Glucagon-Like Peptide 1 Increases Insulin Sensitivity in Depancreatized Dogs

Harmanjit Sandhu, Stephanie R. Wiesenthal, Patrick E. MacDonald, Richard H. McCall, Vaja Tchipashvili, Shirya Rashid, Malathy Satkunarajah, David M. Irwin, Z. Qing Shi, Patricia L. Brubaker, Michael B. Wheeler, Mladen Vranic, Suad Efendic, and Adria Giacca

To determine whether glucagon-like peptide (GLP)-1 increases insulin sensitivity in addition to stimulating insulin secretion, we studied totally depancreatized dogs to eliminate GLP-1's incretin effect. Somatostatin was infused (0.8 µg · kg^{-1} · min^{-1}) to inhibit extrapancreatic glucagon in dogs, and basal glucagon was restored by intraportal infusion (0.65 ng · kg^{-1} · min^{-1}). To simulate the residual intraportal insulin secretion in type 2 diabetes, basal intraportal insulin infusion was given to obtain plasma glucose concentrations of ~10 mmol/l. Glucose was clamped at this level for the remainder of the experiment, which included peripheral insulin infusion (high dose, 5.4 pmol · kg^{-1} · min^{-1}, or low dose, 0.75 pmol · kg^{-1} · min^{-1}) with or without GLP-1(7-36) amide (1.5 pmol · kg^{-1} · min^{-1}). Glucose production and utilization were measured with 3-[^{3}H]glucose, using radiolabeled glucose infusates. In 12 paired experiments with six dogs at the high insulin dose, GLP-1 infusion resulted in higher glucose requirements than saline (60.9 ± 11.0 vs. 43.6 ± 8.3 µmol · kg^{-1} · min^{-1}, P < 0.001), because of greater glucose utilization (72.6 ± 11.0 vs. 56.8 ± 9.7 µmol · kg^{-1} · min^{-1}, P < 0.001), whereas the suppression of glucose production was not affected by GLP-1. Free fatty acids (FFAs) were significantly lower with GLP-1 than saline (375.3 ± 103.0 vs. 524.4 ± 101.1 µmol/l, P < 0.01), as was glycerol (77.9 ± 17.5 vs. 125.6 ± 51.8 µmol/l, P < 0.05). GLP-1 receptor gene expression was found using reverse transcriptase–polymerase chain reaction of poly(A)-selected RNA in muscle and adipose tissue, but not in liver. Low levels of GLP-1 receptor gene expression were also found in adipose tissue using Northern blotting. In 10 paired experiments with five dogs at the low insulin dose, GLP-1 infusion did not affect glucose utilization or FFA and glycerol suppression when compared with saline, suggesting that GLP-1's effect on insulin action was dependent on the insulin dose. In conclusion, in depancreatized dogs, GLP-1 potentiates insulin-stimulated glucose utilization, an effect that might be contributed in part by GLP-1 potentiation of insulin's antilipolytic action. *Diabetes* 48:1045–1053, 1999

Sulfonylureas are common drugs in use for treatment of type 2 diabetes. The insulin secretory mechanism of sulfonylureas is not glucose dependent, however, and may lead to hypoglycemia. The search for drugs that increase insulin secretion in a glucose-dependent manner has led to the investigation of a new agent, glucagon-like peptide (GLP)-1. GLP-1, a peptide derived from intestinal cleavage of the proglucagon molecule (1), has a glucose-dependent insulinotropic effect (1). GLP-1 also has a suppressive effect on glucagon levels (2,3) and delays gastric emptying (4), thus attenuating the postprandial glucose peaks. Furthermore, GLP-1 may improve insulin action.

Studies in vitro gave controversial results as to whether GLP-1 has insulin-like effects in muscle and liver. GLP-1 enhanced glycogen synthesis and glucose oxidation and utilization in rat skeletal muscle (5) and increased insulin-stimulated glycogen synthesis in L6 muscle cells (6). In another study, however, no effect of GLP-1 on glycogen synthesis in rat skeletal muscle was observed (7). A glycogenic effect of GLP-1 was found in rat hepatocytes by some authors (8), but not by others (9). However, the majority of studies do show some insulin-like effect of GLP-1 on adipose tissue. GLP-1 enhanced [^{14}C]acetate incorporation into fatty acids in explants of rat adipose tissue (10) and increased insulin-stimulated glucose uptake in isolated rat adipocytes (11), as well as glucose uptake and incorporation into fatty acids in 3T3-L1 adipocytes (12). Interestingly, GLP-1 was also found to be lipolytic in isolated rat adipocytes (13).

A number of studies have reported insulin-like or insulin-potentiating effects of GLP-1 in vivo. D'Alessio and colleagues found that GLP-1 enhanced the effect of glucose on its own disposal (14,15) and suppressed glucose production (16). Gutniak et al. (17) found that GLP-1 infusion improved insulin sensitivity during euglycemic clamps in subjects with type 1 diabetes. Glucagon levels were not clamped or measured in that study, however, which could be important since GLP-1 is known to suppress glucagon secretion (2,3). Additionally, tracer methods were not used, and therefore it was not possible to determine whether the GLP-1–mediated

From the Departments of Physiology (H.S., S.R.W., P.E.M., R.H.M., V.T., S.R., M.S., Z.Q.S., P.L.B., M.B.W., M.V., A.G.), Medicine (P.L.B., M.B.W., M.V., A.G.), Surgery (Z.Q.S.), and Laboratory Medicine and Pathobiology (D.M.I.), University of Toronto, Toronto, Ontario, Canada; and the Department of Endocrinology (S.E.), Karolinska Hospital, Stockholm, Sweden.

Address correspondence and reprint requests to Adria Giacca, MD, Assistant Professor of Physiology and Medicine, Department of Physiology, Medical Sciences Bldg., Rm. 3363, 1 King's College Circle, University of Toronto, Toronto, Ontario, Canada M5S 1A8. E-mail: adria.giacca@utoronto.ca.

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FFA, free fatty acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; HPLC, high-performance liquid chromatography; ID, inner diameter; MANOVA, multivariate analysis of variance; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RIA, radioimmunoassay; RT, reverse transcriptase; SSC, standard saline citrate; TFA, trifluoroacetic acid.

increase in insulin sensitivity was due to an effect on glucose production or on glucose utilization. In spite of these results, several in vivo studies could not show any insulin-like or insulin-potentiating effects of GLP-1 (2,18–20).

To investigate the extrapancreatic effects of GLP-1, we performed insulin clamps in moderately hyperglycemic depancreatized dogs. In contrast to the study of Gutniak et al. (17), we also clamped glucagon and used tracer methods. Our dog model is advantageous, since complete pancreatectomy can be performed to study the effect of GLP-1 independent of endogenous insulin secretion, extrapancreatic glucagon secretion in depancreatized dogs (21) can be inhibited by high-dose somatostatin, and insulin and glucagon levels can be clamped by direct intraportal infusions, thus simulating the physiologic portal-peripheral insulin and glucagon gradients, which are maintained in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Experimental animals and preparation. The present study was performed on eight postabsorptive, depancreatized mongrel dogs of either sex, weighing 18–35 kg. Total pancreatectomy was performed under general anesthesia induced with sodium thiopental (Abbott Laboratories, Montreal, Quebec, Canada) and maintained with 0.5% halothane (Halocarbon Laboratories, River Edge, NJ) with nitrous oxide (Canox, Toronto, Ontario, Canada) and assisted ventilation. The pancreas was removed completely, and care was taken to preserve duodenal vascularization through the pancreatoduodenal vessels.

A silastic cannula (0.04-inch inner diameter [ID]; Baxter Healthcare, McGraw Park, IL) was inserted into the portal vein through a branch of the splenic vein and advanced until the tip was approximately 1.0 cm beyond the point of confluence of the splenic vein with the portal vein, that is, approximately 5 cm from the branching point of the portal vein into its left and right bifurcations to the liver. Three silastic cannulas (one 0.04-inch ID and two 0.03-inch ID) were inserted into a jugular vein and advanced into the superior vena cava. In addition, a silastic cannula (0.04-inch ID) was inserted into a carotid artery and advanced into the aortic arch. The carotid cannula served for arterial sampling, and the jugular and portal cannulas served for infusions. The cannulas were tunneled subcutaneously and exteriorized at the back of the neck. They were filled with heparin (1,000 U/ml) (Hepalean; Organon Teknika, Toronto, Ontario, Canada) and bandaged around each dog's neck. The cannulas were flushed regularly (every 3–4 days) with saline to maintain patency.

The dogs received a diet of 400 g dry chow (Purina Mills, St. Louis, MO) and 670 g canned meat (Derby Pet Food, Brampton, Ontario, Canada) once a day. Pancreatic enzyme capsules (Cotazym; Organon Teknika), iron, and folic acid were mixed with food. Regular and NPH porcine insulin (Eli Lilly, Indianapolis, IN) were injected subcutaneously at meal times to maintain glycosuria <1%. Porcine insulin does not induce the formation of anti-insulin antibodies for at least 2 months in dogs (21), thus allowing accurate measurements of plasma insulin. Body weight, body temperature, hematocrit, stools, and food intake were monitored regularly.

Only dogs with a hematocrit >35% and at least 2 days of relatively well-controlled diabetes (blood glucose 8–10 mmol/l) were allowed to undergo experiments. The dogs received the normal amount of food the day before the experiment. The regular insulin dose was unaffected, whereas the NPH insulin was reduced to one-half or one-third the previous day's dose so as to obtain early morning hyperglycemia and thus facilitate the control of blood glucose levels by intravenous insulin. The experiments were performed after an 18 h overnight fast. All procedures were in accordance with the Canadian Council on Animal Care standards and were approved by the Animal Care Committee of the University of Toronto.

Experimental design. The depancreatized dogs were hyperglycemic (24.7 ± 2.3 mmol/l) at the onset of the experiment. Regular porcine insulin was infused intraportally, initially starting at a high dose (20 pmol · kg^{-1} · min^{-1}). The dose was then gradually reduced to basal levels to obtain constant moderate hyperglycemia (9–11 mmol/l). When glucose levels declined <17 mmol/l, a bolus of high-performance liquid chromatography (HPLC)-purified 3-[^{3}H]glucose (7.77×10^7 dpm; New England Nuclear, Boston, MA) was given and a continuous 3-[^{3}H]glucose infusion (5.55×10^5 dpm/min) was initiated (time –150 min) to enable the measurement of glucose turnover. At the same time the tracer infusion was initiated, somatostatin (Bachem, Torrance, CA) and glucagon (Eli Lilly) were infused at 0.8 µg · kg^{-1} · min^{-1} and 0.65 ng · kg^{-1} · min^{-1}, respectively. Somatostatin infusion was used to inhibit glucagon secretion from the gastric mucosa, an extrapancreatic supply of glucagon in the dog (21), while glucagon was infused intraportally to maintain basal glucagon levels throughout the experiment. The tracer equilibration period was at least 120 min long. After at least 30 min of steady-state glucose levels with a fixed low portal insulin dose, basal samples were taken every 10 min (from –30 to 0 min). At time 0, insulin was infused peripherally at either a high dose (5.4 pmol · kg^{-1} · min^{-1}) or a low dose (0.75 pmol · kg^{-1} · min^{-1}), with or without simultaneous GLP-1(7-36) amide (Saxon Biochemicals, Bachem) infusion (1.5 pmol · kg^{-1} · min^{-1}). The high-dose insulin treatment was performed to achieve postprandial insulin levels, and the low-dose insulin treatment was performed to achieve insulin levels close to the fasting range. All insulin infusions were prepared in saline containing approximately 3% (vol/vol) of the dog's own plasma. Plasma glucose was clamped at the initial preclamp glycemic levels for 3 h with a variable exogenous 25% dextrose infusion that was adjusted according to plasma glucose concentrations determined every 5 min. 3-[^{3}H]Glucose tracer was added to the glucose infusate as described previously (22) to prevent the decline in glucose specific activity during the glucose clamp and thus minimize errors associated with the use of the one-compartment, fixed-pool volume model method (23) for calculations of glucose production (24,25). The amount of tracer in the dextrose infusate was based on estimates of suppression of glucose production and dextrose requirements. The following equation by Finegood et al. (25), modified as in Giacca et al. (22) to account for partial suppression of glucose production, was used to calculate the specific activity of the dextrose infusate:

$$\text{SAginf} = I \times \frac{[\text{GINF(ss)}/R\text{a(b)}] - F}{\text{GINF(ss)}} \times \frac{1{,}000}{\text{BW}}$$

where SAginf is specific activity of the dextrose infusate (dpm per micromole), I is constant tracer infusion rate (dpm per minute), GINF(ss) is steady-state glucose infusion rate (micromoles per kilogram per minute), Ra(b) is basal glucose production (rate of appearance in micromoles per kilogram per minute), BW is weight of the dog (kilograms), and F is steady-state suppression of glucose production derived from

$$F = \frac{R\text{a(b)} - R\text{a(ss)}}{R\text{a(b)}}$$

where Ra(ss) is steady-state glucose production (micromoles per kilogram per minute). The SAginf for the high dose was based on initial GINF, Ra(b), and F estimates of 22.2 µmol · kg^{-1} · min^{-1}, 19.5 µmol · kg^{-1} · min^{-1}, and 0.85, respectively. The SAginf for the low dose was based on initial GINF, Ra(b), and F estimates of 6.67 µmol · kg^{-1} · min^{-1}, 15.0 µmol · kg^{-1} · min^{-1}, and 0.4, respectively. These initial values were based on previous studies (22,26) and were continuously updated according to experimental results.

Arterial samples were taken every 10 min in the 1st and 3rd h and every 15 min in the 2nd h of the hyperinsulinemic clamp. The blood samples for 3-[^{3}H]glucose and insulin analysis were collected in tubes containing sodium fluoride (Fisher, Fair Lawn, NJ) and dried heparin. The samples for glucagon, GLP-1, and free fatty acid (FFA) analysis were collected in tubes containing ethylenediamine tetraacetic acid (Sigma, St. Louis, MO) and Trasylol (2,000 kallikrein IU) (Bayer, Etobicoke, Ontario, Canada). Blood samples for glycerol were collected in tubes containing an equal volume of 10% perchloric acid (BDH, Toronto, Ontario, Canada). Within an hour after collection, the samples were centrifuged at 800g, 4°C. The supernatant was stored at –20°C for later analysis.

Laboratory methods. Plasma glucose concentrations were measured on a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). For the determination of 3-[^{3}H]glucose specific activity, plasma was deproteinized in equal volumes of 5% (wt/vol) zinc sulfate and 0.3 N barium hydroxide that had been titrated. A 1-ml aliquot of the supernatant was then evaporated to dryness to eliminate tritiated water. After addition of water and liquid scintillation solution (Ready Safe; Beckman), the radioactivity from 3-[^{3}H]glucose was measured in a beta-scintillation counter (Camberra Packard, Meriden, CT). Aliquots of the infused glucose tracer and of the labeled glucose infusate were diluted with nonradioactive plasma of the same dog and assayed together with the plasma samples.

Plasma insulin levels were determined using a double-antibody radioimmunoassay kit (Pharmacia AB, Uppsala, Sweden). The interassay coefficient of variation was <7%.

Plasma glucagon levels were determined using a radioimmunoassay (RIA) kit (Diagnostic Products, Los Angeles, CA). The interassay coefficient of variation was <16%.

The GLP-1 levels were determined as reported (27): in brief, 2 ml of 1% trifluoroacetic acid (TFA), pH adjusted to 2.5 with diethylamine, was added to 1 ml plasma; the peptides were then collected by adsorption to C18 silica (Sep Pak; Waters Association, Milford, MA), eluted with 80% isopropanol and 0.1% TFA, and dried in vacuo for GLP-1 RIA. The antibody was obtained from Affinity Research Products, Nottingham, U.K., and was specific for the COOH-terminal 36-NH_2 moiety. The assay range was 3–800 pg/tube, and the interassay coefficient of variation was 16%.

FFA levels were determined with the fluorometric method of Miles et al. (28). Glycerol levels were determined using enzymatic spectrofluorometric methods (29).

Isolation of dog GLP-1 receptor cDNA. RNA was isolated from dog pancreas using RNAsol (Gibco BRL, Gaithersburg, MD). First-strand cDNA was synthesized using random hexamer primers and the first-strand cDNA synthesis kit from Canadian Life Technologies. GLP-1 receptor cDNA was amplified by nested polymerase chain reaction (PCR) using two pairs of degenerate primers designed

to conserved regions of the GLP-1, glucagon, and glucose-dependent insulinotropic polypeptide (GIP) receptors. The 5′ primers were located at residues 86–91 (Rec5.1: GCG GAT CCN TGG TAY YTN CCN TGG) and 243–248 (Rec5.2: GAG GAT CCT GGY TNY TNG TNG ARG G). Locations of primers are relative to the human GLP-1 receptor sequence. The 3′ primers were complementary to residues 402–407 (Rec3.3: GAG ATT TCT TRT TNA NRA ARC ART A) and 363–368 (Rec3.4: GAG ATT TCG CRA ANA YNA CYT CRT G). The first round of PCR used primers Rec5.1 and Rec3.3, and the second amplification used primers Rec5.2 and Rec3.4. Template for the nested PCR reaction was 1 µl of the first PCR reaction. PCR products of the appropriate length (~375 bp) were cloned into the pCR2.1 (Invitrogen, Carlsbad, CA) for further analysis.

The initial sequence was extended in a 3′ direction using two nested gene-specific primers (corresponding to nucleotides 562–585 and then 745–766 of the dog GLP-1 receptor sequence; see below) and the 3′ degenerate primer Rec3.3. The 5′ end of receptor cDNA was sequenced in two ways: first with a primer complementary to residues 173–179 (Rec5.3: TCG AYT GYA CNM GNA AYT AYA TYC A) and two gene-specific primers (1,123–1,100 and then 1,076–1,055; see below), and then by 5′ rapid amplification of cDNA ends (5′ RACE) using the same two primers with the Marathon cDNA kit (Clontech, Palo Alto, CA). Using these methods, an additional 204 and 195 bp of the cDNA were sequenced, respectively.

Studies of GLP-1 receptor gene expression. To determine whether the GLP-1 receptor is expressed in extrapancreatic tissue, pancreas, or visceral or subcutaneous adipose tissue, skeletal muscle and liver were obtained from three normal dogs. Total cellular RNA was isolated from each tissue using phenol-chloroform extraction as described previously (30). Total RNA was then subjected to poly(A) selection using an oligo-dT magnetic bead kit from PerSeptive Biosystems (Framingham, MA) according to the manufacturer's instructions.

Northern analysis of 3.5–4 µg of poly(A) RNA was performed using a denaturing formaldehyde gel, charged nylon membrane (Amersham Life Sciences, Little Chalfont, Buckinghamshire, U.K.), and an [α-^{32}P]dCTP-labeled probe as described previously (30). The probe was prepared using a Random Primer Labeling Kit (Gibco BRL), from a dog GLP-1 receptor PCR fragment obtained using 5′ primer: 562-ATCCTCCGAGCGCTGTCCGTCTTC-585 and 3′ primer: 1123-CTTGTTCATC CATCACGAAGGCAA-1100. Prehybridization was carried out using a standard hybridization solution containing 50% formamide (31) at 42°C for ~5 h followed by probe hybridization at 40°C overnight and washes with 1× standard saline citrate (SSC)/0.1% sodium dodecyl sulphate (SDS) at 65°C. Autoradiography was performed using Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) and an exposure time of 7 h (pancreas) or 60 h and 14 days to visualize low-abundance transcripts (other tissues). The membrane was then probed for insulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as described above. The membrane was washed with 1× SSC/0.1% SDS, and autoradiography was performed for 30 min (muscle) or 3 h (other tissues).

Reverse transcriptase (RT)-PCR was carried out on 1 µg of poly(A)-selected RNA from each tissue using a kit from Perkin Elmer (Branchburg, NJ) according to the manufacturer's instructions. PCR primers were the same as above (5′ primer: 562-ATCCTCCGAGCGCTGTCCGTCTTC-585 and 3′ primer: 1123-CTTGTTCATC CATCACGAAGGCAA-1100). Amplification of dog GLP-1 receptor cDNA was performed in 35 temperature cycles of 94°C for 30 s, 60°C for 35 s, 72°C for 45 s, yielding a 562-bp product. Southern blot analysis was performed using 1 µl PCR product run on a 1.4% agarose gel, transferred to a nylon membrane, and probed as described (30). Probe preparation and hybridization was performed as described above, using a dog GLP-1 receptor fragment prepared by PCR using primers (5′: 745-ATGTACCTGTACACGCTGCTGG-766, 3′: 1076-AGGGGGATAA GCGTCAGTGTGG-1055) that amplified a 332-bp fragment internal to the primer set used above. The blot was washed with 0.5× SSC/0.1% SDS at 55°C, and autoradiography was performed as above for 30 min at room temperature.

Calculations. Glucose production was calculated as the endogenous rate of appearance measured with 3-[^{3}H]glucose. A modified one-compartmental model of Steele (25) was used to account for the exogenously infused mixture of labeled and unlabeled glucose. Data were smoothed with the optimal segments routine (32).

Statistics. The data were expressed as mean ± SE. The calculations were performed on data from the last 90 min of the experiment, when a new steady state was obtained. Since the dog experiments were paired, two-way analysis of variance was used to detect differences between treatments. Because significant differences in basal variables were found at the low insulin dose, the glucose clamp results were analyzed as percentage change from basal at both doses. Whenever necessary, the data were log-transformed to equalize variances. Data were also analyzed within each group for detection of differences between the experimental periods. Multivariate analysis of variance (MANOVA) was performed to isolate the effect of treatment from effects of potentially confounding variables, such as the prevailing plasma glucose levels, basal intraportal insulin infusion rates, and plasma glucagon levels. Calculations were performed with SAS software (Statistical Analysis System, Cary, NC).

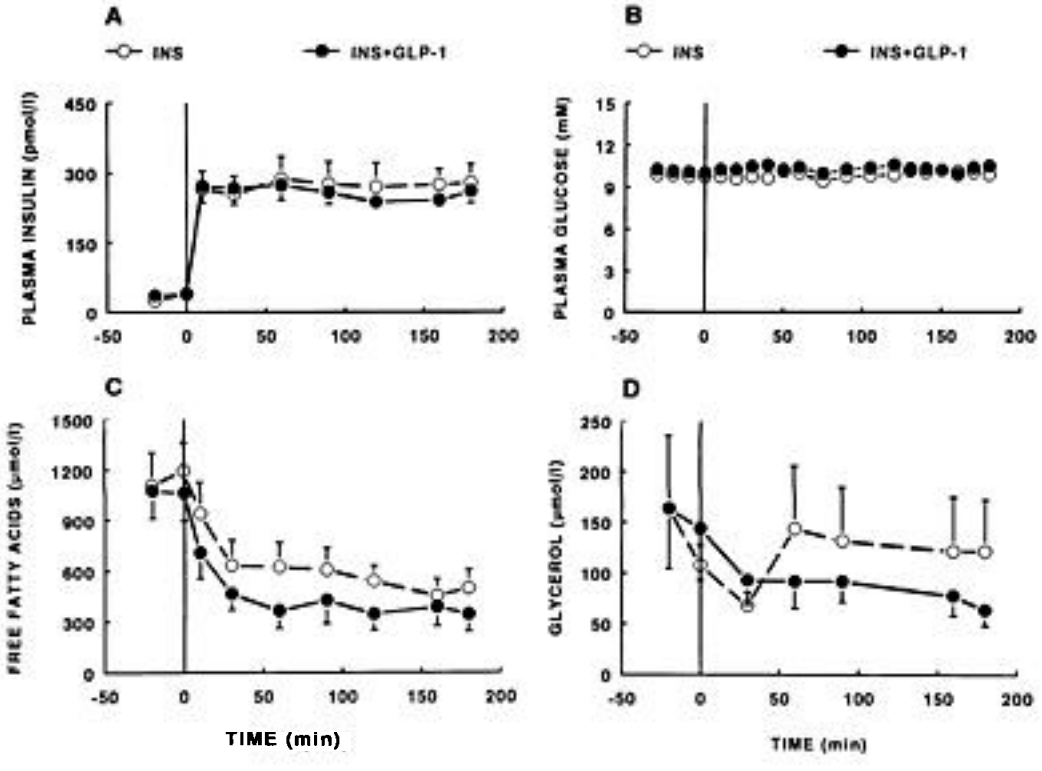

FIG. 1. High-dose insulin infusion study. Plasma insulin (*A*), glucose (*B*), free fatty acid (*C*), and glycerol (*D*) levels. Measurements were taken before (basal) and during peripheral insulin infusion of 5.4 pmol · kg^{-1} · min^{-1} with either GLP-1(7-36) amide at 1.5 pmol · kg^{-1} · min^{-1} (●) or saline (○) in hyperglycemic depancreatized dogs. A glucose clamp was maintained by infusing a mixture of labeled and unlabeled glucose, as described in the text. Values are presented as means ± SE from six paired experiments with GLP-1 or saline. Free fatty acids and glycerol were significantly lower in the experiments with GLP-1 ($P < 0.01$ and $P < 0.05$, respectively).

RESULTS

High insulin dose. The following results are based on a peripheral insulin dose of 5.4 pmol · kg^{-1} · min^{-1} with $n = 6$ for both GLP-1 and saline infusion experiments in the same dog. The basal portal insulin dose required to achieve and maintain moderate hyperglycemia in these dogs was not significantly different between the two groups (GLP-1, 2.16 ± 0.54; saline, 1.92 ± 0.30 pmol · kg^{-1} · min^{-1}). Plasma insulin rose to levels comparable to postprandial values (Fig. 1*A*), while plasma glucose was maintained at basal hyperglycemic levels throughout the clamp (Fig. 1*B*). The insulin-induced suppression of FFAs was greater with GLP-1 than saline ($P < 0.01$; Fig. 1*C*), as was the insulin-induced suppression of glycerol ($P < 0.05$; Fig. 1*D*). The plasma glucagon levels were maintained at basal levels throughout the clamp with both GLP-1 and saline (Table 1). With GLP-1 infusion, the GLP-1 levels rose to pharmacologic levels, whereas in controls they remained at basal levels (Table 1).

The plasma glucose specific activity was maintained near constant throughout the experiment (Fig. 2*A*). The glucose infusion rate was higher with GLP-1 than saline ($P < 0.001$; Fig. 2*B*). The insulin-induced suppression of glucose production was not significantly different between groups (Fig. 2*C*). However, the insulin-induced stimulation of glucose utilization was greater with GLP-1 than saline ($P < 0.001$ for both absolute values and percentage increase over basal; Fig. 2*D*). No difference in the suppression of glucose production and a highly significant difference ($P < 0.001$) in the insulin-induced stimulation of glucose utilization between GLP-1 and saline were also found when using MANOVA in a model that took into account the effects of potentially confounding variables, such as the prevailing plasma glucose levels and basal intraportal insulin infusion rates.

TABLE 1
Plasma glucagon and GLP-1 levels during high-dose peripheral insulin infusion (5.4 pmol · kg^{-1} · min^{-1}) with GLP-1 (1.5 pmol · kg^{-1} · min^{-1}) or saline

	GLP-1	Saline
Glucagon (ng/l)		
Basal	81.8 ± 19.6	83.6 ± 20.6
Clamp	70.6 ± 11.4	72.3 ± 14.4
GLP-1 (pmol/l)		
Basal	6.2 ± 1.9	6.0 ± 2.4
Clamp	83.0 ± 19.0	4.2 ± 0.5

Data are means ± SE.

Low insulin dose. The following results are based on a peripheral insulin dose of 0.75 pmol · kg^{-1} · min^{-1} with $n = 5$ for both GLP-1 and saline infusion experiments in the same dog. The basal portal insulin dose required to achieve and maintain moderate hyperglycemia was not significantly different between the two groups (GLP-1, 1.14 ± 0.54; saline, 1.50 ± 0.90 pmol · kg^{-1} · min^{-1}). Plasma insulin rose to levels comparable to fasting values (Fig. 3*A*), while plasma glucose was maintained at basal hyperglycemia throughout the clamp (Fig. 3*B*). The insulin-induced suppression of FFAs was not significantly different between GLP-1 and saline (Fig. 3*C*). Glycerol was not significantly suppressed, and levels were similar between GLP-1 and saline (Fig. 3*D*). Although plasma glucagon was different between groups ($P < 0.01$), the

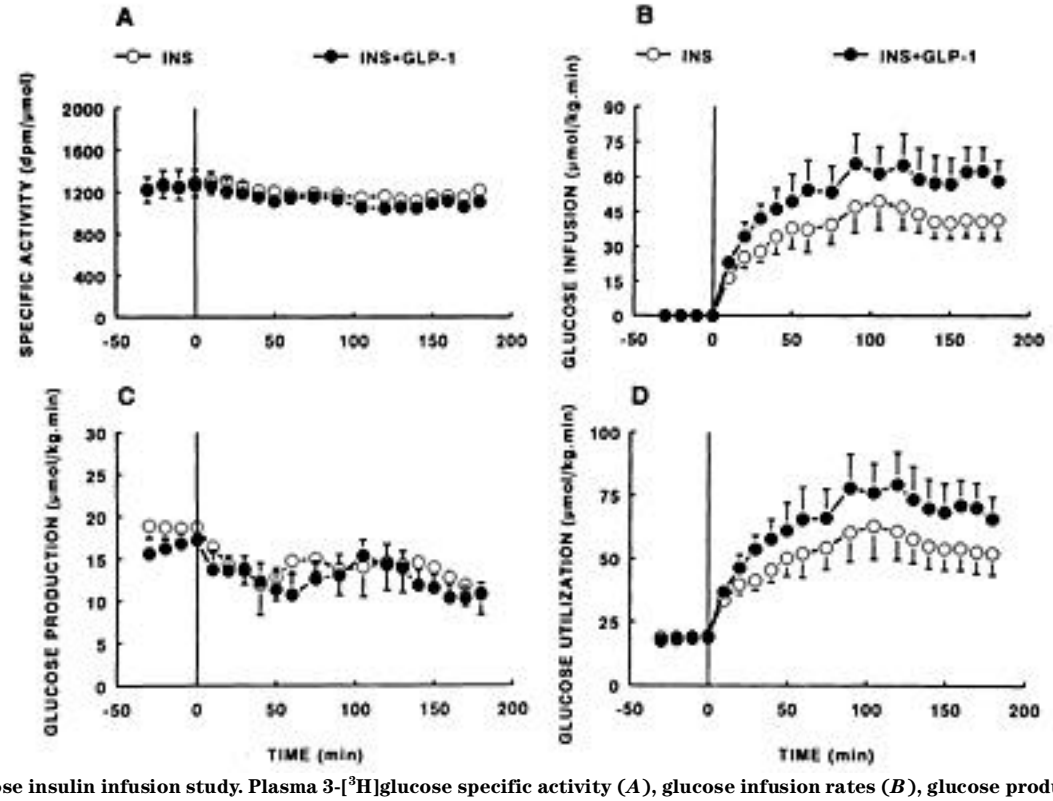

FIG. 2. High-dose insulin infusion study. Plasma 3-[^{3}H]glucose specific activity (*A*), glucose infusion rates (*B*), glucose production (*C*), and glucose utilization (*D*) with GLP-1 or saline. Experimental design is outlined in Fig. 1. Values are presented as means ± SE from six paired experiments with GLP-1 or saline. Glucose infusion rates and glucose utilization were significantly higher in the experiments with GLP-1 ($P < 0.001$).

glucagon levels were maintained at basal levels throughout the clamp with both GLP-1 and saline (Table 2). With GLP-1 infusion, the GLP-1 levels rose to pharmacologic levels (comparable to levels attained in the high-dose study), whereas with saline, they remained at basal levels (Table 2).

The plasma glucose specific activity was maintained constant throughout the experiment (Fig. 4*A*). The glucose infusion rate was not significantly different between GLP-1 and saline (Fig. 4*B*). Although basal levels of glucose production and utilization were different between GLP-1 and saline ($P < 0.01$), neither the insulin-induced suppression of glucose production (Fig. 4*C*) nor the insulin-induced stimulation of glucose utilization (Fig. 4*D*) (percentage changes over basal) were significantly different. The results did not change when MANOVA was used to isolate the effect of treatment on the glucose turnover response to the insulin clamp from the effects of the prevailing glucose, basal intraportal insulin infusion rates, and prevailing glucagon levels.

GLP-1 receptor sequence. RT-PCR and 5′ RACE yielded a fragment of the dog GLP-1 receptor of 831 bp. The sequenced region showed 90.5% sequence identity with the human GLP-1 receptor on alignment and 62.9% sequence identity with the human glucagon receptor. The high degree of sequence identity predicts that the probability (n) of the sequenced cDNA not corresponding to the GLP-1 receptor is extremely low (7.1×10^{-121}).

GLP-1 receptor gene expression. The expression of GLP-1 receptor mRNA in various tissues was examined in normal nondiabetic dogs. Northern blot analysis of poly(A) RNA at high stringency revealed the presence of two transcripts in the pancreas after a 7-h exposure to autoradiographic film (Fig. 5). These transcripts correspond to the 2.7-kb and less abundant 3.6-kb transcripts described previously in the rat (31). On longer autoradiographic exposure (60 h), identically sized transcripts were initially observed in fat (data not shown). After 14 days, these transcripts were more clearly observed (Fig. 5) but could not be resolved in liver or muscle (Fig. 5). These observations indicate that GLP-1 receptor mRNA is expressed at lower but detectable levels in dog fat tissue. Rehybridization of the membrane to [α-^{32}P]dCTP-labeled probes for insulin and GAPDH under high stringency revealed transcripts at 0.6 kb in the pancreas only (corresponding to the insulin message) and 1.4 kb in all tissues (corresponding to the GAPDH message) (Fig. 5). To further visualize GLP-1 receptor gene expression, the more sensitive RT-PCR–based approach was used. RT-PCR of poly(A) RNA at an annealing temperature of 60°C followed by Southern analysis at high stringency yielded DNA fragments of 562 bp complementary to the dog GLP-1 receptor probe in skeletal muscle, fat, and pancreas (Fig. 6). No transcripts complementary to the dog GLP-1 receptor were detected in liver (Fig. 6) or water blanks (data not shown). These results complement the above Northern analysis in regard to the pancreas and fat tissues. However, the presence of GLP-1 receptor PCR product in skeletal muscle may indicate that mRNA transcripts are present in this tissue at levels below that detectable by standard Northern blotting.

DISCUSSION

The results of the present study obtained with the high insulin dose indicate that, in depancreatized, moderately hyperglycemic (~10 mmol/l) dogs, GLP-1 potentiated insulin

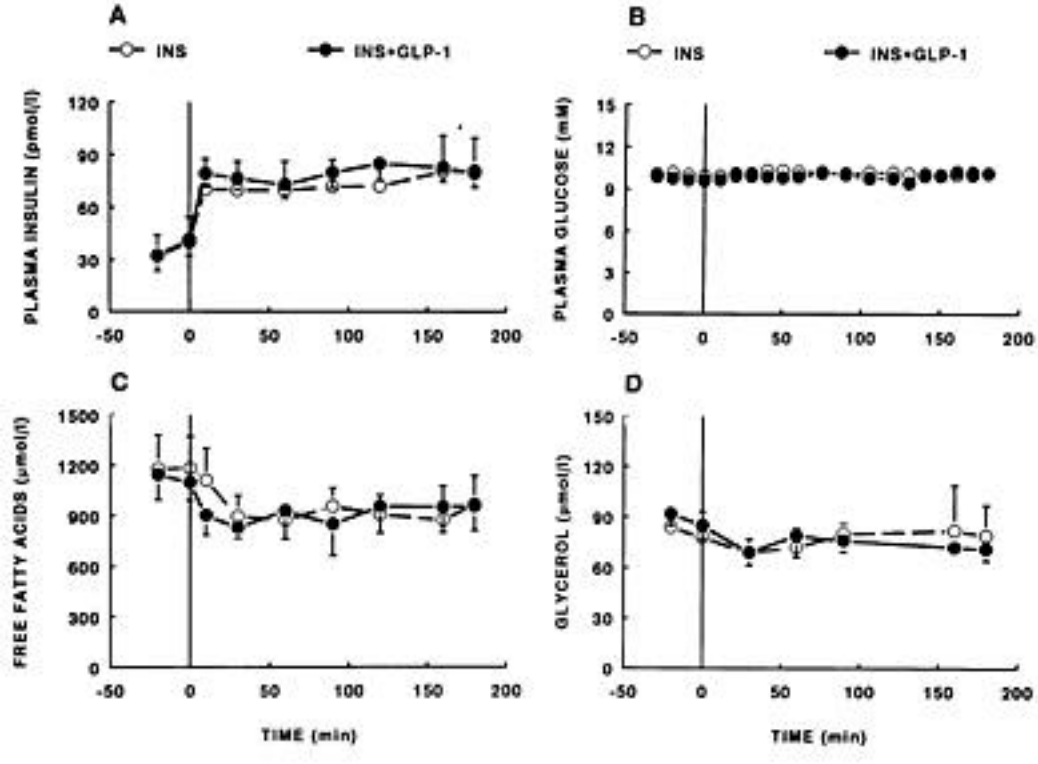

FIG. 3. Low-dose insulin infusion study. Plasma insulin (*A*), glucose (*B*), free fatty acid (*C*), and glycerol (*D*) levels. Measurements were taken before (basal) and during peripheral insulin infusion of 0.75 pmol · kg^{-1} · min^{-1} with either GLP-1(7-36) amide at 1.5 pmol · kg^{-1} · min^{-1} (●) or saline (○) in hyperglycemic depancreatized dogs. A glucose clamp was maintained by infusing a mixture of labeled and unlabeled glucose, as described in the text. Values are presented as means ± SE from five paired experiments with GLP-1 or saline.

TABLE 2
Plasma glucagon and GLP-1 levels during low-dose peripheral insulin infusion (0.75 pmol · kg^{-1} · min^{-1}) with GLP-1 (1.5 pmol · kg^{-1} · min^{-1}) or saline

	GLP-1	Saline
Glucagon (ng/l)		
Basal	93.6 ± 18.0	71.5 ± 15.3
Clamp	81.9 ± 19.9	60.4 ± 7.9
GLP-1 (pmol/l)		
Basal	9.1 ± 3.2	4.3 ± 0.3
Clamp	74.8 ± 26.7	3.7 ± 0.3

Data are means ± SE.

action during hyperinsulinemic clamps at constant glucagon levels. This was due to GLP-1's effect of enhancing insulin-stimulated glucose utilization, while there was no effect of GLP-1 on the insulin-induced suppression of glucose production. The insulin-induced suppression of both FFAs and glycerol was greater with GLP-1 than saline, which indicates that GLP-1 potentiated insulin's antilipolytic effect. In contrast to the results obtained at the high insulin dose, GLP-1 did not potentiate insulin action at levels of insulin close to the fasting range, suggesting that the effect of GLP-1 on glucose utilization is dependent on the insulin levels. Our results were confirmed after taking into account the effects on glucose kinetics of possible confounding variables, such as the prevailing glucose levels, the basal intraportal insulin infusion rates, and the prevailing glucagon levels (which might affect the glucose production response to the clamp).

Muscle is the major site of insulin-mediated glucose utilization during hyperinsulinemic clamps, and a direct effect of GLP-1 in enhancing insulin-mediated glucose utilization in muscle may be suggested by the presence of low levels of GLP-1 receptor mRNA (detectable by RT-PCR) in our dog muscle samples. In addition, part of GLP-1's enhancement of insulin-mediated glucose utilization during the late periods of the clamp may be due to GLP-1's effect in lowering FFA levels. The impairing effect of FFA on glucose utilization through Randle's cycle is delayed in humans but is quicker in dogs (33,34). Our finding that GLP-1 enhances insulin's antilipolytic effect is also supported by the detection of GLP-1 receptor mRNA by Northern blot in dog adipose tissue. A recent study performed using RNase protection and Western blot analysis also shows that functional GIP receptors are present on rat adipocytes (35). The presence of GLP-1 receptor mRNA expression at extrapancreatic sites, as detected in this study, may be species-specific. Studies in rats and preliminary studies in humans have failed to provide any evidence for the expression of GLP-1 receptor mRNA in rat adipose tissue or skeletal muscle by RNase protection assays, RT-PCR/Southern blot analysis, and in situ hybridization (36–38). However, we cannot exclude that differences in methodology, such as the use of poly(A)-selected RNA, may also explain our ability to detect the receptor transcript, since low levels of GLP-1 receptor mRNA expression in rat fat and muscle and in murine 3T3-adipocytes have been reported previously (12,31). Both PCR and Northern blotting were done at relatively high stringency such that the probability is extremely small that the product amplified or the transcript that hybridized to the probe is the product of another receptor gene.

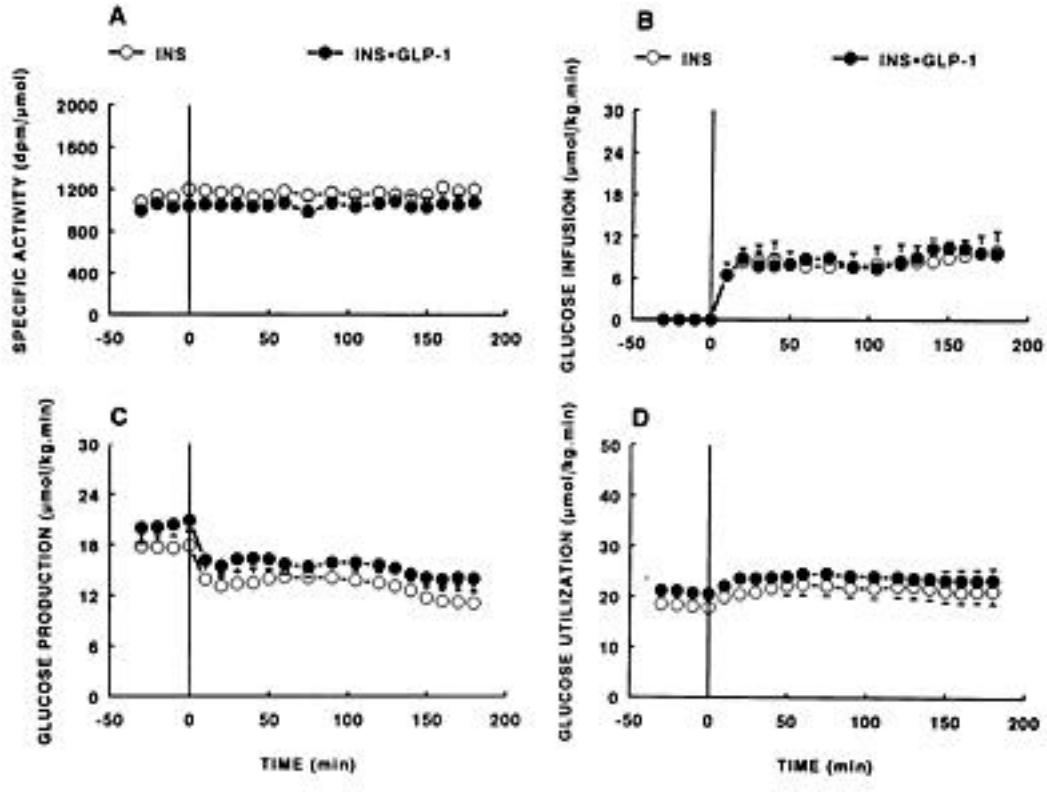

FIG. 4. Low-dose insulin infusion study. Plasma 3-[^{3}H]glucose specific activity (*A*), glucose infusion rates (*B*), glucose production (*C*), and glucose utilization (*D*) with GLP-1 or saline. Experimental design is outlined in Fig. 3. Values are presented as means ± SE from five paired experiments with GLP-1 or saline.

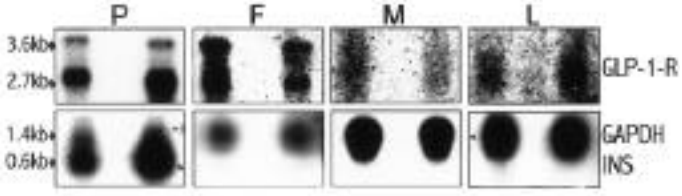

FIG. 5. Northern blot hybridization analysis of GLP-1 receptor distribution in the dog. Poly(A) RNA (4 µg) from several tissues (3.5 µg from the second fat sample [*right lane*]) from normal dogs was analyzed using a [α-^{32}P]dCTP-labeled probe made from a 562-bp GLP-1 receptor fragment (see METHODS). Blots were exposed to autoradiographic film at –70°C with two intensifying screens for 7 h (pancreas) or 14 days (other tissues). Transcripts for insulin at 0.6 kb (present in pancreas only) and GAPDH at 1.4 kb were visualized by hybridization of the membrane to [α-^{32}P]dCTP-labeled probes and exposure to autoradiographic film for 30 min (muscle) or 3 h (other tissues). P, pancreas; F, fat; M, muscle; L, liver.

It should be noted that part of the effect of GLP-1 on muscle glucose utilization may not necessarily be mediated by GLP-1 receptors in insulin-sensitive tissues. Since increased blood flow may increase muscle glucose utilization, it can be speculated that GLP-1 may act by increasing blood flow. While there is no direct evidence of this, the possibility of GLP-1–induced hemodynamic effects is supported by reports that GLP-1 increases blood pressure and heart rate (39).

The absence of an effect of GLP-1 in suppressing glucose production is supported by the absence of GLP-1 receptor mRNA in the dog liver. However, at the high insulin dose, GLP-1 enhanced the insulin-induced decrease in FFAs, which would be expected to reduce glucose production. The similar glucose production with saline or GLP-1 may indicate that FFAs are less important than previously thought for the regulation of glucose production in the depancreatized dog. In a previous study (22), we used the same experimental model with the same insulin dose but no glucagon clamp and found that suppression of glucose production was much greater than in the present study. Thus, it is possible that in depancreatized dogs, glucagon is more important than FFAs in the regulation of glucose production. In contrast, infusion of GLP-1 in humans during a pancreatic clamp was found to decrease glucose production (16). However, the decline in glucose production may have been, in part, a time-dependent effect, since during fasting glucose production decreases over time. Orskov et al. (19) found no effects of GLP-1 on hepatic or peripheral insulin sensitivity during a hyperinsulinemic-euglycemic clamp using somatostatin in healthy humans. Myers et al. (18) also found no such effect in normal dogs during a hyperglycemic (~8.3 mmol/l) hyperinsulinemic clamp. In diabetic states, however, reduced insulin sensitivity is a feature that may allow for a modest increase in insulin sensitivity to be detected (depancreatized dogs are insulin resistant compared with normal dogs [40]). Furthermore, GLP-1 binding in adipose tissue was reported to be increased in diabetic versus nondiabetic subjects (41). In addition to our study and that by Gutniak et al. (17) in subjects with type 1 diabetes, preliminary studies by Myers are also positive for an enhancing effect of GLP-1 on insulin sensitivity in depancreatized dogs (personal communication).

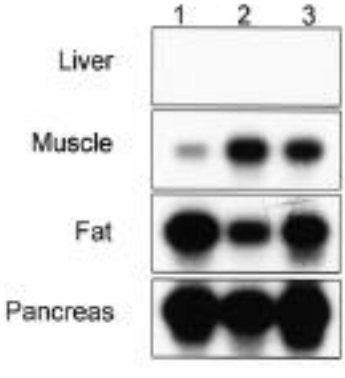

FIG. 6. Southern blot hybridization analysis of GLP-1 receptor cDNA distribution in the dog following RT-PCR of poly(A) RNA (1 µg) for a 562-bp fragment of the dog GLP-1 receptor. PCR product was analyzed using an antisense [α-^{32}P]dCTP-labeled probe made from a 332-bp GLP-1 receptor fragment internal to the PCR product (see METHODS). Blots were exposed to autoradiographic film for 30 min at room temperature.

In type 2 diabetic subjects, GLP-1 had no effect on insulin sensitivity during a euglycemic clamp (20). In this study, however, insulin sensitivity was determined as the ratio of glucose infusion rate over the plasma insulin concentration (42). A crucial assumption of this approach to determine insulin sensitivity is that the two parameters are linearly related (42). Since the insulin levels in the GLP-1 and control groups were substantially different and type 2 diabetic subjects are insulin resistant, such a linear relationship may not hold. Therefore, it is possible that any effect of GLP-1 may have been undetectable using this method.

In addition to increasing insulin sensitivity, it has been reported that GLP-1 may independently affect glucose disposal as well as increase glucose effectiveness (effect of glucose per se in enhancing its own disposal). In vitro studies have shown that GLP-1 increases muscle glucose uptake in an insulin-independent manner (5). However, in vivo there were no effects of GLP-1 on glucose uptake in insulin-deprived depancreatized dogs (2). These negative results in dogs are consistent with the results of our low insulin dose study, in which GLP-1 had no effect in the presence of low insulin. Our protocol does not allow us to study the action of GLP-1 on glucose effectiveness, which is currently controversial (14,15,43).

The mechanism of the peripheral effect of GLP-1 is unclear. Miki et al. (11) found that GLP-1 improves insulin-stimulated glucose utilization in rat adipocytes while decreasing the intracellular cAMP content. This is in contrast to the well-known effect of GLP-1 in increasing cAMP in pancreatic β-cells but is consistent with a recent report that the GLP-1 receptor can also couple to inhibitory G proteins (44). In addition, in transfected COS cells, the GLP-1 receptor was coupled to phospholipase C (31). Thus, GLP-1's effect may be mediated via Ca^{2+}/protein kinase C. Although the predominant effect of Ca^{2+}/protein kinase C on insulin action is inhibitory, stimulatory effects have also been reported (45,46). Furthermore, the effect of GLP-1 may be due to the presence of other receptors, to which GLP-1 could bind at pharmacologic levels (6,44,47). Our results would indicate that the effect of GLP-1, however mediated, would selectively enhance insulin signaling, since the effect was dependent on the insulin level.

In conclusion, the results of the present study indicate that GLP-1 has an insulin-potentiating action on glucose utilization in vivo in depancreatized dogs, an action independent

of GLP-1–induced changes in pancreatic hormone secretion. It remains to be determined whether this insulin-potentiating effect of GLP-1 can also be shown in type 2 diabetes. A dual effect of GLP-1 on insulin secretion and action would make it an excellent candidate for the treatment of type 2 diabetes, a condition characterized by both reduced insulin secretion and insulin resistance.

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