminal amino acids of GLP-1-(7–36) are rapidly cleaved even during continuous intravenous infusion. Thus, despite continuous intravenous infusion of high concentrations of GLP-1-(7–36), virtually all of the active peptide was metabolized rapidly.

To be certain that the assay for the NH₂-terminal fragment of GLP-1 recognized GLP-1-(7–36) in the plasma, we sampled blood from the RA, into which GLP-1-(7–36) or GLP-1-(9–36) were infused during acute GLP-1-(9–36) infusion, indicating that the two NH₂-terminal amino acids of GLP-1-(7–36) are rapidly cleaved even during continuous intravenous infusion. Thus, despite continuous intravenous infusion of high concentrations of GLP-1-(7–36), virtually all of the active peptide was metabolized rapidly.

To be certain that the assay for the NH₂-terminal fragment of GLP-1 recognized GLP-1-(7–36) in the plasma, we sampled blood from the RA, into which GLP-1-(7–36) or GLP-1-(9–36) were infused during acute GLP-1-(9–36) infusion, indicating that the two NH₂-terminal amino acids of GLP-1-(7–36) are rapidly cleaved even during continuous intravenous infusion. Thus, despite continuous intravenous infusion of high concentrations of GLP-1-(7–36), virtually all of the active peptide was metabolized rapidly.

Table 1. Hemodynamics in conscious dogs at baseline and after development of DCM

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GLP-1-(7–36)</th>
<th>GLP-1-(9–36)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>DCM</td>
<td>Baseline</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>118±3</td>
<td>108±3*</td>
<td>116±1</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>11±1</td>
<td>26±1*</td>
<td>9±1</td>
</tr>
<tr>
<td>LV dP/dt, mmHg/s</td>
<td>2,744±81</td>
<td>1,423±116*</td>
<td>2,747±67</td>
</tr>
<tr>
<td>Heart rate beats/min</td>
<td>77±1</td>
<td>104±7*</td>
<td>80±3</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>92±3</td>
<td>88±2*</td>
<td>92±2</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>2.1±0.2</td>
<td>1.5±0.1*</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>28±2</td>
<td>16±2*</td>
<td>27±3</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>38±1</td>
<td>24±2*</td>
<td>40±3</td>
</tr>
<tr>
<td>Coronary blood flow, ml/min</td>
<td>28±1</td>
<td>30±2</td>
<td>27±2</td>
</tr>
<tr>
<td>MVO₂, ml of O₂/min</td>
<td>2.7±0.2</td>
<td>2.4±0.1*</td>
<td>2.7±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Control dogs (n = 7) were randomized to receive 48-h infusion of saline; glucagon-like peptide-1 (GLP-1)-(7–36) dogs (n = 9), to receive 48-h infusion of GLP-1-(7–36); and GLP-1-(9–36) dogs (n = 7), to receive 48-h infusion of GLP-1-(9–36). DCM, dilated cardiomyopathy; LV, left ventricular; LV dP/dt, first derivative of LV pressure; LVEF, LV ejection fraction; MVO₂, myocardial oxygen consumption. *P < 0.01 compared with baseline.
Figure 3 illustrates that both GLP-1-(7–36) and GLP-1-(9–36) amide significantly improved LV SV, CO, and LV ejection fraction to a similar degree, whereas saline infusion had no effect. The effects of the respective infusions of GLP-1 on mean arterial pressure, although significant, were modest.

Figure 4 illustrates that there was no difference in left circumflex CBF among the three interventions, yet posterior wall thickening improved significantly in both the GLP-1-(7–36) or GLP-1-(9–36)-treated groups compared with saline control. Thus continuous infusions of both the parent peptide...
and its metabolite had comparable hemodynamic benefits in conscious dogs with pacing-induced DCM compared with saline control.

The effects of GLP-1 infusions on basal and insulin-stimulated myocardial glucose uptake. Table 2 illustrates the effects of the respective treatments in DCM on myocardial substrate availability and glucoregulatory hormones. The development of DCM was associated with significant increases in plasma NEFA, plasma insulin, and glucagon levels. There was no significant difference in plasma glucose levels that remained normal. Plasma norepinephrine levels were increased significantly in DCM. Saline infusion had no effect on plasma hormone levels. GLP-1-(7–36) amide increased plasma insulin levels modestly from 65 ± 6 to 75 ± 5 pmol/l. GLP-1-(9–36) had no effect on plasma insulin levels. Both peptides decreased plasma glucagon levels and significantly suppressed plasma norepinephrine levels.

Figure 5 illustrates the effects of the respective treatments on basal (preclamp) and insulin-stimulated myocardial glucose uptake measured at the end of a 2-h hyperinsulinemic-euglycemic clamp. In normal dogs before pacing, myocardial glucose uptake was stimulated sixfold during hyperinsulinemic-euglycemic clamp. In contrast, after the development of DCM at matched levels of hyperinsulinemia (~1,000 pmol/l), there was a marked attenuation in myocardial glucose uptake consistent with myocardial insulin resistance. However, after a 48-h infusion of either GLP-1-(7–36) or GLP-1-(9–36), both basal and insulin-stimulated glucose uptakes were enhanced significantly in DCM compared with the saline control group. There was an attenuated CBF response to hyperinsulinemia in DCM that was partially restored during GLP-1-(7–36) or GLP-1-(9–36) infusion.

Figure 6 illustrates the time course of the improvement in LV dP/dt and myocardial glucose uptake during infusion of the respective forms of GLP-1. There was no significant hemodynamic improvement in LV dP/dt during the first 6 h, but there was significant improvement at 24 and 48 h. In addition, the benefits were maintained for at least 24 h after cessation of the infusions. The effects of each form of the peptide on LV dP/dt were indistinguishable from each other. Figure 6 illustrates that myocardial glucose uptake was not altered at 1 h but was increased significantly at 6, 24, and 48 h during infusion of both GLP-1-(7–36) or GLP-1-(9–36). The augmentation of myocardial glucose uptake waned after the cessation of the infusions.

DISCUSSION

In the present study, we compared the effects of GLP-1-(7–36) amide and its metabolite GLP-1-(9–36) amide on LV function and myocardial glucose uptake in conscious dogs with pacing-induced DCM. When compared with the saline control group, both treatments significantly improved LV pressures and contractility as well as ventricular performance in DCM. Notably, the magnitude of the benefit was similar between the two peptides. Furthermore, both peptides increased basal and insulin-stimulated glucose uptake during hyperinsulinemic-euglycemic clamps. The augmented basal myocardial glucose uptake seen in association with improved LV and systemic hemodynamics was independent of the increases in plasma

Table 2. Effects of GLP-1-(7–36) or GLP-1-(9–36) on plasma substrates and hormones in DCM

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GLP-1-(7–36)</th>
<th>GLP-1-(9–36)</th>
<th>GLP-1-(7–36) + saline</th>
<th>GLP-1-(9–36) + saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM/l</td>
<td>4.6 ± 0.3</td>
<td>5.1 ± 0.7</td>
<td>5.0 ± 0.8</td>
<td>4.4 ± 0.4</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>NEFA, µM/l</td>
<td>312 ± 76</td>
<td>667 ± 98*</td>
<td>557 ± 113</td>
<td>354 ± 26</td>
<td>617 ± 48*</td>
</tr>
<tr>
<td>Plasma insulin, pM/l</td>
<td>34 ± 5</td>
<td>65 ± 6*</td>
<td>57 ± 9</td>
<td>27 ± 5</td>
<td>67 ± 5*</td>
</tr>
<tr>
<td>Plasma glucagon, pg/ml</td>
<td>18 ± 2</td>
<td>37 ± 5*</td>
<td>33 ± 4</td>
<td>17 ± 2</td>
<td>34 ± 4*</td>
</tr>
<tr>
<td>Plasma norepinephrine, mM/l</td>
<td>0.44 ± 0.09</td>
<td>1.08 ± 0.10*</td>
<td>0.98 ± 0.12</td>
<td>0.34 ± 0.10</td>
<td>0.98 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Control dogs (n = 7) were randomized to receive 48-h infusion of saline; GLP-1-(7–36) dogs (n = 9), to receive 48-h infusion of GLP-1-(7–36); and GLP-1-(9–36) dogs (n = 7), to receive 48-h infusion of GLP-1-(9–36). NEFA, nonesterified fatty acids. *P < 0.05 compared with baseline; †P < 0.05 compared with saline control.
insulin, indicating an insulinomimetic effect. These data suggest that the GLP-1-(9–36) peptide has potent metabolic and hemodynamic effects that are comparable to those seen with continuous GLP-1-(7–36) infusion.

There is a growing body of evidence supporting the efficacy of GLP-1-(7–36) amide as a potent stimulus to insulin release (insulinotropic) in Type 2 diabetes mellitus (10, 15, 19). In addition, several studies have demonstrated cardiovascular effects of GLP-1-(7–36) amide in rodent models, namely, increases in heart rate and blood pressure (3–6, 27). However, there remains a paucity of data examining myocardial effects of GLP-1 in cardiovascular disease states in experimental animal models or humans. Prior work from our laboratory (22) demonstrated that GLP-1-(7–36) amide improved LV and systemic hemodynamics in conscious dogs with DCM induced by rapid pacing. The significant hemodynamic improvement was accompanied by marked enhancement in basal and insulin-stimulated myocardial glucose uptake. Most notably, the improvement in basal myocardial glucose uptake occurred in the absence of a significant increase in plasma insulin with GLP-1-(7–36) amide, because these conscious dogs manifest insulin resistance but had normal plasma glucose levels (24). These findings confirm an important insulinomimetic effect of GLP-1-(7–36) to stimulate glucose uptake without an increase in plasma insulin and suggest its efficacy in insulin-resistant states as well as Type 2 diabetes mellitus. It is conceivable that the improvement in basal myocardial glucose uptake in the absence of significant increases in plasma insulin was due to suppression of plasma glucagon, which has been noted previously with these incretins (10).

We have reported previously that a continuous infusion of GLP-1-(7–36) amide had a significant insulinotropic effect and improved glucose homeostasis and LV performance in patients after acute myocardial infarction (23). Notably, these patients had frank hyperglycemia with fasting plasma glucose of 170 mg/dl that facilitated the insulinotropic actions of GLP-1-(7–36).

Although the above findings were observed during continuous intravenous administration, GLP-1-(7–36) amide in both experimental animal models and humans have...
yielded conflicting results. Some studies (2) have suggested that GLP-1-(9–36) amide is a competitive inhibitor of the GLP-1 receptor, and coadministration antagonizes the insulinotropic actions of GLP-1-(7–36) amide. However, recent studies (25) in normal humans demonstrated that GLP-1-(9–36) did not attenuate the insulinotropic effects of GLP-1-(7–36) amide in the face of an intravenous glucose challenge. In addition, these investigators found that GLP-1-(9–36) amide had no effect on glucose homeostasis during intravenous glucose challenge. In contrast, studies (12) in anesthetized pigs demonstrated that GLP-1-(9–36) amide increased glucose disposal, independent of insulin effects. To date, there have been no studies examining the effects of GLP-1-(9–36) in cardiovascular disease states in general or on myocardial glucose uptake in particular.

In the present investigation, it is noteworthy that GLP-1-(7–36) amide increased myocardial glucose uptake with only a modest increase in plasma insulin. This modest insulinotropic effect is consistent with the fact that plasma glucose levels were not elevated and that the insulinotropic effects of the incretin are dependent on the ambient glucose concentration. However, we (22, 24) have shown previously that pacing-induced heart failure is an insulin-resistant state with more marked insulin resistance evident in the myocardium compared with whole body glucose uptake. As such, the observed increase in basal myocardial glucose uptake is attributable to an insulinnimetic effect of both GLP-1-(7–36) and GLP-1-(9–36).

Similarly, at matched levels of hyperinsulinemia during euglycemic clamp, either GLP-1-(7–36) amide or GLP-1-(9–36) amide increased myocardial glucose uptake compared with responses before GLP-1 treatment.

It is possible that the responses would have been different in a model of Type 2 diabetes with attendant hyperglycemia. Prior studies (25) comparing the action of the two forms of GLP-1 have done so after glucose challenge, creating a clinical circumstance in which there is at least transient hyperglycemia and, as such, the insulinnimetic actions may be more pronounced. Our data suggest that the effects of the two infusions on basal and insulin-stimulated myocardial glucose uptake were similar in an insulin-resistant, but euglycemic, state. Under such circumstances, the insulinnimetic and glucagonostatic properties of the peptides might be more manifest.

In the present investigation, we observed virtually no measurable concentrations of the active COOH-terminal peptide in aortic plasma samples, despite continuous infusion or escalating doses of GLP-1-(7–36). Furthermore, continuous infusion of GLP-1-(9–36) produced similar hemodynamic and metabolic effects as GLP-1-(7–36). We did not test the efficacy of GLP-1-(7–36) amide in the presence of DPP IV inhibition where the effects of endogenous metabolism would be attenuated, nor did we determine whether the effects of GLP-1-(9–36) amide were mediated by the GLP-1 receptor. Rather, we sought to determine whether the cardiovascular effects of GLP-1-(7–36) and GLP-1-(9–36) were similar. In a relevant large animal model of LV dysfunction and insulin resistance, the effects of the infusions of either agent were qualitatively similar, suggesting that the insulinnimetic effects of GLP-1 may be mediated by the GLP-1-(9–36). Further study is needed to determine whether the parent compound and its active metabolite act via similar cellular mechanisms in the myocardium.

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REFERENCES


