Effect of intraportal glucagon-like peptide-1 on glucose metabolism in conscious dogs

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Nishizawa, Makoto, Mary Courtney Moore, Masakazu Shiota, Stephanie M. Gustavson, Wanda L. Snead, Doss W. Neal, and Alan D. Cherrington. Effect of intraportal glucagon-like peptide-1 on glucose metabolism in conscious dogs. Am J Physiol Endocrinol Metab 284: E1027–E1036, 2003.—Arteriovenous difference and tracer ([3-3H]glucose) techniques were used in 42-h-fasted conscious dogs to identify any insulin-like effects of intraportally administered glucagon-like peptide 1-(7–36)amide (GLP-1). Each study consisted of an equilibration, a basal, and three 90-min test periods (P1, P2, and P3) during which somatostatin, intraportal insulin (3-fold basal) and glucagon (basal), and peripheral glucose were infused. Saline was infused intraportally in P1. During P2 and P3, GLP-1 was infused intraportally at 0.9 and 5.1 pmol·kg⁻¹·min⁻¹ in eight dogs, at 10 and 20 pmol·kg⁻¹·min⁻¹ in seven dogs, and at 0 pmol·kg⁻¹·min⁻¹ in eight dogs (control group). Net hepatic glucose uptake was significantly enhanced during GLP-1 infusion at 20 pmol·kg⁻¹·min⁻¹ [21.8 vs. 13.4 μmol·kg⁻¹·min⁻¹ (control), P < 0.05]. Glucose utilization was significantly increased during infusion at 10 and 20 pmol·kg⁻¹·min⁻¹ [87.3 ± 8.3 and 105.3 ± 12.8, respectively, vs. 62.2 ± 5.3 and 74.7 ± 7.4 μmol·kg⁻¹·min⁻¹ (control), P < 0.05]. The glucose infusion rate required to maintain hyperglycemia was increased (P < 0.05) during infusion of GLP-1 at 5.1, 10, and 20 pmol·kg⁻¹·min⁻¹ (22, 36, and 32%, respectively, greater than control). Nonhepatic glucose uptake increased significantly during delivery of GLP-1 at 5.1 and 10 pmol·kg⁻¹·min⁻¹ (25 and 46% greater than control) and tended (P = 0.1) to increase during GLP-1 infusion at 20 pmol·kg⁻¹·min⁻¹ (24% greater than control). Intraportal infusion of GLP-1 at high physiological and pharmacological rates increased glucose disposal primarily in nonhepatic tissues.

incretin; net hepatic glucose uptake; muscle glucose uptake; blood glucose

Type 2 diabetes (T2DM) is characterized by abnormal insulin secretion from pancreatic β-cells and insulin resistance (13). Sulfonylureas, commonly used in treatment of T2DM, have a strong insulinotropic action, but they are prone to cause fasting hypoglycemia because their action is not glucose dependent. Therefore, a drug that effectively stimulates insulin secretion only under hyperglycemic conditions is desirable. Recently, glucagon-like peptide 1-(7–36)amide (GLP-1) has been shown to have a glucose-dependent insulinotropic effect (2).

GLP-1 is derived from proglucagon in the L cells in the distal ileum and colon and is rapidly released into the portal vein after meal ingestion (2). It lowers postprandial glucose levels in both healthy and T2DM subjects (24, 50). Its actions include stimulation of insulin secretion via a specific receptor on pancreatic β-cells (46), inhibition of glucagon secretion from α-cells, and delay of gastric emptying (2).

Whether GLP-1 has any effects on glucose disposal, outside of its actions on pancreatic hormone secretion and gastric emptying, remains unclear. Although some in vitro data indicate that GLP-1 can stimulate glucose uptake by adipocytes (18, 33) and skeletal muscle (51), other investigations have found no GLP-1 stimulation of glucose transport or glycogen synthesis in isolated muscle (22). Similarly, some in vivo investigations have suggested that GLP-1 enhances either insulin-dependent (43) or -independent (8) glucose uptake, whereas others have failed to find such effects (20, 21, 40, 42). In these previous in vivo studies, GLP-1 was delivered via a peripheral or central vein, in contrast to its normal route of secretion into the hepatic portal vein. Recent evidence suggests that the peptide may act within the hepatoporal region (5, 36, 38, 39). Because GLP-1 is degraded very rapidly by dipeptidyl-peptidase IV (DPP-IV) in plasma (9, 10), it would be optimal to deliver the peptide into the portal vein to evaluate its physiological effects fully. Some of the divergent results obtained in earlier in vivo studies may have arisen because of the difficulty in delivering an effective dose of GLP-1 to the liver, especially in human subjects, where the portal vein is inaccessible as an infusion route. In the present study, therefore, we examined the physiological and pharmacological effects of GLP-1 on glucose metabolism by infusing it into the portal vein of conscious dogs. We hypothesized that intraportal delivery would stimulate hepatic and nonhepatic glucose uptake independently of changes in pancreatic hormone secretion. We performed the studies under a somatostatin-controlled pancreatic clamp.

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because of GLP-1’s effect on pancreatic hormone secretion, and we employed hyperglycemic and hyperinsulinemic conditions to mimic the postprandial state, when most GLP-1 release occurs.

RESEARCH DESIGN AND METHODS

**Animals and surgical procedures.** Experiments were performed on seventy-three 42-h-fasted conscious mongrel dogs (23.5 ± 0.6 kg) of either sex that had been fed once daily a standard meat and chow diet (31% protein, 52% carbohydrate, 11% fat, and 6% fiber based on dry weight; Kal Kan, Vernon, CA and Purina Lab Canine Diet no. 5006, Purina Mills, St. Louis, MO). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. At least 16 days before experimentation, a laparotomy was performed with animals under general anesthesia. Silastic catheters (Dow Corning, Midland, MI) for blood sampling were placed into the portal vein, a hepatic vein, and a femoral artery, and infusion catheters were inserted into a jejunal vein and a splenic vein, as previously described (35). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the portal vein and the hepatic artery. On the day of the experiment, the catheters were exteriorized under local anesthesia, and intravenous access was established in three peripheral veins (35). Dogs were used for an experiment only if they met established criteria (35).

**Experimental design.** Each experiment in the three protocols consisted of a tracer equilibration period (−120 to −20 min), a basal period (−20 to 0 min), and three test periods during which hyperglycemia and hyperinsulinemia existed (0−120 min, 120−210 min, and 210−300 min). At −120 min, a primed (1.2 μCi/kg), continuous (0.17 μCi/min) infusion of [3-14C]glucose (New England Nuclear, Boston, MA) and a continuous infusion of indocyanine green dye (0.08 mg/min; Sigma Chemical, St. Louis, MO) were started. At time 0, a peripheral infusion of somatostatin (0.8 μg·kg−1·min−1; Bachem, Torrance, CA) was begun to inhibit endogenous pancreatic insulin and glucagon secretion. Intraportal infusions of insulin (1.2 mU·kg−1·min−1; Eli Lilly, Indianapolis, IN), to achieve hyperinsulinemia, and glucagon (0.5 ng·kg−1·min−1; Bedford Laboratory, Bedford, OH), to maintain basal insulin, were also started. A 50% dextrose solution was infused peripherally at variable rates starting at time 0 to clamp the arterial plasma glucose level at 220 mg/dl. The infusion rate of glucose was adjusted in response to the plasma glucose concentration, which was measured every 5 min. In the second and third test periods in one protocol, GLP-1-(7−36)amide (Sigma) was infused into the portal vein at 0.9 and 5.1 pmol·kg−1·min−1, respectively [low-dose GLP-1 (LGLP), n = 8]. In the second and third test periods of another protocol, GLP-1 was infused intraportally at 10 and 20 pmol·kg−1·min−1, respectively [high-dose GLP-1 (HGLP), n = 7]. These GLP-1 infusion rates were chosen to create a wide range of physiological and pharmacological plasma GLP-1 concentrations. In the third protocol, saline was infused intraportally during the second and third test periods [control group (CONT), n = 8]. Blood samples were taken periodically at the time points as previously described (35). Diprotin A (50 nmol/ml; Sigma) was added to the blood as soon as it was obtained to inhibit DPP-IV activity.

**Analytical procedures.** Plasma glucose and glucose radioactivity (3H), insulin, glucagon, cortisol, nonesterified fatty acids (NEFA), and blood lactate, glycerol, and alanine were measured as previously described (6, 35). Plasma GLP-1 levels were determined by an RIA method that specifically determines the biologically active form of GLP-1 [i.e., GLP-1(7−36)amide or GLP-1(7−37)] and binds to the NH2-terminal region (Linco Research, St. Charles, MO) (52).

**Calculations.** Net hepatic substrate balance (NHB) was calculated using the formula [H−Ft − (A−Ft + P−Ft)], where A, F, and P are the arterial, portal vein, and hepatic vein substrate concentrations, and Fp, Fe, and Fa are hepatic arterial, portal vein, and total hepatic blood or plasma flows (as appropriate), respectively. Hepatic substrate load was calculated as A−Ft + P−Ft. Net hepatic fractional extraction (FE) was calculated as NHB/ hepatic load. For all calculated data, plasma glucose concentrations were converted to blood concentrations with correction factors compiled from extensive data from our laboratory (25). During the basal period, arterial, portal vein, and hepatic vein plasma concentrations were multiplied by 0.74, 0.74, and 0.73 to convert them to blood glucose concentrations, and during the experimental period all plasma concentrations were multiplied by 0.73. Use of blood, rather than plasma, glucose concentrations ensures accurate NHB calculations regardless of the characteristics of glucose entry into the erythrocyte.

Sinusoidal hormone concentrations were calculated, as was hepatic load, by use of plasma flows and division by total hepatic flow. Nonhepatic glucose uptake was the difference between the glucose infusion rate (GIR) and net hepatic glucose uptake (NHGU). Net hepatic carbon retention, an indicator of the carbon available for glycogen synthesis, was calculated as NHGU minus net hepatic lactate output. This omitted the contribution of gluconeogenic substrates other than lactate and also of the carbon utilized in hepatic oxidation. Glucolysis would not have contributed in a net sense to glycogen synthesis, because its net hepatic uptake during the test periods was not different from zero. Amino acid uptake (as evidenced by net hepatic alanine uptake) did not change throughout the study, was not different between protocols, and would have contributed a maximum of 0.3 μmol glucose equivalents·kg−1·min−1 in each protocol. This would have been partially offset by hepatic glucose oxidation of 0.5 μmol glucose equivalents·kg−1·min−1 (44). Thus net hepatic carbon retention should provide a reasonably close index of net hepatic glycogen synthesis. Glycogen balance data in the text are reported as means of values during the last 30 min of the relevant experimental period, and changes in data are calculated as differences between the mean value of the last two time points in the first test period and the values during the last 30 min of the other periods. Tracer-determined glucose production (Rg) and glucose utilization (Rg) were determined with a two-compartment model by use of dog parameters (15).

**Statistical analysis.** Data are expressed as means ± SE and analyzed by SigmaStat (SPSS, Chicago, IL). Two-way repeated-measures analysis of variance was used to compare the time-course data of groups. Classification factors were treatment group and time period, as well as their interaction. “Dog within group” was used for an error term. For significant F values, the Student-Newman-Keuls multirange test was employed as a post hoc analysis. Differences were considered significant when P < 0.05.

RESULTS

**Hormone concentrations.** The mean arterial insulin concentrations increased to approximately threefold basal and were not different among the groups (Table
1). The plasma glucagon concentrations remained basal and similar in all of the groups. Likewise, the plasma cortisol concentrations remained basal in all of the groups.

Neither the arterial nor the portal plasma GLP-1 concentrations changed significantly during saline infusion (Fig. 1). In LGLP, the mean arterial plasma GLP-1 concentration increased from 12 ± 5 pmol/l during period 1 to 17 ± 6 and 51 ± 16 pmol/l during the last 30 min of periods 2 and 3, respectively (P < 0.05 for period 3 vs. period 1). The portal plasma GLP-1 concentrations in LGLP were 12 ± 5, 25 ± 8, and 102 ± 31 pmol/l during periods 1, 2, and 3, respectively (P < 0.05 for period 3 vs. period 1). In HGLP, arterial plasma GLP-1 levels increased from 22 ± 4 pmol/l in period 1 to 383 ± 84 and 480 ± 70 pmol/l during the last 30 min of periods 2 and 3, respectively (P < 0.05 for periods 2 and 3 vs. period 1). The portal plasma GLP-1 concentrations in HGLP were 26 ± 2, 588 ± 90, and 914 ± 158 pmol/l during periods 1, 2, and 3, respectively (P < 0.05 for periods 2 and 3 vs. period 1).

In response to somatostatin, portal and total hepatic blood glucose concentrations increased to 8.7 mmol/l, where they were maintained (Fig. 2). The hepatic glucose loads (HGL) during period 1 averaged 252 ± 17, 207 ± 13, and 221 ± 11 µmol·kg⁻¹·min⁻¹ in CONT; 28.2 ± 6.4, 22.0 ± 3.3, and 19.4 ± 7.9 in HGLP; and 18.1 ± 3.2, 15.1 ± 3.1, and 14.1 ± 3.3 in LGLP, respectively (P < 0.05 vs. CONT).

Table 1. Plasma insulin, glucagon, and cortisol concentrations and hepatic blood flows before and during intraportal infusion of GLP-1 or saline in the presence of hyperglycemia and hyperinsulinemia in 42-h-fasted conscious dogs given somatostatin, 3-fold basal intraportal insulin, and basal glucagon infusions

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Test Period 1</th>
<th>Test Period 2</th>
<th>Test Period 3</th>
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<tbody>
<tr>
<td>CONT</td>
<td>53 ± 5</td>
<td>132 ± 14</td>
<td>125 ± 12</td>
<td>130 ± 11</td>
</tr>
<tr>
<td>LGLP</td>
<td>46 ± 7</td>
<td>134 ± 13</td>
<td>135 ± 14</td>
<td>115 ± 7</td>
</tr>
<tr>
<td>HGLP</td>
<td>46 ± 7</td>
<td>109 ± 6</td>
<td>108 ± 15</td>
<td>117 ± 6</td>
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</table>

Hepatic blood flow and cardiovascular parameters. In response to somatostatin, portal and total hepatic blood flows decreased in each group (Table 1). Arterial blood flow was similar in LGLP and CONT during the test periods. High-dose GLP-1 infusion increased hepatic artery flow significantly, with the mean for the third period being 46% greater than that in CONT (P < 0.05). The increase in hepatic artery flow brought about an increase (P < 0.05) in total hepatic blood flow during periods 2 and 3 in HGLP (29 ± 1 ml·kg⁻¹·min⁻¹ in both periods vs. 26 ± 1 ml·kg⁻¹·min⁻¹ during period 1). Neither the CONT nor the LGLP group exhibited an increase in total hepatic blood flow during periods 2 and 3. GLP-1 did not change heart rate or blood pressure at any infusion rate (data not shown).

Blood glucose levels and hepatic glucose balance. In response to the peripheral glucose infusion, arterial blood glucose concentrations increased to ~8.7 mmol/l, where they were maintained (Fig. 2). The hepatic glucose loads (HGL) during period 1 averaged 252 ± 17, 207 ± 13, and 221 ± 11 µmol·kg⁻¹·min⁻¹ in CONT.
LGLP, and HGLP, respectively. The HGL remained relatively stable in CONT (Δ12 ± 6 and −1 ± 10 μmol·kg⁻¹·min⁻¹ for periods 2 and 3, respectively, vs. period 1), and it did not change significantly during low-dose GLP-1 infusion (Δ15 ± 14 and 19 ± 19 μmol·kg⁻¹·min⁻¹ vs. period 1). However, it increased significantly with the 10 and 20 pmol·kg⁻¹·min⁻¹ infusion rates (Δ28 ± 10 and 31 ± 12 μmol·kg⁻¹·min⁻¹ for periods 2 and 3, respectively, vs. period 1, P < 0.05), primarily as a result of the rise in hepatic artery blood flow.

Hyperglycemia combined with hyperinsulinemia switched net hepatic glucose balance from output to uptake in all of the groups (Fig. 3). In response to saline infusion (CONT), NHGU averaged 9.3 ± 2.1, 10.9 ± 1.5, and 13.4 ± 1.2 μmol·kg⁻¹·min⁻¹ in test periods 1, 2, and 3, respectively. In the low-dose GLP-1 study, NHGU increased from 9.4 ± 1.3 μmol·kg⁻¹·min⁻¹ in test period 1 to 11.3 ± 1.1 and 14.8 ± 3.3 μmol·kg⁻¹·min⁻¹ in test periods 2 and 3, respectively (NS vs. CONT). The corresponding rates in the group receiving high-dose GLP-1 were 8.6 ± 2.4, 10.2 ± 1.7, and 21.8 ± 4.2 (P < 0.05 vs. CONT during period 3) μmol·kg⁻¹·min⁻¹, respectively. Net hepatic FE of glucose followed the same pattern; only during delivery of GLP-1 at 20 pmol·kg⁻¹·min⁻¹ was FE significantly greater than in CONT (0.086 ± 0.016 vs. 0.055 ± 0.007; P < 0.05).

The mean GIRs required to maintain hyperglycemia were similar in the three groups during the first test period (Fig. 4). Thereafter, the GIR increased significantly in all groups. The GIR in CONT was 45.6 ± 6.2, 58.6 ± 6.7, and 69.4 ± 7.6 μmol·kg⁻¹·min⁻¹ during the first, second, and third test periods, respectively. The GIR in LGLP was 51.4 ± 6.6, 69.2 ± 6.1, and 84.7 ± 5.2 (P < 0.05 vs. CONT) μmol·kg⁻¹·min⁻¹ during the first, second, and third test periods, respectively, and the corresponding rates in HGLP were 56.2 ± 7.1, 79.7 ± 7.5 (P < 0.05 vs. CONT), and 91.5 ± 9.8 (P < 0.05 vs. CONT) μmol·kg⁻¹·min⁻¹.

Nonhepatic glucose uptake (non-HGU) increased significantly during test periods 2 and 3 compared with test period 1 in all groups (Fig. 4). In CONT, the rates were 36.3 ± 5.8, 47.7 ± 6.4, and 56.1 ± 7.8 μmol·kg⁻¹·min⁻¹ during the first, second, and third test periods, respectively. In LGLP, the rates averaged 42.0 ± 7.5, 47.9 ± 6.2, and 70.0 ± 6.3 (P < 0.05 vs. CONT) μmol·kg⁻¹·min⁻¹ during the three test periods. In HGLP, the rates of non-HGU were 47.7 ± 6.6, 69.6 ± 8.5 (P < 0.05 vs. CONT), and 69.7 ± 10.3 (P = 0.02).
0.10 vs. CONT) μmol·kg⁻¹·min⁻¹ during the three test periods.

Glucose $R_a$ was reduced to a rate no different from zero in all groups during the test periods (data not shown). Tracer-determined glucose $R_d$ during GLP-1 delivery at 0.9 and 5.1 pmol·kg⁻¹·min⁻¹ was not significantly different from that during saline infusion (Fig. 4). However, during infusion of GLP-1 at 10 and 20 pmol·kg⁻¹·min⁻¹, glucose $R_d$ was significantly greater than the rate evident with saline infusion, 87.3 ± 8.3 vs. 62.2 ± 5.3 μmol·kg⁻¹·min⁻¹ (GLP-1 at 10 pmol·kg⁻¹·min⁻¹ vs. CONT) and 105.3 ± 12.8 vs. 74.7 ± 7.4 μmol·kg⁻¹·min⁻¹ (GLP-1 at 20 pmol·kg⁻¹·min⁻¹ vs. CONT; $P < 0.05$ for both).

NEFA and glycerol concentrations and net hepatic uptake. Concomitant hyperinsulinemia and hyperglycemia reduced the arterial NEFA levels substantially from 811 ± 161, 761 ± 70, and 724 ± 67 μmol/l at 0 min to 96 ± 19, 102 ± 15, and 52 ± 11 μmol/l at 120 min in CONT, LGLP, and HGLP, respectively (Table 2). NEFA concentrations then remained low throughout each protocol. Net hepatic NEFA uptake fell to near zero in response to hyperglycemia and hyperinsulinemia and did not change significantly thereafter in any protocol.

The arterial blood glycerol levels decreased from 91 ± 14, 90 ± 8, and 78 ± 7 μmol/l in the basal period to 40 ± 11, 25 ± 4, and 29 ± 3 μmol/l at 120 min in CONT, LGLP, and HGLP, respectively (Table 2), after which they remained reduced. Net hepatic glycerol uptake was at a minimum in all groups by 120 min and did not differ among groups.

Lactate and alanine concentrations and balance data and net hepatic glycogen synthesis. After initiation of the hyperglycemic hyperinsulinemic clamp, arterial blood lactate levels increased (Fig. 5), and net hepatic lactate balance (NHILB) changed from uptake to output in all groups. NHLB declined to near 0 μmol·kg⁻¹·min⁻¹ in all groups by 150 min, and the CONT group then returned to a low rate of net hepatic lactate uptake. In the LGLP and HGLP groups, a low rate of net hepatic lactate output was evident during period 3, consistent with a stimulation of glycolysis by GLP-1. During infusion of GLP-1 at 20 pmol·kg⁻¹·min⁻¹, net hepatic glycogen storage was significantly enhanced, as indicated by comparison of net hepatic carbon retention with CONT during test period 3 (20.7 ± 3.2 vs.
Table 2. Arterial NEFA and glycerol concentrations and net hepatic uptake of NEFA and glycerol before and during intraportal infusion of GLP-1 or saline in the presence of hyperglycemia and hyperinsulinemia in 42-h-fasted conscious dogs given somatostatin, 3-fold basal intraportal insulin, and basal glucagon infusions

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th>Test Period 1</th>
<th>Test Period 2</th>
<th>Test Period 3</th>
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<tr>
<td></td>
<td>90</td>
<td>120</td>
<td>150</td>
<td>180</td>
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<tr>
<td>CONT</td>
<td>81 ± 161</td>
<td>101 ± 21</td>
<td>96 ± 19</td>
<td>100 ± 18</td>
</tr>
<tr>
<td>LGLP</td>
<td>76 ± 70</td>
<td>118 ± 16</td>
<td>102 ± 15</td>
<td>102 ± 14</td>
</tr>
<tr>
<td>HGLP</td>
<td>72 ± 67</td>
<td>94 ± 14</td>
<td>52 ± 11</td>
<td>51 ± 9</td>
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<tr>
<th></th>
<th>Arterial plasma NEFA, µmol/l</th>
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<tr>
<td>CONT</td>
<td>3.1 ± 0.8</td>
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<tr>
<td>LGLP</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>HGLP</td>
<td>3.5 ± 0.5</td>
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<tr>
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<th>Net hepatic NEFA uptake, µmol·kg⁻¹·min⁻¹</th>
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<tbody>
<tr>
<td>CONT</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>LGLP</td>
<td>0.0 ± 0.1</td>
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<tr>
<td>HGLP</td>
<td>0.2 ± 0.1</td>
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<table>
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<tr>
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<th>Arterial blood glycerol, µmol/l</th>
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<tbody>
<tr>
<td>CONT</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>LGLP</td>
<td>1.2 ± 0.2</td>
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<tr>
<td>HGLP</td>
<td>1.4 ± 0.4</td>
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<th>Net hepatic glycerol uptake, µmol·kg⁻¹·min⁻¹</th>
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<tbody>
<tr>
<td>CONT</td>
<td>0.7 ± 0.4</td>
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<tr>
<td>LGLP</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>HGLP</td>
<td>0.4 ± 0.3</td>
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</table>

Values are means ± SE. Test periods are shown in minutes. NEFA, nonesterified fatty acid. All groups are as described in Table 1. There were no significant differences among the groups. Values during the test periods were lower (P < 0.05) than the corresponding values during the basal period.

12.9 ± 1.6 µmol·kg⁻¹·min⁻¹, P < 0.05). No significant changes were observed in the arterial blood alanine levels, net hepatic alanine uptakes, or net hepatic FE throughout the study (data not shown).

DISCUSSION

GLP-1 was infused into the portal vein at four different rates. GLP-1 infusion at 0.9 pmol·kg⁻¹·min⁻¹ increased the arterial plasma GLP-1 from a basal value of 12 ± 5 to 17 ± 6 pmol/l. The levels achieved during the lowest GLP-1 infusion rate are consistent with the concentrations of intact GLP-1 reported for conscious dogs after an oral glucose load (2 g/kg) (32) or intragastric delivery of a mixed meal (12) and for healthy humans after mixed meal ingestion (52). These concentrations are also similar to the arterialized venous concentrations obtained in individuals with type 1 diabetes (T1DM) and T2DM during peripheral GLP-1 infusion at 1.2 pmol·kg⁻¹·min⁻¹ (49, 50). The infusion of GLP-1 at 5.1 pmol·kg⁻¹·min⁻¹ resulted in arterial GLP-1 plasma levels of 51 ± 16 pmol/l, approximately twofold higher than those normally reported after a mixed meal in young adults (52) but similar to those attained in elderly subjects with T2DM ingesting a mixed meal (14). The portal GLP-1 concentrations at the 0.9 and 5.1 pmol·kg⁻¹·min⁻¹ infusion rates were 25 ± 8 and 102 ± 31 pmol/l, respectively. Because there are few reports in the literature that include portal vein GLP-1 concentrations, we have measured intact arterial and portal vein GLP-1 in three overnight-fasted dogs under basal conditions and for 3 h after an intragastric glucose bolus of 1.5 g/kg (Dardevet D, Moore MC, and Cherrington AD, unpublished data). The basal GLP-1 concentrations in those animals were 17 ± 3 pmol/l in both the artery and portal vein. Peak values (20 min after glucose administration) were 39 ± 12 (artery) and 65 ± 24 (portal vein) pmol/l, with a gradual decline thereafter. Two hours after glucose delivery, the levels had reached 21 ± 5 and 25 ± 10 pmol/l in the artery and portal vein, respectively. Thus the two lowest infusion rates in the current investigation resulted in physiological or near-physiological concentrations of GLP-1. On the other hand, intraportal infusion of GLP-1 at 10 and 20 pmol·kg⁻¹·min⁻¹ resulted in arterial GLP-1 levels that were clearly pharmacological (383 ± 82 and 480 ± 70 pmol/l, respectively). The portal concentrations achieved in our study (588 ± 90 and 914 ± 158 pmol/l with GLP-1 at 10 and 20 pmol·kg⁻¹·min⁻¹, respectively) were somewhat lower than what can be predicted on the basis of our infusion rates and portal flow (~800 and 1,600 pmol/l, respectively). This probably resulted from the rapid degradation of GLP-1 in the circulation (29). Also, GLP-1 was prepared in saline that contained 3% (vol/vol) of the dog’s own plasma, as were all of the other hormones, to reduce binding of the hormones to the containers used for preparing them and to the infusion lines. No DPP-IV inhibitor was added to the GLP-1 solution, and thus it is possible that there was some degradation of GLP-1 during the infusion period.

The dogs studied tolerated all GLP-1 infusion rates without apparent discomfort or other side effects. Gastrointestinal side effects, such as nausea, have been reported in human subjects receiving GLP-1, especially when the peptide was delivered intravenously at rates as high as 2.7 pmol·kg⁻¹·min⁻¹ (41). GLP-1 receptor agonists have also caused nausea in some investigations (1, 19) but not in others (17, 37), and calves have...
tolerated short-term GLP-1 infusion rates as high as 35 pmol·kg⁻¹·min⁻¹ (16). It is likely that the gastrointestinal side effects are related to the well-known ability of GLP-1 to delay gastric emptying (17), and thus the fact that the current studies were conducted in fasted dogs may have helped to reduce any discomfort that might otherwise have accompanied GLP-1 treatment.

In vitro data suggest that GLP-1 can directly stimulate glucose uptake by nonhepatic tissues (18, 33, 51), although not all investigations have confirmed this effect (22). Whether GLP-1 has an independent effect on tissue glucose uptake in vivo has also been difficult to determine, largely because physiological doses of GLP-1 stimulate insulin secretion (26, 47). Using the minimal-model technique to evaluate the response of normal subjects to a frequently sampled intravenous glucose tolerance test, D’Alessio et al. (7) concluded that GLP-1 infusion increased glucose effectiveness and insulin-independent glucose disposal. In a study using the same technique in mice (3), GLP-1 was found to augment insulin secretion but not insulin sensitivity. In agreement with this, Freyse et al. (21) observed that intravenous infusion of GLP-1 at 10 pmol·kg⁻¹·min⁻¹ did not change insulin sensitivity in C-peptide-negative 90% pancreatectomized dogs. Similarly, Ryan et al. (42) found no evidence of enhancement of insulin sensitivity by GLP-1 in normal volunteers during a euglycemic clamp. Somatostatin was not used, but each subject underwent a control experiment during which insulin was infused at a rate to mimic the levels observed during the infusion of GLP-1. Even in studies utilizing the pancreatic clamp technique to eliminate changes in insulin and glucagon secretion, differing conclusions have been reached regarding the insulin-independent effects of GLP-1. Healthy volunteers exhibited no augmentation of glucose disposal associated with GLP-1 infusion during a hyperinsulinemic euglycemic clamp in which somatostatin was used to suppress pancreatic hormone secretion and glucagon and growth hormone were replaced at basal levels (40). Similar conclusions were obtained with euinsulinemic and hyperinsulinemic clamp studies in hyperglycemic subjects with T2DM (50). On the other hand, a delayed enhancement (during the 4th h of GLP-1 infusion) of whole body but not splanchnic glucose disposal was observed during intraduodenal glucose infusion under pancreatic clamp conditions in individuals with T1DM (49). Sandhu et al. (43) reported that GLP-1 potentiated insulin-stimulated glucose utilization during a hyperinsulinemic hyperglycemic clamp in depancreatized dogs but had no effect in the presence of a euinsulinemic hyperglycemic clamp. GLP-1 appeared to augment the suppression of NEFA levels during the hyperinsulinemic but not the euinsulinemic clamp, and this could at least partly account for the stimulation of glucose utilization by GLP-1 (43). In contrast to the normal route of GLP-1 secretion, i.e., the hepatic portal vein, a common feature of all of the previous in vivo studies cited is that GLP-1 was administered via a peripheral or central vein. The degradation of GLP-1 in vivo is very rapid (plasma half-life 2–4 min) (11, 28). Consequently, portal vein GLP-1 concentrations during peripheral GLP-1 administration were undoubtedly lower than the peripheral concentrations. Thus these previous studies beg the question of what effect GLP-1 might have on glucose metabolism when delivered via its endogenous secretion route.

The current data are unique in that they were obtained in conscious animals during the intraportal infusion of GLP-1. Recent reports highlight the importance of the hepatoporal area in the physiological activity of GLP-1. Nakabayashi et al. (36) reported that intraportal GLP-1 infusion at a physiological dose stimulated afferent vagal nerve activity in rats. This activation, in turn, stimulated efferent signaling in the pancreatic branch of the vagus nerve, suggesting a neural component of GLP-1’s stimulation of insulin secretion (36). This hypothesis was verified by a recent investigation (4). In addition, the afferent limb of the
inhibitory effect of GLP-1 on gastric emptying is mediated by the vagus nerve (27). Moreover, portal but not peripheral infusion of the GLP-1 antagonist exendin (9–39) inhibited the increase in glucose clearance observed during portal glucose infusion in normal mice (5). Also, glucose clearance did not increase during portal glucose infusion in GLP-1 receptor knockout mice, although it did so in wild-type mice. Despite the strong evidence in the literature linking the hepatoportal region with GLP-1 action, the current findings clearly show that the 0.9 pmol·kg\(^{-1}\)·min\(^{-1}\) infusion rate, which resulted in physiological circulating GLP-1 levels, did not bring about an enhancement of glucose uptake by either the liver or the nonhepatic tissues. During infusion of GLP-1 at 5.1 pmol·kg\(^{-1}\)·min\(^{-1}\), there were no discernible effects on the liver, but the GIRs required to maintain the hyperglycemic clamp and the non-HGU were elevated over the rate evident in CONT. On the other hand, during delivery of pharmacological doses of GLP-1, NHGU, Rd, GIR, and non-HGU were increased. Because all of the GLP-1 infusion rates associated with enhancement of glucose uptake by the peripheral tissues resulted in high physiological or frankly pharmacological circulating GLP-1 levels, it is impossible to distinguish between two possible interpretations of the data: 1) GLP-1 does not have a direct effect on peripheral tissues but instead acts within the portal vein to stimulate non-HGU indirectly, or 2) GLP-1 can act via a hepatoportal receptor but also may have direct peripheral effects at higher concentrations. In regard to the first possibility, release of a humoral factor from the liver has been hypothesized to enhance insulin sensitivity in skeletal muscle (30), but it remains unclear whether our current observation relates to that factor.

A second unique feature of the current results is that they clearly indicate, by two independent measures (GIR and glucose R\(_d\)), that GLP-1 stimulated whole body glucose disposal in normal conscious dogs in which pancreatic hormone concentrations were fixed and hyperglycemia was present. The plasma NEFA levels were low, and there were no significant differences among the groups. This suggests that the combination of hyperglycemia and hyperinsulinemia brought about a maximum antilipolytic effect, so that we were able to examine the effect of GLP-1 on glucose disposal independent of an indirect effect resulting from suppression of lipolysis. During infusion of GLP-1 at 5.1 and 10 pmol·kg\(^{-1}\)·min\(^{-1}\), non-HGU was significantly increased, and non-HGU tended to be increased during delivery of GLP-1 at 20 pmol·kg\(^{-1}\)·min\(^{-1}\). In fact, there were no differences among the rates of non-HGU during GLP-1 infusion at 5.1, 10, and 20 pmol·kg\(^{-1}\)·min\(^{-1}\), but the increased variance evident during the highest infusion rate precluded statistical significance. The time course of GLP-1’s action is unclear. If infused over a longer period, it is possible that it would have had larger effects at lower dosages (49). This is consistent with the enhancement of GIR and non-HGU by GLP-1 at 5.1 pmol·kg\(^{-1}\)·min\(^{-1}\), because that infusion rate occurred during the third test period.
glucose $R_3$ and increased the GIR required to maintain the hyperglycemic clamp. This was primarily a consequence of an increase in glucose uptake by the nonhepatic tissues, because significant stimulation of NHGU was observed only during the highest dosage of GLP-1 and in a setting in which hepatic artery flow changed, consistent with stimulation of NHGU by either the change in blood flow or a factor that contributed to the change in flow. These data demonstrate that there is a potential for GLP-1 to affect glucose uptake in both the liver and the nonhepatic tissues (presumably primarily skeletal muscle), but they leave unresolved the issue of dose dependence and time course of action. Nevertheless, our findings support a role for GLP-1 as a tool for the reduction of postprandial hyperglycemia in individuals with diabetes.

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