Glucagon-Like Peptide-1 Limits Myocardial Stunning following Brief Coronary Occlusion and Reperfusion in Conscious Canines

Lazaros A. Nikolaidis, Aaron Doverspike, Teresa Hentosz, Lee Zourelias, You-Tang Shen, Dariush Elahi, and Richard P. Shannon

Cardiovascular Research Institute, Department of Medicine, Allegheny General Hospital, Pittsburgh, Pennsylvania (L.A.N., A.D., T.H., L.Z., Y.-T.S., R.P.S.); and University of Massachusetts School of Medicine, Worcester, Massachusetts (D.E.)

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ABSTRACT
We have recently demonstrated the benefits of glucagon-like peptide-1 (GLP-1) in enhancing regional and global myocardial function after reperfusion in the clinical setting of acute myocardial infarction. We hypothesized that GLP-1 facilitates recovery from myocardial stunning after an ischemic event. To investigate this, we administered GLP-1 (1.5 pmol/kg/min) to six dogs undergoing 10-min occlusion of the left circumflex coronary artery, followed by 24-h reperfusion. We compared the responses of coronary blood flow and regional thickening of the posterior wall with a group of eight vehicle-treated dogs undergoing the same occlusion-reperfusion protocol. Although recovery of coronary blood flow was identical, regional wall motion recovery occurred significantly (*p < 0.05) earlier (92 ± 4 versus 57 ± 5% at 15 min) and was complete in the GLP-1-treated dogs, whereas residual contractile dysfunction persisted in the control group (99 ± 4 versus 78 ± 3%* at 24 h). This phenomenon was independent of changes in systemic hemodynamics or global systolic function. However, isovolumic left ventricular relaxation improved significantly in GLP-1-treated dogs. GLP-1 caused an insulinotropic effect, but no hypoglycemia. We conclude that GLP-1 enhances recovery from ischemic myocardial stunning after successful reperfusion.

Although nonesterified fatty acids (NEFA) are preferred as metabolic substrate for normal myocardium, glucose remains the most efficient source of myocardial ATP under conditions of limited oxygen supply (Taegtmeyer and DeVillalobos, 1995; Depre et al., 1999). The approach of enhancing myocardial energetic efficiency by increasing glucose availability and utilization has led to glucose-insulin-potassium (GIK) therapy in acute myocardial infarction (MI) (Malmberg et al., 1995; Fath-Ordoubadi and Beatt, 1997; Diaz et al., 1998; Van der Horst et al., 2003). GIK clinical trials in the prethrombolytic era (Fath-Ordoubadi and Beatt, 1997) or as an adjunct to thrombolysis (Malmberg et al., 1995; Diaz et al., 1998) demonstrated mortality benefits, notwithstanding hypoglycemia, hypokalemia, and volume overload (Opie, 1999; Sack and Yellon 2003). Recently, the first GIK trial as an adjunct to primary coronary intervention also demonstrated beneficial effects overall (Van der Horst et al., 2003). However, these benefits did not extend to patients presenting with heart failure after their myocardial infarction (Killip II-IV), presumably due to increased volume infusion requirements of this metabolic strategy (Apstein, 2003). Such practical concerns of GIK can be obviated using metabolic agents sharing the physiological properties of insulin, without causing systemic hypoglycemia or electrolyte imbalance.

Glucagon-like peptide-1-[7-36] amide (GLP-1) is a natural incretin with insulinomimetic, insulinotropic, and glucagonostatic actions whose metabolic effects favor glucose uptake (D’Alessio et al., 1994; Ritzel et al., 1995; Ahren et al., 1997). Because its insulinotropic activity is glucose-dependent (Elahi et al., 1994; Ryan et al., 1998) and ceases at glucose levels <4 mM (70 mg/dl), the risk of systemic hypoglycemia with GLP-1 is minimal, as confirmed in trials of GLP-1 for type 2 diabetes (Todd et al., 1997; Zander et al., 2002). Another pharmacological advantage of GLP-1 is the ability to administer therapeutic concentrations at minimal infusion volumes (3–6 ml/day), although its short half-life mandates continuous infusion.

ABBREVIATIONS: NEFA, nonessential fatty acid(s); GIK, glucose-insulin-potassium; GLP-1, glucagon-like peptide-1; LV, left ventricular; LCX, left circumflex; CBF, coronary blood flow; WTh, wall thickening; LVEDD, left ventricular end-diastole; FS, fractional shortening; dL/dt, diastolic myocardial segment length change; CAO, coronary artery occlusion; CAR, coronary artery reperfusion; C, control.
We recently demonstrated the safety and efficacy of 72-h infusions of GLP-1 in hospitalized patients with acute myocardial infarction and left ventricular (LV) dysfunction, undergoing successful primary coronary intervention reperfusion (Nikolaidis et al., 2004b). However, it is unclear whether GLP-1 ameliorates recovery from postischemic contractile dysfunction. The purpose of this study was to investigate whether continuous infusion of GLP-1 attenuates postischemic regional contractile dysfunction in normal conscious dogs undergoing brief (10-min) coronary artery occlusion and subsequent reperfusion.

Materials and Methods

Instrumentation. Adult mongrel dogs were anesthetized and instrumented as described previously (Nikolaidis et al., 2001), including LV pressure gauges, aortic, left and right atrial catheters, as well as transonic flow probes in the proximal aorta and the left circumflex (LCX) coronary artery for continuous recording of cardiac output and coronary blood flow (CBF), respectively. In addition, a hydraulic occluder implanted in the LCX proximal to the flow probe was used to induce reversible coronary arterial occlusion. Regional wall thickening (WTh) of the posterior wall and LV dimensions at end-diastole (LVEDD) and end-systole (LVESD) were measured by sonomicrometry. Global LV systolic function was evaluated using LV dP/dt and LV fractional shortening [FS = (LVEDD – LVESD)/LVEDD]. The rate of diastolic myocardial segment length change (dL/dt) of the LV short-axis diameter was used to estimate of diastolic LV filling, and the isovolumic relaxation half-time (t1/2), defined as the time required for LV pressure at end-systole to be reduced by 50%, was used to assess isovolumic relaxation (Ihara et al., 1994). All procedures were performed according to the National Institutes of Health protocols for humane use of experimental animals and approved by the institutional animal care committee.

Coronary Occlusion-Reperfusion. Animals recovered from surgery for 3 weeks before experimentation. All experiments were performed after 12-h overnight fasting. Heparin was avoided due to its lipolytic effects. The dogs were conscious during experimentation; however, morphine sulfate (2 mg i.v.) was administered before coronary artery occlusions (CAO) to mitigate ischemic pain. After baseline hemodynamic recordings, the proximal LCX was occluded for 10 min, by inflating the balloon of the hydraulic occluder, as described previously (Kim et al., 1997). Total occlusion was confirmed by absence of CBF at the flow probe distal to the occluder. The balloon was then deflated to establish coronary artery reperfusion (CAR).

To prevent ventricular arrhythmias, lidocaine (1%) was administered intravenously as follows: 2 ml at 5 min before occlusion, 1 ml at 9 min into occlusion, and 1 to 2 ml as needed if there was ventricular ectopy during the first 5 min after reperfusion. Serial hemodynamic and ECG recordings were obtained for the first 3 h of CAR (at 15, 30, 60, 120, and 180 min) and subsequently 24 h post-CAR, in a fasting, conscious state.

Metabolic Intervention. Six dogs receiving 24-h continuous i.v. infusion of GLP-1 (1.5 pmol/kg/min) were compared with eight dogs receiving placebo. The dose of GLP-1 was determined based upon the effective dose used in human studies of postischemic contractile dysfunction (Nikolaidis et al., 2004b) and has been demonstrated to increase plasma GLP-1 levels 10-fold (Nikolaidis et al., 2004a). Infusion of either GLP-1 or placebo was initiated 1 min before CAR. The total volume for either infusion was 3 ml/day using a microinfusion system (Medtronic Minimed, Northridge, CA). Fasting arterial glucose, insulin, and NEFA levels were measured at initiation and completion of the infusion.

Effect of GLP-1 in Normal Dogs. To exclude the possibility of GLP-1 having an intrinsic inotropic or vasodilator effect, we administered GLP-1 intravenously to five normal dogs, in the absence of ischemia, at doses ranging from 25 to 400% of the dose administered in the current study. We compared the dose response to GLP-1 to the respective CBF and WTh responses to the inotropic agonist dobutamine (1–10 μg/kg/min).

Statistical Analysis. Parameters obtained at multiple time points from each animal were compared between the two groups (GLP-1 versus controls) using repeated measures analysis of variance. Dose-response curves to either GLP-1 or dobutamine in normal dogs were compared by repeated measures analysis of variance. Serum concent-

<table>
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<tr>
<th>TABLE 1</th>
<th>Systemic hemodynamic parameters at baseline</th>
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<td>Baseline Hemodynamics</td>
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<tr>
<td>n</td>
<td>8</td>
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<tr>
<td>LVP (mm Hg)</td>
<td>113 ± 4</td>
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<tr>
<td>LVEDP (mm Hg)</td>
<td>12 ± 1</td>
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<tr>
<td>LV dP/dt (mm Hg/s)</td>
<td>2355 ± 85</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>85 ± 4</td>
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<tr>
<td>HR (min⁻¹)</td>
<td>74 ± 4</td>
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<tr>
<td>CO (l/min)</td>
<td>1.9 ± 0.3</td>
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<tr>
<td>CBF (ml/min)</td>
<td>21 ± 1</td>
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<tr>
<td>PWTh (mm)</td>
<td>2.6 ± 0.3</td>
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<tr>
<td>FS (%)</td>
<td>14 ± 1</td>
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<tr>
<td>dL/dt (mm/s)</td>
<td>11.8 ± 1</td>
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<tr>
<td>t1/2 (ms)</td>
<td>21.6 ± 0.7</td>
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CO, cardiac output; HR, heart rate; LVP, left ventricular pressure, LVEDP, left ventricular end-diastolic pressure; LV dP/dt: first derivative of LVP during systole; MAP, mean arterial pressure; PWTh: posterior wall thickening.

Fig. 1. CBF and respective regional wall thickening responses to CAO and CAR. The CBF response (top) is characterized by reactive hyperemia followed by gradual return of flow to preocclusion levels in both control (solid line) and GLP-1-treated animals (dotted lines). The different patterns of recovery of regional contractile function between the two groups are illustrated (bottom).
trations of glucose, insulin, and NEFA were compared by Student's *t* test. A two-tailed *p* < 0.05 was considered statistically significant.

**Results**

Baseline hemodynamics, global LV systolic and diastolic function, CBF, and regional myocardial WTh before CAO were comparable between groups (Table 1). As depicted in Fig. 1A, reperfusion resulted in an early phase of reactive hyperemia during the first 15 min, followed by a gradual return of CBF to baseline levels. Responses of CBF to CAO and CAR during reactive hyperemia were identical in the two groups (GLP-1, 553 ± 81%; and C, 548 ± 48%). Figure 1B illustrates the significant dissociation between normalization of CBF and re-

![Graphs showing mean arterial pressure, heart rate, left ventricular end-diastolic pressure, and isovolumic relaxation half-time during reperfusion.](image)

**Fig. 2.** Effects of GLP-1 (dotted lines) versus controls (solid lines) on mean arterial pressure, heart rate, left ventricular end-diastolic pressure, global fractional shortening, LV dP/dt, and isovolumic relaxation half-time during reperfusion.
covery of the respective posterior WTh, in the control group, consistent with myocardial stunning, even 24 h post-CAR, when regional WTh had only recovered to 78 ± 4% of baseline. In contrast, GLP-1-treated dogs demonstrated rapid and complete recovery of regional function in the ischemic zone after CAR. The difference was statistically significant as early as 15 min after reperfusion and persisted through 24 h.

As depicted in Fig. 2, no major differences were observed in regard to heart rate, mean arterial pressure, or global LV systolic function (+LV dP/dt, FS) response during CAO or CAR, although GLP-1-treated dogs demonstrated a trend (p of ~0.06) toward lower LVEDP. Isovolumic LV relaxation (t_{1/2}) was prolonged during CAO in both groups (C, 21.6 ± 0.8 to 29.9 ± 1.0***; GLP-1, 21.9 ± 0.8 to 28.8 ± 1.1*** ms; ***p < 0.001). After CAR, t_{1/2} gradually returned to baseline in GLP-1-treated dogs, whereas recovery was delayed in controls. dL/dt declined during CAO, subsequently increased during reactive hyperemia (C, 11.8 ± 1 to 9.7 ± 1.1* to 12.4 ± 1.7* mm/sec, GLP-1: 11.3 ± 1 to 10.1 ± 1.4* to 12.7 ± 1.9* mm/sec; *p < 0.05), and returned to baseline levels at 24 h in both groups. However, this recovery occurred rapidly in GLP-1-treated dogs, whereas it was significantly delayed in controls (dL/dt at 3-h CAR: C, 64 ± 7% versus GLP-1, 90 ± 8%** of baseline; p < 0.03).

**ACUTE DOSE-RESPONSES OF NORMAL DOGS TO GLP-1 COMPARED TO DOBUTAMINE**

**CORONARY BLOOD FLOW AND REGIONAL WALL THICKENING RESPONSES OF NORMAL DOGS TO CONTINUOUS GLP-1 (1.5 pmol/kg/min) FOR 24-48 HOURS**

Fig. 3. Effects of GLP-1 on CBF and WTh in normal dogs. Top, comparison of the acute (10-min) dose-response effects of normal dogs (n = 5) to a dose range of GLP-1 (D1 = 0.075 pmol/kg/min, D2 = 1.5 pmol/kg/min, D3 = 3 pmol/kg/min, and D4 = 6 pmol/kg/min) and dobutamine (D1 = 1 µg/kg/min, D2 = 2.5 µg/kg/min, D3 = 5 µg/kg/min, and D4 = 10 µg/kg/min). Bottom, depiction of the lack of effect of intravenous GLP-1 on CBF or wall thickening of normal dogs (n = 4), even after 24 to 48 h of continuous administration at the exact same dose (1.5 pmol/kg/min) used in the occlusion-reperfusion experiment.
Although all dogs received prophylactic lidocaine, three controls developed nonsustained ventricular tachycardia, yet no reperfusion arrhythmia occurred in GLP-1-treated dogs. Despite a trend toward lower glucose levels in both groups, normoglycemia was maintained at 24 h (C, 92 ± 4 to 87 ± 4 mg/dl; GLP-1, 98 ± 5 to 86 ± 3 mg/dl). Plasma insulin significantly (*p < 0.05) increased after 24 h of GLP-1 infusion (52 ± 8 to 62 ± 4 pmol/l), whereas it decreased in controls (62 ± 15 to 42 ± 10 pmol/l). NEFA tended to increase in controls (625 ± 102 to 712 ± 120 μM) compared with GLP-1-treated animals (661 ± 97 to 648 ± 39 μM).

In contrast to the effects of GLP-1 in the posts ischemic myocardium, GLP-1 had no demonstrable effects on either CBF or myocardial thickening in normal dogs (n = 5) either acutely (10–15 min) or after 24 to 48 h of continuous intravenous administration. The lack of intrinsic GLP-1 effect in normal dogs was in stark contrast to the dose-dependent augmentation of both CBF and regional WTh in response to conventional inotropic therapy with dobutamine (Fig. 3).

Discussion

Postischemic contractile dysfunction often limits complete recovery of regional function after successful reperfusion by coronary angioplasty. The mechanisms of myocardial stunning are complex, and several plausible strategies to overcome or attenuate stunning have been investigated (Gross et al., 1999; Verma et al., 2002). On a cellular level, myocardial stunning is associated with increased free radical production, intracellular calcium overload, and altered myocardial calcium sensitivity due to troponin I degradation (Heyndrickx, 2003; Kim et al., 2003). As such, therapeutic efforts have focused on free radical scavengers and calcium sensitizers. Most commonly, adrenergic agonists such as dobutamine have been used to support global ventricular function after stunning (McFalls et al., 2003) as well as to distinguish stunned from irreversibly injured myocardium (Shironi et al., 2003).

The role of metabolic adaptation has been less well defined. PET studies in dogs have correlated myocardial glucose uptake with recovery of function in the first 24 h after brief coronary occlusion (Di Carli et al., 2000). Metabolic interventions indirectly augmenting glucose utilization, such as dichloroacetate administration, facilitate recovery from stunning in a porcine model of partial coronary occlusion (Kudej et al., 2002). The clinical success of GIK as an adjunct to thrombolysis (Malmberg et al., 1997; Diaz et al., 1998), primary angioplasty (Van der Horst et al., 2003), or coronary bypass surgery (Coleman et al., 1989; Lazar et al., 1997) corroborates the utility of metabolic intervention in attenuating posts ischemic contractile dysfunction.

Although the metabolic effects of GLP-1 in diabetes have been investigated, less is known regarding the mechanisms whereby GLP-1 mediates its cardioprotective effects (Nikolaidis et al., 2004b). GLP-1 receptors are expressed in the human heart as well as the pancreas, lung, kidney, stomach, and hypothalamus (Wei and Mojsov, 1995; Wei and Mojsov, 1996). Although some studies have demonstrated modest increases in blood pressure and heart rate with GLP-1 in rats (Barragan et al., 1994; Yamamoto et al., 2002), others have shown antihypertensive effects in a salt-sensitive rat strain (Yu et al., 2003), whereas in calves, GLP-1 was hemodynamically neutral (Edwards et al., 1997). Similarly, there is no consensus regarding effects on cardiac output, since GLP-1 has been reported to exert negative inotropic effects on rat cardiomyocytes in vitro (Vila Petroff et al., 2001), whereas others have described positive inotropy (Barragan et al., 1994; Yu et al., 2003), particularly in the presence of β-blockers.

Our study is the first to demonstrate the utility of a novel metabolic agent, GLP-1, in attenuating myocardial stunning after ischemia-reperfusion in vivo, in a large conscious animal model. Furthermore, it is the first to investigate the effects of GLP-1 specifically on diastolic function in this setting, since diastolic relaxation is an ATP-dependent process conceivably influenced by myocardial substrate metabolism (Diamant et al., 2003). The precise cellular effects of GLP-1 on stunned myocardium remain to be determined. In this study, GLP-1 had a modest insulinotropic effect, consistent with the fact that plasma glucose levels were normal (98 mg/dl). We have shown that GLP-1 increases myocardial glucose uptake in conscious dogs with pacing induced heart failure (Nikolaidis et al., 2004a), which is a model of chronic myocardial stunning (Nikolaidis et al., 2001). In that model, we have shown that the increase in myocardial glucose uptake is independent of the insulinotropic effects of GLP-1, using hyperinsulinemic, euglycemic clamps (Nikolaidis et al., 2004a). The precise effects of GLP-1 on the myocardial insulin-signaling cascade remain to be determined.

Our findings are in contrast to Kavianipour et al. (2003), who observed no effects on hemodynamics or infarct size in an open-chest porcine model of prolonged (60 min) myocardial ischemia, despite increased pyruvate kinetics. However, our study investigated the effects of GLP-1 in a model of brief myocardial ischemia, as opposed to a complete infarct.

Conclusion

In summary, GLP-1 attenuated posts ischemic regional contractile dysfunction after brief CAO in conscious dogs. This was accompanied by improvement in LV diastolic relaxation. The effects could not be replicated in normal, nons ischemic dogs. The salutary effects of GLP-1 in experimental myocardial ischemia were associated with modest increases in plasma insulin, but not hypoglycemia.

References


