Direct Effects of Glucagon-Like Peptide-1 on Myocardial Contractility and Glucose Uptake in Normal and Postischemic Isolated Rat Hearts

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ABSTRACT

Recent evidence suggests that glucagon-like peptide-1 (GLP-1) enhances recovery of left ventricular (LV) function after transient coronary artery occlusion. However, it is uncertain whether GLP-1 has direct effects on normal or ischemic myocardium and whether the mechanism involves increased myocardial glucose uptake. LV function and myocardial glucose uptake and lactate production were measured under basal conditions and after 30 min of low-flow ischemia and 30 min of reperfusion in the presence and absence of GLP-1(7–36) amide. The response was compared with standard buffer alone or buffer containing insulin (100 μU/ml). GLP-1 decreased the left ventricular developed pressure (baseline: 100 ± 2 mm Hg; GLP-1: 75 ± 3 mm Hg, p < 0.05) and LV dP/dt (baseline: 4876 ± 65 mm Hg/s; GLP-1: 4353 ± 76 mm Hg/s, p < 0.05) in normal hearts. GLP-1 increased myocardial glucose uptake (baseline: 33 ± 3 μmol/min/g; GLP-1: 81 ± 7 μmol/min/g, p < 0.05) by increasing nitric oxide production and glucose transporter (GLUT)-1 translocation. GLP-1 enhanced recovery after 30 min of low-flow ischemia with significant improvements in LV end-diastolic pressure (control: 13 ± 4 mm Hg; GLP-1: 3 ± 2 mm Hg, p < 0.05) and LV developed pressure (control: 66 ± 6 mm Hg; GLP-1: 98 ± 5 mm Hg, p < 0.05). GLP-1 increased LV function, myocardial glucose uptake, and GLUT-1 and GLUT-4 translocation during reperfusion to an extent similar to that with insulin. GLP-1 has direct effects on the normal heart, reducing contractility, but increasing myocardial glucose uptake through a non-Akt-1-dependent mechanism, distinct from the actions of insulin. However, GLP-1 increased myocardial glucose uptake and enhanced recovery of cardiac function after low-flow ischemia in a fashion similar to that of insulin.

Glucagon-like peptide-1 (GLP-1) is a member of the proglucagon incretin-family implicated in the control of appetite and satiety (Drucker, 1998; Kieffer and Habener, 1999; Doyle and Egan, 2001). GLP-1 has insulinotropic, insulinnemimetic, and glucagonostatic effects, thereby exerting multiple complementary actions to lower blood glucose in subjects with type 2 diabetes mellitus (Drucker, 1998; Kieffer and Habener, 1999; Doyle and Egan, 2001). A major advantage over conventional insulin is the fact that the insulinotropic actions of GLP-1 are dependent on the ambient glucose concentration, mitigating the risks of hypoglycemia.

Although receptors for GLP-1 have been found in a variety of extrapancreatic tissues including the heart (Bullock et al., 1996; Wei and Mojsov, 1996), whether there are direct cardiovascular actions of GLP-1 remains controversial. For example, GLP-1 increased heart rate and blood pressure in intact rodents through sympathostimulatory effects (Barragan et al., 1994, 1996; Yamamoto et al., 2002) but depressed myocardial contractility in isolated rat ventricular myocytes (Vila Petroff et al., 2001). Recently, infusion of GLP-1 was associated with improvements in myocardial glucose uptake and left ventricular (LV) systolic function in conscious dogs with dilated cardiomyopathy but not in normal dogs (Nikolaidis et al., 2004a). Our laboratory has also shown that GLP-1 infusion improved regional and global function in patients with myocardial infarction and severe LV systolic dysfunction after successful primary angioplasty (Nikolaidis et al., 2004a). This work was supported in part by United States Public Health Service Grants DA-10480 and AG-023125.

ABBREVIATIONS: GLP-1, glucagon-like peptide-1; LV, left ventricular; KH, Krebs-Henseleit; LVEDP, left ventricular end-diastolic pressure; LVDevP, left ventricular developed pressure; GLUT, glucose transporter; MAP, mitogen-activated protein; MAPKAP, MAP kinase-activated protein kinase; NOx, nitrite/nitrate; NO, nitric oxide; DU, density units.
GLP-1 Depresses Contractility but Reduces Stunning

et al., 2004b). However, neither hemodynamics nor consequent infarct size was altered by GLP-1 infusion in an open-chest, anesthetized porcine model of ischemia (Kavianipour et al., 2003). By contrast, GLP-1 infusion reduced infarct size in an isolated isovolumic rodent model of regional ischemia, but ventricular function was unaffected and glucose uptake was not measured (Bose et al., 2005). As such, whether the actions of GLP-1 on myocardial function and glucose uptake are mediated by direct effects and whether they are evident in both normal and ischemic myocardium remain unresolved.

Accordingly, the purpose of the present study was to determine whether GLP-1 had direct effects on myocardial contractility and glucose uptake in the isovolumic rat heart. A second goal was to examine the cellular effects of GLP-1 on the translocation of glucose transport proteins. A third goal was to compare these effects with those observed after 30 min of low-flow global ischemia and to determine whether GLP-1 hastened the recovery of ventricular function during reperfusion.

Materials and Methods

Isovolumetrically Heart Perfusion

Seventy-five male Wistar-Kyoto rats (Charles River Laboratories, Wilmington, MA) were anesthetized with a 1-ml injection of 60 mg/ml pentobarbital sodium. After cessation of peripheral nervous function, hearts were quickly excised and arrested in cold heparin-containing Krebs-Henseleit (KH) buffer. The care and use of animals were conducted under the Guidelines on Human Use and Care of Laboratory Animals for Biomedical Research published by the National Institutes of Health and according to experimental protocol approved by the Institutional Animal Care Use Committee of Allegheny General Hospital.

The methodology of Langendorff-perfused rat heart preparation has been described previously in detail (Zhao et al., 2001). Briefly, hearts were cannulated via aortic arch for retrograde perfusion at 37°C under constant pressure (70 mm Hg) using KH buffer containing 119 mM NaCl, 5.4 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 25 mM NaHCO3, 0.25 mM EDTA, and 5.0 mM glucose. All buffer components were obtained from Sigma Chemical (St. Louis, MO). A left atrial incision was made to expose the mitral annulus through which a water-filled latex balloon was passed into the left ventricle. The balloon was attached via polyethylene tubing to a pressure transducer (model Px 272, Baxter Scientific Products, Miami, FL) that was connected to Triton System I. The initial balloon volume was set to generate left ventricular end-diastolic pressure (LVEDP) ~5 mm Hg. Myocardial function was measured, including left ventricular developed pressure (LVDP), LVEDP, and LV dp/dt. LVDP was calculated by subtracting LVEDP from the peak systolic pressure. Coronary flow was calculated by a timed collection of the effluent.

The perfusate leaving the heart was collected over 1 min for the measurement of glucose and lactate during steady state every 10 to 15 min during the respective protocols. Glucose and lactate in perfusate was measured using a YSI glucose analyzer. Glucose uptake was calculated by the following equation (Tada et al., 2000): Myocardial lactate production (micromoles per minute per gram) was calculated by the following equation (Tada et al., 2000): Myocardial lactate production (micromoles per minute per gram) was calculated by the following equation (Tada et al., 2000):

control group (n = 15), hearts were continuously perfused with KH buffer for an additional 30 min; 2) GLP-1 group (n = 15), hearts were switched into KH buffer containing GLP-1 (500 pmol/l) for an additional 30 min; 3) insulin group (n = 15), hearts were switched into KH buffer containing insulin (100 μU/ml) for an additional 30 min. GLP-1-(7–36) amide was synthesized in the protein/peptide core facility of the Endocrine Unit of the Massachusetts General Hospital. The peptide content was 99% pure and gave a single peak on high-performance liquid chromatography. The peptide was lyophilized in vials under sterile conditions for single use and was certified to be both pyrogen-free and sterile. Net peptide content was used for all calculations. The peptide used in this protocol was from a single lot. Novolin U-100 insulin was purchased from NovoNordisk (Princeton, NJ).

Experimental Protocol 2. The goal of these experiments was to determine whether GLP-1 mitigates postischemic contractile dysfunction after brief periods of low-flow ischemia. After 30 min of perfusion with normal KH buffer, hearts were subjected to low-flow global ischemia (5% of baseline flow by reducing coronary perfusion pressure to 25–30 mm Hg) for 30 min followed by 30 min of reperfusion. The control group (n = 10) received KH buffer during low-flow ischemia and recovery; The GLP-1 treatment group (n = 10) received KH buffer plus GLP-1 (500 pmol/l), administrated 1 min before induction of low-flow ischemia and throughout reperfusion. To compare the direct effects of GLP-1 to insulin on postischemic contractile dysfunction, a third group (n = 10) was studied in which insulin (100 μU/ml) was added 1 min before the induction of low-flow ischemia and throughout reperfusion. Ventricular function, myocardial glucose uptake, and lactate production were measured every 10 to 15 min during the protocol. Creatine phosphokinase levels were measured on effluent collected at 20 min of reperfusion.

Myocardial Insulin Signaling Cascade

LV myocardium from 10 control, 10 GLP-1-treated, and 10 insulin-treated hearts from protocol 1 and seven control, eight GLP-1-treated, and eight insulin-treated hearts from protocol 2 were snap-frozen in liquid nitrogen and stored at ~70°C. Purified sarcolemmal membranes were prepared using density gradient centrifugation as described previously (Nikolaides et al., 2004c). Translocation of respective GLUT transporters (GLUT-1 and GLUT-4) was analyzed by assessing the protein content in the purified sarcolemmal membrane preparations as a percentage of the total protein expression in the sarcolemma and cytosolic membrane fractions under the respective experimental circumstances.

LV myocardium was homogenized in a buffer free of phosphatase inhibitors and subjected to electrophoresis separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Nikolaides et al., 2004c). Resolved proteins were transferred onto a polyvinylidene difluoride membrane (Immobilon-P™; Millipore Corp, Bedford, MA) at a constant voltage (100 V) for 1 to 2 h at 4°C. Nonspecific membrane protein binding sites were blocked for at least 1 h at room temperature, and then membranes were probed with the specific primary antibody overnight at 4°C. The blots were washed in appropriate secondary antibody-horseradish peroxidase conjugate. The immunoreactive proteins were detected by use of an enhanced horseradish peroxidase/luminol chemiluminescence reaction kit (NEN Life Science Products, Boston, MA) and exposed to X-ray film (Hyperfilm ECL; Amersham Pharmacia Biotech, Piscataway, NJ). Denitometric analysis of the bands was carried out using a Personal Densitometer SI and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Adjustments for protein loading were accomplished by normalizing bands based upon Coomassie Blue staining of the blots.

Goat anti-rabbit IgG-horseradish peroxidase conjugate, anti-Akt-1, anti-phospho-Akt-1 (Ser-473) and (Thr-308) were purchased from BD Transduction Laboratories (San Diego, CA). Anti-GLUT4, anti-GLUT-1, and protein A/G PLUS-agarose were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phospho-Akt-1...
specificity was confirmed using phosphorylated and nonphosphorylated NIH/3T3 cell extracts purchased from Cell Signaling Technology, Inc. (Beverly, MA). Anti-GLP-1 receptor antibody was purchased from Alpha Diagnostics International (San Antonio, TX).

The AMP-activated protein kinase activity in the LV myocardium was determined using the method of Musi et al. (2001). In brief, the LV lysates containing 200 μg of protein were immunoprecipitated with a specific antibody to AMP-activated protein kinase α2 and protein A/G PLUS-agarose beads. Kinase reactions were performed in 40 mM HEPES, pH 7.0, 0.2 mM AMP, 80 mM NaCl, 0.8 mM dithiothreitol, 5 mM MgCl₂, 0.2 mM ATP (containing 2 μCi of γ-[32P]ATP), and 0.2 mM substrate for AMP-activated protein kinase peptide in a final volume of 40 μl for 20 min at 30°C. At the end of the reaction, a 20-μl aliquot was removed and spotted on Whatman P81 paper. Radioactivity was quantitated with a scintillation counter. Activity was expressed as incorporated ATP (picomoles) per milligram of protein per minute.

The p38α MAP kinase activity in the LV myocardium was determined using a p38α/SAPK2α assay kit purchased from Upstate Biotechnology (Charlottesville, VA) with slight modifications. In brief, the LV lysates containing 200 μg of protein were immunoprecipitated with a specific antibody to p38α MAPK (Chemicon, Temecula, CA) and protein A/G PLUS-agarose beads. The activation of MAPKAP kinase 2 was carried out by adding 5 μl (200 ng) of inactive MAPKAP kinase 2 and then 5 μl of ice-cold 200 μM ATP-30 mM MgCl₂ in assay dilution buffer 1 and incubating for 15 min at 30°C. The activation reaction was stopped by adding 40 μl of ice-cold ADBI and 10 μl of 0.886 mM MAPKAP 2 kinase substrate peptide. The phosphorylation of MAPKAP kinase 2 substrate peptide was performed by adding 10 μl of 75 mM MgCl₂ and 500 μM ATP (containing 5 μCi of γ-32P]ATP) in a final volume of 80 μl for 10 min at 30°C. At the end of the reaction, a 20-μl aliquot was removed and spotted on Whatman P81 paper. The papers were washed three times in 1% phosphoric acid and one time with acetone. Radioactivity was quantitated with a scintillation counter. Activity was expressed as incorporated ATP (picomoles) per milligram of protein per minute.

Total nitric oxide production in the isolated preparation was determined by measuring NOx in the effluent after control, GLP-1, and insulin infusions, respectively. The measurements of total nitric oxide levels were carried out using a kit purchased from R&D Systems, Inc. (Minneapolis, MN). The reaction buffer was ultrafiltered through a 10,000 molecular weight cutoff filter to eliminate proteins. Because most of the NO is oxidized to nitrite and nitrate, the concentrations of these anions are used as a quantitative measure of NO production. After the enzymatic conversion of nitrite to nitrate was demonstrated, a significant difference between groups. A paired t-test was used to compare any pair of pre- and post-treatment values for the same parameter. p < 0.05 was considered significant.

Results

Effects of GLP-1 on Left Ventricular Function in Normal Hearts. The baseline functional and metabolic parameters were comparable between groups (Table 1). In the control group, continuous perfusion with KH buffer did not alter baseline LVDP, LV dP/dt, heart rate, and coronary flow (Fig. 1). When hearts were switched at 30 min from normal KH buffer to the buffer containing GLP-1 (500 pmol/l), GLP-1 caused a rapid decrease in left ventricular developed pressure (baseline: 100 ± 2 mm Hg; GLP-1: 75 ± 3 mm Hg; p < 0.05), associated with a decline in LV dP/dt max (baseline: 4876 ± 65 mm Hg/s; GLP-1: 4353 ± 76 mm Hg/s; p < 0.05) (Fig. 1). GLP-1 increased coronary flow (baseline: 14 ± 1 ml/min; GLP-1: 18 ± 2 ml/min; p < 0.05) but did not change left ventricular diastolic pressure or heart rate.

To compare the effects of GLP-1 to insulin, we studied a third group using a similar protocol, but added insulin (100 μU/ml) instead of GLP-1 at 30 min. There was a significant increase in LVDP (baseline: 98 ± 3 mm Hg; insulin: 108 ± 3 mm Hg; p < 0.05) and LV dP/dt max (baseline: 4981 ± 98 mm Hg/s; insulin: 5101 ± 78 mm Hg/s; p < 0.05) in contrast to the findings with GLP-1 (Fig. 1). Additionally, insulin administration increased coronary flow (baseline: 14 ± 3; insulin: 19 ± 2 ml/min; p < 0.05) but did not change LVEDP or heart rate (Fig. 1).

Both GLP-1 and insulin administration increased myocardial glucose uptake compared with control (Fig. 2). The increase in glucose uptake was associated with increased glucose extraction and was greater than the observed increase in coronary flow. The increase in myocardial glucose uptake was accompanied by increased myocardial lactate production compared with control, although the magnitude of lactate production was less and occurred later than the increase in glucose uptake.

Effects of GLP-1 on Glucose Transporters. Figure 3 illustrates the effects of GLP-1 on selective components of the insulin-signaling cascade. Importantly, the 43-kDa isoform of the GLP-1 receptor was present in the myocardium and did not differ significantly between control- (30 ± 8 DU), GLP-1- (42 ± 10 DU), and insulin- (50 ± 12 DU) treated groups. Despite the observed increase in myocardial glucose uptake, GLP-1 treatment was not associated with either increased Akt-1 abundance or activation (phospho-Akt-1) compared with control, whereas insulin treatment was associated with a marked increase in the serine kinase activity.

Statistics

All measurements were expressed as means ± S.E.M. The data were analyzed by either unpaired Student’s t test or one-way analysis of variance. The Student-Newman-Keuls post hoc test was further used for pairwise comparisons after analysis of variance dem

Table 1

<table>
<thead>
<tr>
<th>Baseline hemodynamics</th>
<th>Control (n = 10)</th>
<th>GLP-1 (n = 10)</th>
<th>Insulin (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>101 ± 2</td>
<td>104 ± 3</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>LV end diastolic pressure (mm Hg)</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>LV dP/dt (mm Hg/s)</td>
<td>4976 ± 76</td>
<td>4876 ± 65</td>
<td>4981 ± 98</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td>95 ± 2</td>
<td>100 ± 3</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>251 ± 16</td>
<td>247 ± 13</td>
<td>236 ± 18</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>13 ± 1</td>
<td>14 ± 2</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Myocardial glucose uptake (μmol/min/g)</td>
<td>35 ± 4</td>
<td>33 ± 3</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>Myocardial lactate production (μmol/min/g)</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
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473 and threonine 308 phosphorylation of Akt-1. As expected, insulin stimulated GLUT-4 translocation to a greater extent than in either control or GLP-1-treated groups. In contrast, GLP-1 significantly increased myocardial NOx production, p38 MAP kinase activity, and GLUT-1 translocation to a greater extent than in the control or insulin-treated groups (Fig. 3).

The Effect of GLP-1 on Left Ventricular Function during Low-Flow Ischemia. GLP-1 significantly improved the recovery of function after 30 min of low-flow ischemia (Fig. 4). LVDevP (control: 66 ± 6; GLP-1: 84 ± 5; p < 0.05) and LV dP/dt\text{max} (control: 2345 ± 112 mm Hg/s; GLP-1: 4081 ± 165 mm Hg/s; p < 0.05) recovered faster and to a greater extent than control. GLP-1 treatment was associated with a lower postischemic LVEDP (control: 13 ± 4 mm Hg; GLP-1: 4 ± 2 mm Hg; p < 0.05). Heart rate and coronary flow responses did not change significantly with GLP-1 administration. The effects of GLP-1 did not differ significantly from the effects of insulin on the recovery of LV function after ischemia.

GLP-1 was associated with an increase in postischemic myocardial glucose uptake compared with control and a significantly greater increase in ischemic lactate production (Fig. 5). The effects of GLP-1 were similar to the effects of insulin.

Given that the ischemic insult involved global as opposed to regional myocardial perfusion, we measured creatine phosphokinase release after reperfusion. The creatinine phosphokinase release was less (p < 0.05) in the GLP-1-treated group (187 ± 43 U/l) and in the insulin-treated group (197 ± 35 U/l) compared with control (358 ± 31 U/l).

The Effects of GLP-1 on Postischemic GLUT Translocation. Figure 6 illustrates the effects of GLP-1 on
GLUT-1 and GLUT-4 translocation after 30 min of reperfusion compared with insulin or control. There was no difference in AMP kinase activity among the groups (control: 26 ± 5 pmol ATP/mg/min; GLP-1: 28 ± 7 pmol ATP/mg/min; insulin: 31 ± 10 pmol ATP/mg/min), although activity was higher (p < 0.05) in the postischemic myocardium compared with normal hearts (control: 13 ± 5 pmol ATP/mg/min; GLP-1: 17 ± 8 pmol ATP/mg/min; insulin: 15 ± 7 pmol ATP/mg/min). Notably, both the GLP-1- and insulin-treated groups demonstrated increased GLUT-4 and GLUT-1 translocation compared with control but in the absence of increased Akt-1 expression or activation. However, both GLP-1- and insulin-treated groups showed increased myocardial NO production and increased p38 MAP kinase activity in contrast to the selective effects on glucose transporters observed in normal hearts.

Discussion

In the present study, we determined that GLP-1 had direct negative effects on myocardial contractility but stimulated myocardial glucose uptake and lactate production in normally perfused, isolated rat hearts. The effects on myocardial contractility were distinct from those of insulin, despite comparable effects on coronary flow and myocardial glucose uptake. We identified the GLP-1 receptor in the myocardium and demonstrated that the effects of GLP-1 to stimulate myocardial glucose uptake were not mediated through the classic insulin-signaling cascade involving Akt-1 activation and GLUT-4 translocation but rather through increased myocardial nitric oxide production, p38 MAP kinase activity, and GLUT-1 translocation. Finally, GLP-1 enhanced functional recovery of the postischemic myocardium in contrast to
its negative inotropic effects on the normally perfused heart. Both the functional and the cellular effects on glucose uptake and lactate production were similar to those of insulin in the postischemic heart.

There have been a limited number of studies examining the effects of GLP-1 on infarct size or recovery from a postischemic injury with conflicting results. Kavianipour et al. (2003) reported that GLP-1-(7–36) did not alter myocardial glucose utilization, hemodynamics, or infarct size in an acute porcine model of infarction, despite the fact that GLP-1 increased insulin secretion and decreased lactate uptake. In contrast, our laboratory (Nikolaidis et al., 2005) has demonstrated that a 24-h infusion of GLP-1 (2.5 pmol/kg/min) mitigated postischemic contractile dysfunction and improved ventricular relaxation in conscious dogs after brief periods (10 min) of complete coronary artery occlusion. Similar beneficial effects have been observed in humans treated with a 72-h infusion of a similar dose of GLP-1 after acute myocardial infarction complicated by cardiogenic shock (Nikolaidis et al., 2004b). Nonetheless, these studies do not address whether the effects of GLP-1 were attributable to direct myocardial actions or indirect effects associated with the systemic insulinotropic actions of GLP-1.

Bose et al. (2005) investigated the effects of GLP-1 infusion (4.8 pmol/kg/min) in vitro in Sprague-Dawley rats subjected
to 30 min of left anterior descending artery occlusion and 120 min of reperfusion and observed that GLP-1 had no salutary effects on hemodynamics. The absence of hemodynamic benefits was surprising given that the investigators found a 50% reduction in infarct size in the GLP-1-treated group. A major difference between our findings and those of Bose et al. (2005) in the isolated heart models during ischemia may relate to the difference between complete and partial ischemia in the two models. The low-flow global ischemia used in our model may have facilitated GLP-1 perfusion into the ischemic zone, whereas Bose et al. (2005) used a model of complete coronary artery occlusion. Although the results differ, both studies support a direct effect of GLP-1 on myocardial function and preservation.

We observed abundant expression of GLP-1 receptors in the rat myocardium. However, the mechanisms whereby GLP-1 receptors couple to intracellular effectors in extraparenchymatic tissues, such as the heart, remain largely unexplored. We observed opposing effects of GLP-1 and insulin on myocardial contractility in normal hearts. In turn, we observed that GLP-1 and insulin had comparable effects on myocardial glucose uptake, but via different cellular mechanisms. Insulin-mediated glucose uptake was associated with Akt-1 phosphorylation and GLUT-4 translocation. In contrast, GLP-1 did not increase phospho-Akt-1 or GLUT-4 translocation but did result in increased GLUT-4 expression in the sarcolemma. Furthermore, GLP-1 was associated with increased myocardial NO production and increased p38 MAP kinase activity. Prior studies have demonstrated that GLP-1 increases NO production in vascular smooth muscle (Nystrom et al., 2004). However, this is the first illustration of such an effect in myocardium. Importantly, NO has been shown to stimulate p38 MAP kinase (McFalls et al., 2004), and both NO (Van Dyke et al., 2003) and p38 MAP kinase (Denton and Tavare, 1995; Taha et al., 1997) have been shown to increase membrane-associated GLUT-1 in isolated muscle cells. It remains to be determined what the proximal steps are in GLP-1 receptor coupling in the myocardium and where the contractile and glucoregulatory pathways diverge.

During recovery from low-flow ischemia, we showed that both GLP-1 and insulin had comparable effects on myocardial glucose uptake and functional recovery from the ischemic insult. Notably, both GLP-1- and insulin-mediated myocardial glucose uptake did not involve Akt-1 activation in the postischemic myocardium. Our findings on the effects of GLP-1 on Akt-1 activation differ from those reported by Bose et al. (2005). Prior studies (Young et al., 1997; Egert et al., 1999) have shown that low-flow ischemia is associated with increased myocardial GLUT-1 and GLUT-4 expression. Notably, during recovery, all three groups had increased AMP kinase activity consistent with ATP depletion during low-flow ischemia. Although AMP kinase is a potent stimulus to insulin-independent GLUT-4 translocation, this mechanism is insufficient to explain the increases in membrane GLUT-1 expression in the GLP-1- and insulin-treated groups. However, both GLP-1- and insulin-treated groups demonstrated increased NO production and p38 MAP kinase activity in the postischemic hearts compared with control and in contrast to what was observed with insulin in the normal heart. This pathway has been demonstrated previously to be integral to postischemic functional recovery (McFalls et al., 2004). It remains to be elucidated as to how GLP-1 increases nitric oxide production, the isoforms involved, and the intracellular compartment in which nitric oxide synthase is activated. Finally, it is conceivable that the benefits of GLP-1 and insulin to improve postischemic contractile dysfunction are related simply to enhanced glucose uptake rather than to a direct cellular effect on myocardial stunning. Our findings provide new insights into the direct myocardial effects of GLP-1 and novel signaling mechanisms for glucose uptake, independent of the Akt-1 activation.

References


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