Importance of Peripheral Insulin Levels for Insulin-induced Suppression of Glucose Production in Depancreatized Dogs

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Abstract

It is generally believed that glucose production (GP) cannot be adequately suppressed in insulin-treated diabetes because the portal–peripheral insulin gradient is absent. To determine whether suppression of GP in diabetes depends on portal insulin levels, we performed 3-h glucose and specific activity clamps in moderately hyperglycemic (10 mM) depancreatized dogs, using three protocols: (*a*) 54 pmol·kg^{-1} bolus + 5.4 pmol·kg^{-1}·min^{-1} portal insulin infusion (n = 7; peripheral insulin = 170±51 pM); (*b*) an equimolar peripheral infusion (n = 7; peripheral insulin = 294±28 pM, $P < 0.001$); and (*c*) a half-dose peripheral infusion (n = 7), which gave comparable (157±13 pM) insulinemia to that seen in protocol 1. Glucose production, use (GU) and cycling (GC) were measured using HPLC-purified 6-[^{3}H]- and 2-[^{3}H]glucose. Consistent with the higher peripheral insulinemia, peripheral infusion was more effective than equimolar portal infusion in increasing GU. Unexpectedly, it was also more potent in suppressing GP (73±7 vs. 55±7% suppression between 120 and 180 min, $P < 0.001$). At matched peripheral insulinemia (protocols 2 and 3), not only stimulation of GU, but also suppression of GP was the same (55±7 vs. 63±4%). In the diabetic dogs at 10 mM glucose, GC was threefold higher than normal but failed to decrease with insulin infusion by either route. Glycerol, alanine, FFA, and glucagon levels decreased proportionally to peripheral insulinemia. However, the decrease in glucagon was not significantly greater in protocol 2 than in 1 or 3. When we combined all protocols, we found a correlation between the decrements in glycerol and FFAs and the decrease in GP (r = 0.6, $P < 0.01$). In conclusion, when suprabasal insulin levels in the physiological postprandial range are provided to moderately hyperglycemic depancreatized dogs, suppression of GP appears to be more dependent on peripheral than portal insulin concentrations and may be mainly mediated by limitation of the flow of precursors and energy substrates for gluconeogenesis and by the suppressive effect of insulin on glucagon secretion. These results suggest that a portal–peripheral insulin gradient might not be necessary to effectively suppress postprandial GP in insulin-treated diabetics. (*J. Clin. Invest.* 1992. 90:1769–1777.) Key words: portal–peripheral insulin gradient • glucose turnover • gluconeogenic precursors • free fatty acids • glucose cycling

Introduction

With conventional insulin therapy in diabetes, the portal–peripheral insulin gradient is absent. It is widely believed that in the absence of such a gradient, normoglycemia cannot be achieved without peripheral hyperinsulinemia to enhance peripheral glucose uptake and increase hepatic insulinization, thereby inhibiting glucose production (GP). However, insulin could inhibit GP not only through a direct hepatic action, but also indirectly by reducing the availability of substrates and energy for gluconeogenesis. This mechanism might be of particular importance in diabetes where gluconeogenesis is substantially increased (1, 2). Also, peripheral insulin levels may control glucose production by their inhibitory effect on glucagon secretion. The relative importance of the direct and indirect hepatic effects of insulin has not yet been established. In addition, GP might be affected differently by hepatic arterial and portal venous insulin concentrations. The results of most (3–6), but not all (7, 8) of the studies where acute administration of insulin by either the portal or peripheral route was performed are consistent with some inhibitory effect of portal insulin levels on GP, especially at low insulin doses (5, 6). The results of the studies involving chronic peripheral insulin delivery, as with pancreatic transplants (9, 10), could be consistent with suppression of GP by either portal or peripheral insulin levels or both. The recent application of modifications to tracer methods, in which constant specific activity of glucose is provided during a glucose clamp (Matched Step Tracer Infusion method [MSTI] (11, 12), and HPLC-purified tritiated glucose is used (13), has markedly increased the precision of the measurement of GP, particularly when GP is suppressed. The importance of peripheral insulin levels in the suppression of GP has recently been emphasized in obese humans, where low-dose insulin clamps were applied (14). Ader and Bergman (15) have used the method of constant glucose specific activity during normoglycemic glucose clamps for the first time to compare the acute effects of portal and peripheral insulin infusions. These experiments were carried out in normal dogs during infusion of somatostatin with concomitant insulin and glucagon replacement. Suppression of GP was the same at matched peripheral insulin levels, despite much higher hepatic insulinization with portal than peripheral infusions.

The aim of the present study was to determine whether, in diabetes, the inhibition of GP is primarily dependent upon portal or peripheral insulin levels and whether such inhibition

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1. *Abbreviations used in this paper:* GC, glucose cycling; GP, glucose production; GU, glucose use; MCR, metabolic clearance rate; MSTI, Matched Step Tracer Infusion; Rd, rate of disappearance.

could be related to insulin's peripheral effects on hormones and metabolites. Our experiments were done in depancreatized dogs, thus avoiding the need for somatostatin. Although it has been claimed that somatostatin does not have major metabolic effects (16), it is conceivable that under certain metabolic conditions it might enhance the peripheral effects of insulin (17). The depancreatized dog is a model of selective insulin deficiency, because glucagon (IRG 3500) is secreted by the gastric mucosa (18, 19) in dogs. Therefore, this model also allowed us to evaluate the effects of the route of insulin administration on extrapancreatic glucagon levels. For the first time we studied the effects of the portal–peripheral insulin gradient during a glucose clamp in diabetes and we measured GP by combining the MSTI method with the use of HPLC-purified tracers. We also measured hepatic glucose cycling (glucose ⇆ glucose-6-P; GC), which is a more sensitive probe than GP for alterations in hepatic glucose metabolism in diabetes (20), and which, therefore, could be preferentially affected by the absence of a portal–peripheral insulin gradient. The results of the present study refer to the effects of suprabasal insulin infusions, resulting in peripheral insulin levels, as seen physiologically in the postprandial state.

Methods

Experimental animals. Male mongrel dogs weighing 17–28 kg underwent total pancreatectomy and vessel cannulation performed under general anesthesia, induced with thiamylal sodium and maintained with nitrous oxide and halothane and assisted ventilation. Silastic cannulae (silastic medical grade tubings; Dow Corning Corp., Midland, MI) were inserted into the carotid arch via the carotid artery (0.04-inch i.d.), into the superior vena cava via the jugular vein (three 0.03-inch i.d. cannulae) and into the portal vein through a branch of the splenic vein (0.04-inch i.d.). The carotid cannula served for sampling, and the jugular and portal cannulae served for infusions. All cannulae were tunneled subcutaneously and exteriorized at the back of the neck. They were filled with heparin (1,000 U/ml, Hepalean; Organon Canada, Toronto, Ontario, Canada) and were maintained patent by flushing frequently with saline. Catheter position was verified at autopsy. Dogs were fed once daily with a combined diet of 15 g/kg per d chow (25% protein, 9% fat, 38%; Ralston Purina Canada, Mississauga, Ontario, Canada) and 500 g beef (50% protein, 24% fat, 21% carbohydrate; Dr. Ballards, Toronto, Ontario, Canada). Pancreatic enzymes were supplemented (Cotazyme; Organon Canada). Diabetes was treated with a once daily subcutaneous injection of regular (4–15 U) and NPH (8–30 U) porcine insulin (Eli Lilly and Company, Indianapolis, IN) to maintain glycosuria below 1%. Porcine insulin does not induce the formation of insulin antibodies for ≥ 2 mo (21), thus allowing accurate measurements of plasma insulin.

Experimental design. Three protocols were carried out as follows: protocol 1, insulin (54 pmol · kg^{-1} bolus + 5.4 pmol · kg^{-1} · min^{-1}) was given intraportally (n = 7). Protocol 2, the same dose of insulin was infused into a peripheral vein (n = 7), resulting in much higher peripheral insulin levels than with the equimolar portal infusions. In this protocol, portal levels of insulin were estimated to be slightly lower with the peripheral infusion due to dilution. Protocol 3, peripheral (n = 7) infusions of insulin were given, which resulted in the same peripheral insulinemia as with portal infusions. Assuming a 50% hepatic degradation of insulin we infused peripherally one half the dose given portally (27 pmol · kg^{-1} bolus + 2.7 pmol · kg^{-1} · min^{-1}). Thus, matched peripheral insulin levels were obtained, but portal insulin levels were estimated to be even lower than in protocol 2. The experiments were carried out in 14 conscious dogs ≥ 2 wk after pancreatectomy. In protocols 1 and 2, in which the experiments were paired, ≥ 6 d elapsed before repeating the study in the same dog. The order of the paired experiments was random. Insulin was withdrawn for 24 h and food was withdrawn for 18 h before each experiment.

On the day of the study, an intraportal infusion of insulin (20 pmol · kg^{-1} · min^{-1}) was started and gradually reduced until moderately hyperglycemic levels were reached (~ 10 mM). These were maintained by infusing insulin intraportally at a constant basal rate (1.2±0.3 pmol · kg^{-1} · min^{-1}) for ≥ 60 min before and throughout the clamp. When plasma glucose decreased below 14 mM, a primed (155.4 × 10^6 dpm) continuous infusion (1.11 × 10^6 dpm · min^{-1}) of a tracer mixture (New England Nuclear, Boston, MA) containing 50% 2-[^{3}H]glucose and 6-[^{3}H]glucose was started and maintained throughout the study. Both tracers had been submitted to the HPLC purification procedure (13). After ≥ 120 min of tracer equilibration and ≥ 30 min of steady state hyperglycemia, four arterial samples were taken (from time −30 to 0 min) for basal determinations. At time 0 a suprabasal infusion of porcine monocomponent insulin (Iletin II; Eli Lilly and Company) was started and maintained for 180 min through either the portal or the peripheral venous route. During the suprabasal insulin infusions, the plasma glucose concentration was clamped at the mean basal level by adjusting the rate of a variable infusion of glucose according to frequent (every 5 min) glycemic determinations. An aliquot of the 2.22 × 10^7 dpm · ml^{-1} mixture of 2-[^{3}H]- and 6-[^{3}H]glucose was added to the 50% glucose infusate (Dextrose 50%; Abbott Laboratories, Montreal, Quebec, Canada) to minimize the decline in glucose specific activity during the clamp (MSTI method).

The specific activity of the infusate was calculated based on estimation of the parameters of the formula of Finegood et al. (12) modified to allow for incomplete suppression of GP: $SA_{ginf} = I \cdot [(GINF_{ss}/GP_b) - F]/(GINF_{ss} \cdot BW)$; where SA_{ginf} = specific activity of the glucose infusate (dpm · μmol^{-1}); I = constant tracer infusion rate (dpm · min^{-1}); $GINF_{ss}$ = steady state glucose infusion rate (μmol · kg^{-1} · min^{-1}); GP_b = basal glucose production (μmol · kg^{-1} · min^{-1}); BW = body weight (kg); F (fractional suppression) = $(GP_b - GP_{ss})/GP_b$. The following estimates were used in all sets of experiments: GP_b = 19.6 μmol · kg^{-1} · min^{-1}; GP_{ss} = 0.15 GP_b; and $GINF_{ss}$ = 22.4 μmol · kg^{-1} · min^{-1}. GP_b was the mean basal glucose production under identical basal conditions (22), GP_{ss} was assumed from the literature (23, 24), and $GINF_{ss}$ was the mean glucose infusion rate during pilot experiments. Although two tracers were used, we based our estimates primarily on glucose production (rate of appearance of glucose determined with 6-[^{3}H]glucose), since insufficient information was available on glucose output (rate of appearance of glucose determined with 2-[^{3}H]glucose). We also used the same estimates for the three groups, since we could not predict the effect of the route of administration on $GINF$ and F.

During the clamp, arterial samples were taken every 10 min in the first and third hour and every 15 min in the second hour. The total amount of blood sampled in each experiment was 150–160 ml. The procedures for collection of samples have been described before (25).

Laboratory methods. Glucose concentrations were measured by the glucose oxidase method on a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Insulin and glucagon were analyzed by radioimmunoassay (coefficient of variation 12 and 15%, respectively) (26, 27), FFAs by a radiochemical technique (28); lactate, alanine, and glycerol by enzymatic fluorometric methods (29).

For the determination of 2-[^{3}H]glucose- and 6-[^{3}H]glucose–specific activity, plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and run through columns containing ion-exchange resins (Ag 2-X8 and Ag 50W-X8; Bio-Rad Laboratories, Richmond, CA) to remove labeled metabolites. An aliquot of the eluate was then evaporated to dryness to eliminate tritiated water. After addition of water and liquid scintillation solution, the total radioactivity from 6-[^{3}H]glucose and 2-[^{3}H]glucose was measured in a β-scintillation counter. An external standard was used for quench corrections. The radioactivity of 6-[^{3}H]glucose was determined with the dimedone precipitation technique (30). In brief, carrier glucose was added to an aliquot of eluate from ion-exchange chromatography. Glucose was then oxidized with

periodate to five molecules of formic acid (derived from carbons 1–5) and one molecule of formaldehyde (derived from carbon 6). After the addition of dimedone, formaldehyde precipitates as formaldimedone. The radioactivity of the precipitate was determined after filtering, drying, and weighing. The radioactivity of 2-[^{3}H]glucose was calculated as the difference between total radioactivity and that of 6-[^{3}H]glucose, corrected for recovery. Recovery was obtained by running aliquots of 6-[^{3}H]glucose in each assay. Aliquots of the infused mixture of 2-[^{3}H]glucose and 6-[^{3}H]glucose and of the labeled glucose infusate were assayed together with the plasma samples. Aliquots of 2-[^{3}H]glucose passed through the procedure did not display any radioactivity above background.

Calculations. GP and output were calculated as the endogenous rate of appearance measured with 6-[^{3}H]glucose and with 2-[^{3}H]glucose, respectively; glucose use (GU) as the rate of disappearance measured with 6-[^{3}H]glucose (Rd). A modified one-compartmental model (11) was used to account for the exogenously infused mixture of labeled and unlabeled glucose. Data were smoothed with the optimal segments routine (31). With the MSTI method, the monocompartmental assumption becomes minor because the nonsteady state part of the Steele's equation is close to zero. Glucose cycling (GC) was calculated from the difference between glucose output and GP. Rd corresponded to GU and plasma clearance rate of glucose (Rd/glycemia) to glucose metabolic clearance rate (MCR) because plasma glucose levels were below the renal threshold for glucose in dogs (25).

Statistics. The data were expressed as mean±standard error. One- or two-way analysis of variance was carried out, as appropriate, for differences between experimental groups. Data were also analyzed within each group for differences between the experimental periods. In case of unequal variances, data were logarithmically transformed. Pearson's correlation coefficient was calculated with linear regression analysis. Calculations were performed with SAS software (SAS Statistical Analysis System, Cary, NC).

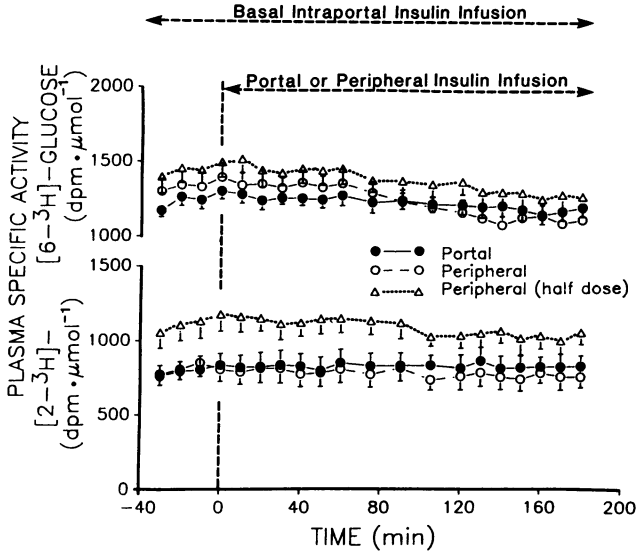

Figure 1. Plasma specific activity of 6-[^{3}H]glucose (*top*) and 2-[^{3}H]-glucose (*bottom*). Measurements are taken before and during portal insulin infusion of 54 pmol·kg^{-1} + 5.4 pmol·kg^{-1}·min^{-1} (● —— ●), peripheral insulin infusion of 54 pmol·kg^{-1} + 5.4 pmol·kg^{-1}·min^{-1} (○ - - - ○), or peripheral insulin infusion of 27 pmol·kg^{-1} + 2.7 pmol·kg^{-1}·min^{-1} (△---△) in moderately hyperglycemic (~ 10 mM) depancreatized dogs infused with basal intraportal insulin (1.2±0.3 pmol·kg^{-1}·min^{-1}). A glucose clamp was maintained by infusing a mixture of labeled and unlabeled glucose, as described in the text. Values are presented as mean±SE from seven experiments in each group.

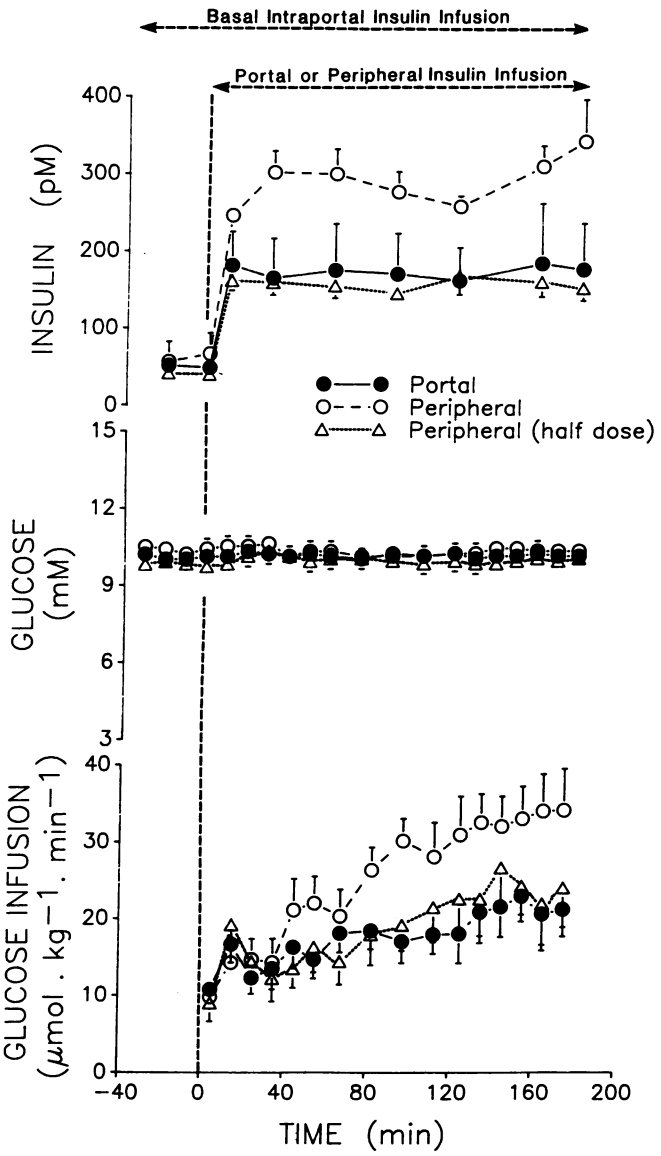

Figure 2. Peripheral insulin levels (*top*), plasma glucose levels (*middle*), and glucose infusion rates (*bottom*) in the three experimental groups. Experimental design is outlined in Fig. 1. Values are presented as mean±SE.

Results

Specific activity of 6-[^{3}H]glucose (Fig. 1) declined slightly ($P < 0.05$) but was at any time within 20% of the basal levels. The decline was not significantly different among the three groups. Specific activity of 2-[^{3}H]glucose remained constant.

Basal insulin levels (Fig. 2) in the preclamp period were in the low normal range and were similar in the three groups (portal: 49.2±11.4; peripheral: 60.6±26.4; half peripheral: 39.6±4.2 pM). With the suprabasal infusions of insulin, plasma insulin levels rose and rapidly plateaued at 170±51 pM with the portal infusion and at 294±28 pM with the equimolar peripheral infusion ($P < 0.001$). With peripheral infusion of insulin given at half the rate, peripheral insulin levels (157±13 pM) were comparable to those obtained with portal infusion. Plasma glucose remained constant at moderately hyperglycemic levels (~ 10 mM), which were equivalent in the three

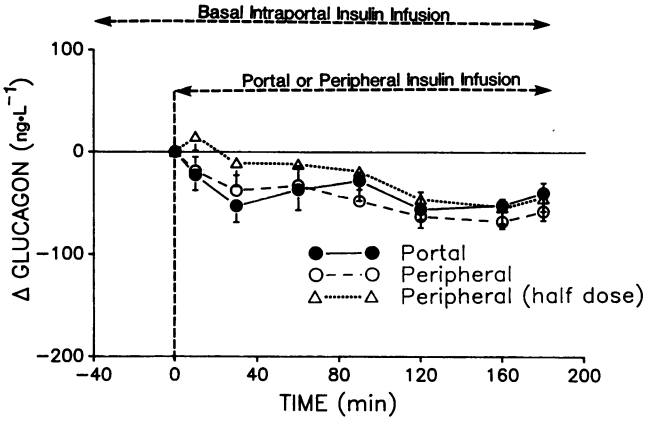

Figure 3. Changes in plasma glucagon levels from basal in the three experimental groups. Experimental design is as outlined in Fig. 1. Values are presented as mean±SE of individual deviations (Δ) from basal. Average basal values are reported in the text.

groups. The rate of exogenous glucose required to clamp plasma glucose at the initial levels increased gradually, up to a steady state rate that was 50% greater with the peripheral than with the portal or half peripheral infusions.

Basal glucagon levels were slightly higher than in normal dogs (32) but were lower in the group of dogs who underwent the half peripheral infusion than in the other groups, despite the same treatment in the basal state (portal: 192±27; peripheral: 196±26; half peripheral: 128±21 ng·L^{-1}). The decline from basal (Fig. 3) was slightly, but not significantly greater with peripheral than with portal or half peripheral infusions (difference from basal in the 90–180-min period: portal: 42±19; peripheral: 60±22; half peripheral: 42±14 ng·liter^{-1}).

Basal glucose turnover (Fig. 4) was in the high normal range (32, 33) and was equivalent in the three groups (portal: 20.4±1.0; peripheral: 20.0±1.1; half peripheral: 19.3±1.5 μmol·kg^{-1}·min^{-1}). During the clamp, when peripheral insulin levels were greater with peripheral than equimolar portal insulin infusion, GU (Fig. 4, *left*) increased more, as expected, to 40.4±3.8 versus 30.4±2.9 μmol·kg^{-1}·min^{-1} ($P < 0.001$), respectively, in the 3rd h of the clamp. GP declined rapidly, but was not suppressed completely with either portal or equimolar peripheral insulin infusion. Unexpectedly, the peripheral infusion resulting in greater peripheral insulin levels induced a greater suppression of GP from 20.0±1.1 to 5.4±1.3 μmol·kg^{-1}·min^{-1} during the 3rd h of the clamp compared with a decline from 20.4±1.0 to 9.0±1.3 μmol·kg^{-1}·min^{-1} with the portal insulin infusion (73±7 vs. 55±7%, $P < 0.001$). The portal and half peripheral insulin infusions that resulted in the same peripheral insulinemia resulted, as expected, in equivalent stimulation of GU (Fig. 4, *right*) to 28.6±2.7 with the portal and 30.5±3.9 μmol·kg^{-1}·min^{-1} with the half peripheral infusion. In spite of greater hepatic insulinization with portal than half peripheral infusion, at matched peripheral insulin levels GP was suppressed to the same extent from 20.4±1.0 to 9.0±1.3 μmol·kg^{-1}·min^{-1} and from 19.3±1.5 to 7.1±0.8

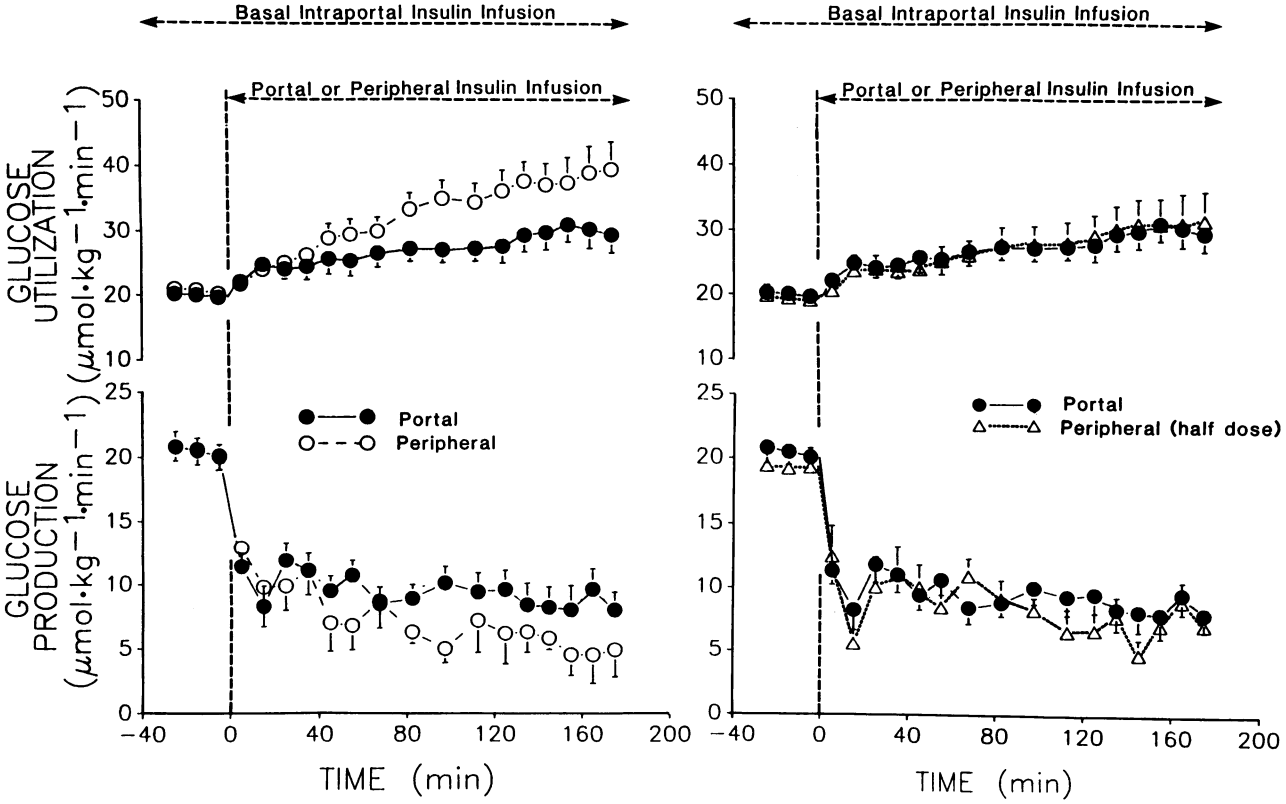

Figure 4. (*Left*) Comparison between portal insulin infusion of 54 pmol·kg^{-1} + 5.4 pmol·kg^{-1}·min^{-1} (● —— ●) and peripheral insulin infusion of 54 pmol·kg^{-1} + 5.4 pmol·kg^{-1}·min^{-1} (○ - - - ○). (*Right*) Comparison between portal insulin infusion of 54 pmol·kg^{-1} + 5.4 pmol·kg^{-1}·min^{-1} (● —— ●) and peripheral insulin infusion of 27 pmol·kg^{-1} + 2.7 pmol·kg^{-1}·min^{-1} (△---△). GP (*top*) and GP (*bottom*). Experimental groups are as outlined in Fig. 1. Values are presented as mean±SE.

$\mu mol \cdot kg^{-1} \cdot min^{-1}$ with the portal and the half peripheral insulin infusions, respectively, during the 3rd h of the clamp (55±7 vs. 63±4% suppression).

Basal MCR (not shown) was moderately lower than in normal dogs (32, 33) and equivalent in the three groups (portal: 2.00±0.13; peripheral: 1.99±0.11; half peripheral: 1.97±0.16 $ml \cdot kg^{-1} \cdot min^{-1}$). During the clamp, MCR increased to 2.84±0.31 with portal, 3.67±0.37 with equimolar peripheral, and 3.13±0.45 $ml \cdot kg^{-1} \cdot min^{-1}$ with half peripheral insulin infusion, levels that are also lower than those observed in normal dogs with similar infusion rates (15).

Under conditions of moderate hyperglycemia and low to normal hepatic insulinization (basal period), GC (not shown) was as much as 51±6% of GP, compared with 15% in normal dogs, as previously reported by us (34) and others (33). GC did not decrease with peripheral or portal infusion of insulin in any of the three groups (basal: portal = 11.7±2.1, peripheral = 11.8±3.0, half peripheral = 7.3±1.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; 3rd h of the clamp: portal = 12.4±2.13, peripheral = 13.5±2.8, half peripheral = 6.8±1.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Basal alanine, glycerol, and FFA levels were higher than in normal dogs (32), but basal alanine and FFAs were lower in the group of dogs who underwent the half peripheral infusion than in the other groups, despite the same treatment in the basal state. Alanine decreased gradually (Fig. 5) from basal levels of 686±131, 661±76, and 495±55 μM to average 90–180 min values of 568±105, 465±70, and 408±50 μM in the portal, peripheral, and half peripheral infusion groups, respectively. The decline was greater with peripheral than equimolar portal ($P < 0.01$) or half peripheral infusion. Glycerol decreased rapidly from 0.12±0.01 to 90–180-min levels of 0.08±0.01 mM with portal infusion, from 0.12±0.02 to 0.06±0.01 with periph-

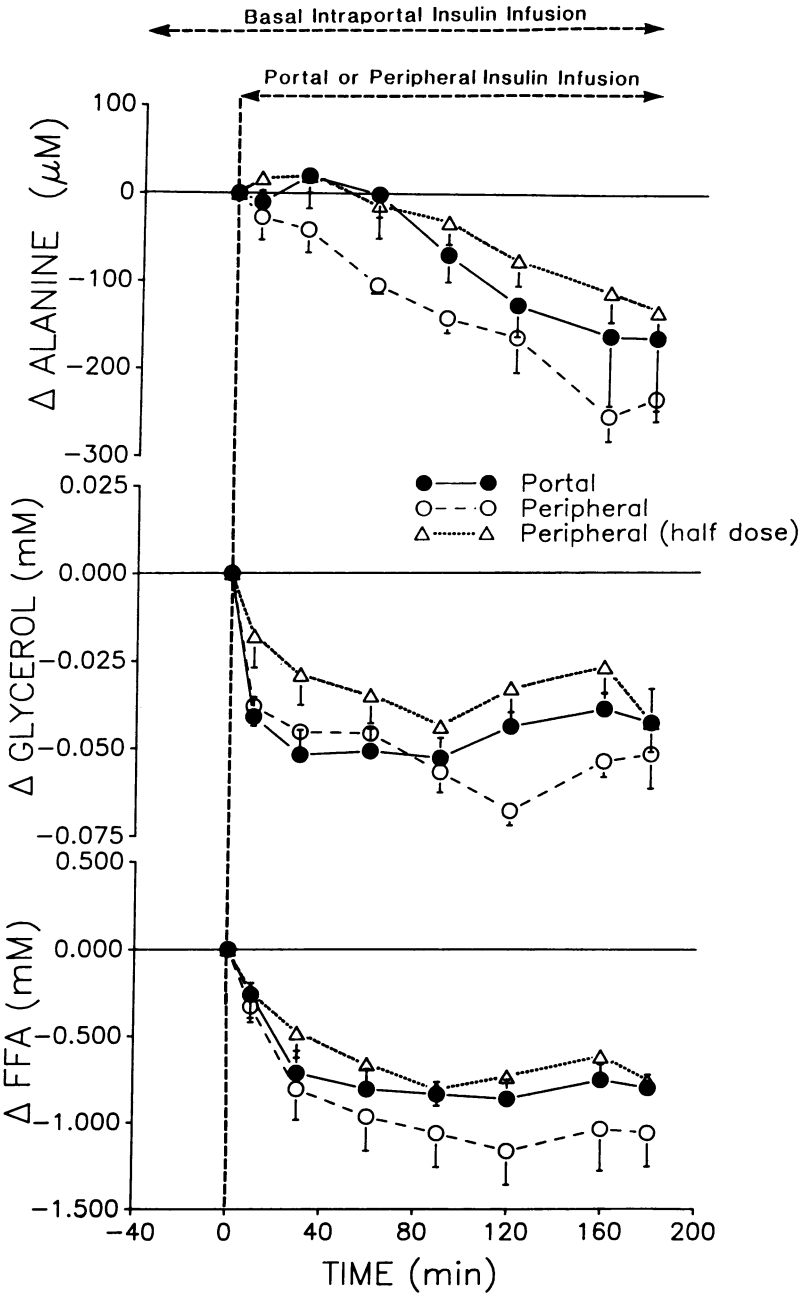

Figure 5. Changes in plasma levels of alanine (*top*), glycerol (*middle*), and FFAs (*bottom*) from basal in the three experimental groups. Experimental design is as outlined in Fig. 1. Values are presented as mean±SE of individual deviations (Δ) from basal. Average basal values are reported in the text.

Table I. Plasma Lactate Levels (mM)

Time	Portal	Peripheral	Half peripheral
−20	1.10±0.21	1.27±0.35	0.69±0.09
0	1.20±0.29	1.31±0.37	0.71±0.11
10	1.22±0.30	1.19±0.30	0.85±0.17
30	1.54±0.37	1.54±0.39	0.84±0.16
60	1.50±0.33	1.47±0.28	0.86±0.14
90	1.12±0.17	1.23±0.17	0.84±0.12
120	1.21±0.17	1.35±0.28	0.88±0.13
160	0.99±0.11	0.98±0.17	0.88±0.14
180	0.96±0.15	1.05±0.18	0.80±0.12

Portal: 54 pmol · kg^{-1} + 5.4 pmol · kg^{-1} · min^{-1} portal insulin infusion. Peripheral: 54 pmol · kg^{-1} + 5.4 pmol · kg^{-1} · min^{-1} peripheral insulin infusion. Half peripheral: 27 pmol · kg^{-1} + 2.7 pmol · kg^{-1} · min^{-1} peripheral insulin infusion. Values are mean±SE from seven experiments in each group.

eral infusion ($P < 0.001$ vs. portal), and from 0.11±0.01 to 0.07±0.01 with half peripheral infusion (NS vs. portal). Also FFAs decreased rapidly from 1.36±0.13 to 90–180-min levels of 0.54±0.08 mM with portal infusion, from 1.42±0.22 to 0.37±0.08 with peripheral infusion ($P < 0.001$ vs. portal), and from 1.19±0.10 to 0.46±0.08 with half peripheral infusion (NS vs. portal).

Basal lactate levels (Table I) were in the high normal range (32) but lower in the group of dogs who received the half peripheral insulin infusion, despite the same treatment in the preclamp period. During the clamp, lactate increased transiently by 30% in the portal and peripheral infusion protocols. In the half peripheral infusion protocol, the increase in lactate was lower but more sustained. When the whole duration of the clamp was considered, the increase was not different from the other two groups.

When the results of all the three protocols were combined, we found a positive correlation between the percentage suppression of GP and the percentage increase in peripheral insulin levels ($r = 0.471$, $P < 0.05$) and GU ($r = 0.664$, $P < 0.01$). There was also a positive correlation between the percentage suppression of GP and the percentage suppression of glycerol ($r = 0.580$, $P < 0.01$) and FFAs ($r = 0.628$, $P < 0.01$). Suppression of GP was not correlated with suppression of alanine or glucagon.

Discussion

In the present study, at suprabasal insulin levels in the physiological postprandial range, insulin-induced suppression of GP in moderately hyperglycemic, depancreatized dogs appeared to be more dependent on peripheral than portal insulin concentrations. GP was evaluated with a glucose clamp + the MSTI method, which consists of using tracer-enriched glucose infusates to keep specific activity constant. In fact, we observed a slow and modest decline in 6-[^{3}H]glucose specific activity. This could not have influenced our steady state calculations of GP, since a constant specific activity was reached in all groups during the 3rd hour of the clamp. With regard to the nonsteady state calculations of GP, we have recently found that a slow decline in specific activity of up to 35% does not cause underestimation of GP (unpublished observations from our laboratory).

Steady state hyperglycemia was maintained with near normal insulinemia in the basal state, indicating insulin resistance. As expected, with suprabasal insulin infusions the increase in peripheral insulin levels was twofold greater with peripheral than with portal or half peripheral. The overall potency of insulin on glucose turnover, as judged by the glucose infusion rate required to maintain the clamp, was proportional to peripheral insulin levels.

In depancreatized dogs, basal to elevated glucagon levels are maintained by gastric secretion of extrapancreatic glucagon into the portal circulation (19). This glucagon is indistinguishable from pancreatic glucagon (18) and normally is suppressible by insulin (2). With intraportal insulin infusion downstream from the site of glucagon production, insulin reaches the glucagon-producing cells at peripheral concentrations. In this study, glucagon was suppressed to a greater extent with equimolar peripheral than with portal or half peripheral insulin infusions. The difference did not reach statistical significance but might reflect a significant difference in portal glucagon levels.

GU and MCR increased to a greater extent with peripheral than equimolar portal or half peripheral infusion, as expected from the difference in peripheral insulin levels. It is estimated that the increase in portal insulin levels is slightly lower with equimolar peripheral than portal infusion because of dilution of insulin in the general circulation before it reaches the portal circulation (15). With peripheral insulin infusion at half the dose, the increase in portal insulin levels is less than half that obtained with a full-dose portal infusion. Approximately 70–80% of hepatic insulinization is determined by portal insulin levels (35). Unexpectedly, suppression of GP was greater with equimolar peripheral than portal infusion and was the same when peripheral insulin levels were matched. Thus, it was apparently independent of hepatic insulinization. In addition to GP, we also determined glucose cycling (glucose ⇆ glucose-6-P; GC). Basal GC was more than threefold higher than normal (33, 34) whereas GP was near normal, confirming the sensitivity of GC as a marker of diabetes (20). In agreement with Bell et al. (36), insulin failed to decrease GC when glycemia was not allowed to fall. This was not due to the nonphysiological route of insulin administration, since portal infusion also had no effect.

Elevated basal levels of gluconeogenic precursors and FFAs also suggest insulin resistance. Insulin resistance was probably less marked in the group of dogs that eventually underwent the half peripheral infusion, as indicated by the lower basal levels of these metabolites (as well as the difference in basal glucagon). During the clamp, lactate increased transiently in two out of three groups, as previously described (37). This was likely due to an overlap of two effects of insulin on lactate production: an increase due to stimulation of glycolysis and a decrease due to stimulation of pyruvate oxidation. Conceivably, this dual action of insulin may explain the absence of differences related to different peripheral insulin levels. Alanine reflects both carbohydrate and protein metabolism and thus is variably affected during insulin clamps (37). In depancreatized dogs, insulin decreases alanine levels (38). In our study, the greater suppression of alanine with peripheral than portal or half peripheral infusion can be explained by higher

peripheral insulin levels. The rapid drop in FFAs and glycerol indicates the sensitivity of lipolysis to inhibition by insulin (39). The greater suppression of both glycerol and FFAs with peripheral than with portal or half peripheral infusion is also consistent with higher peripheral insulinization.

These data demonstrate that peripheral insulin levels are important for inhibition of GP in our animal model of diabetes, as has recently also been shown in the normal dog (15). With regard to the apparent independence of suppression of GP from portal insulin levels, which is the most unexpected finding of this study, a few considerations apply. The extent of suppression of GP achieved in the present study was marked, even with half peripheral infusion (60%). However, GP was not completely suppressed, in contrast to previous studies carried out in humans at the same insulin levels (40). This might be related to the use of the MSTI method and HPLC-purified tracers and/or to the diabetic state of our dogs. If suppression of GP was close to maximum, then the effect of a further elevation in portal insulin levels might have been hard to detect. However, the peripheral insulin levels that we obtained were in the physiological postprandial range. In this range, an increase in peripheral insulin levels suppressed GP by a further 10–15%. This finding suggests that, postprandially, the direct suppressive effect of increased hepatic insulin levels may rapidly reach a maximum, however, GP still remains sensitive to peripheral regulation by insulin. In contrast, at basal insulin levels, a portal–peripheral insulin gradient could be more important because suppression of GP may be mainly regulated by the direct effect of insulin on the liver (5).

Another factor that could have diminished the effect of portal insulin concentrations on GP is laminar flow in the portal circulation, resulting in nonhomogeneous insulin delivery and, therefore, regions of hepatic underinsulinization. In our study, the hepatic fractional excretion of insulin was 50%, which indicates that insulin did not bypass the liver, although lesser degrees of nonhomogeneous insulin delivery might be manifest in terms of GP but not of insulin extraction.

With regard to the predominance of peripheral insulin levels in regulating GP, peripheral actions of insulin that might be involved are suppression of the flow of precursors and energy substrates (FFAs) for gluconeogenesis and suppression of glucagon secretion. In previous studies, the exaggerated suppression of recycling with inhibition of GP by peripheral insulin infusion (5) indicates the possibility of a substrate-mediated inhibition of gluconeogenesis. In normal subjects, GP did not increase with an increasing load of gluconeogenic precursors (41) and FFAs (42). In diabetes, the effects of FFA infusion are controversial (43, 44). However, combined reduction of gluconeogenic precursors such as was seen in the present study did decrease GP in normal subjects (41) and, in addition, lowering of FFAs suppressed GP in non–insulin-dependent diabetes (44). In our study, suppression of GP was correlated with suppression of FFAs and glycerol ($P < 0.01$). The correlation with alanine was not significant but can be masked, since hepatic extraction of alanine decreases when gluconeogenesis is inhibited (2), which leads to a rise in plasma alanine levels. However, correlations do not necessarily indicate cause–effect relationships. Also, the differences in metabolites seen in this study, as well as their correlations with the difference in suppression of GP, are not striking. We did not quantitate the hepatic load of metabolites but, from the different decreases in the arterial concentrations of alanine and glycerol ($\sim$ 80 and 20 μM greater with peripheral than equimolar portal infusion), their hepatic fractional extraction under similar conditions (0.4 and 0.8, respectively) (2), and an assumed hepatic blood flow of 30 $ml \cdot kg^{-1} \cdot min^{-1}$ (45), it is calculated that the difference in these gluconeogenic precursors could account for a GP rate of $\sim$ 0.5 and 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, assuming complete conversion of these metabolites (two molecules) to glucose. Lactate was slightly higher from 90 to 180 min in the peripheral infusion group, but the baseline value was also higher. After the peak that occurred at 30 min, lactate decreased by $\geq$ 60 μM more with equimolar peripheral than with portal infusion, which for a liver fractional extraction of 0.3 (2) would add another 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. If we omit this last calculation as it results from a nonsignificant difference, the measured changes in precursors could account for a rate of GP of $\sim$ 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. This is only 20% of the difference in the decrease ($\sim$ 3.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) in GP observed between equimolar portal and peripheral infusion. The amount of ATP provided by the difference in FFAs ($\sim$ 200 μM) can be calculated assuming a plasma hepatic flow of 18 $ml \cdot kg^{-1} \cdot min^{-1}$ (corresponding to a blood flow of 30 $ml \cdot kg^{-1} \cdot min^{-1}$ for a hematocrit of 40%), a liver fractional extraction of 0.2 (2), and oxidation of 30% of the hepatic FFA uptake (46). From simple stoichiometry, 129 molecules of ATP are generated from complete oxidation of palmitate and six high-energy phosphate bonds are used up for gluconeogenesis from pyruvate. This would give a rate of gluconeogenesis of 4.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which would account for the entire difference in GP. However, it is still unclear how much gluconeogenesis is related to changes in energy supply in vivo and how dependent it is on other sources of ATP than FFA oxidation.

Under basal conditions, up to 75% of GP is glucagon driven (47). Therefore, even small changes in glucagon (48, 49), which may not be detected with significance in immunoassays, might still be significant at the level of the liver. The lack of correlation between suppression of GP and suppression of glucagon could also be explained in terms of variability of the immunoassay. Since about one third of glucagon immunoreactivity represents cross-reacting material (50), $\sim$ 130 $ng \cdot liter^{-1}$ of 3,500 mol wt glucagon was probably responsible for 15 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ of basal GP in the dogs that eventually underwent equimolar portal or peripheral insulin infusion. Assuming a linear relationship between changes in glucagon levels and changes in GP, it is calculated that a quantitative difference in glucagon similar to that observed between the equimolar portal and peripheral infusions (18 $ng \cdot liter^{-1}$) could account for $\sim$ 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which is more than half the difference in suppression of GP. However, in the absence of statistical significance, the extent of this difference in glucagon remains uncertain. The effects of peripheral insulin levels on GP might also be at least in part independent of insulin's peripheral actions. In this study, suppression of GP correlated with peripheral insulin levels, but less than with some peripheral actions of insulin (i.e., suppression of FFAs and glycerol, stimulation of GU). However, this might only reflect the greater precision of measurement of glucose turnover and metabolites than of immunoreactive insulin. Insulin concentrations in the hepatic artery are the same as in the peripheral circulation. In the liver, different hepatic zones have a func-

tional subspecialization, the periportal hepatocytes being more glycogenolytic–gluconeogenic (glucose-producing cells) and the perivenous cells being more glycolytic–glycogen synthetic (glucose-using cells) (51). It might be postulated that if the blood mixture supplying the periportal hepatocytes had a higher ratio of arterial to portal blood than the blood supplying the perivenous hepatocytes, GP may be preferentially inhibited by insulin reaching the liver from the hepatic artery. Current evidence suggests that the mixture of blood supply is the same in all hepatic areas (35). However, the hemodynamics of the liver are complex and it is still possible that insulin reaching the liver from the hepatic artery is sensed differently from that carried through the portal vein. Also, insulin in the systemic circulation could inhibit GP by activating peripheral or central neural pathways, influencing the activity of hepatic nerves (35).

Another intriguing possibility is that although glucose levels were kept unchanged during the clamp, GP may have been inhibited by the higher glucose infusion rate necessary to clamp plasma glucose in the presence of higher peripheral insulin levels. Recently, a number of studies have shown a fall in GP during glucose infusion in the absence of a detectable rise in plasma glucose or insulin (42, 52). This could account for the correlation between stimulation of GU and suppression of GP.

In conclusion, at the suprabasal but physiological insulin levels tested in our depancreatized dogs, portal insulin infusion suppressed GP less than equimolar peripheral infusion. With portal infusion, the extent of GP suppression was the same as with peripheral infusion at half the rate, resulting in equal peripheral insulinemia. These results suggest that the absence of a portal-peripheral insulin gradient in insulin-treated diabetics might not be important for postprandial suppression of GP. The importance of the portal-peripheral insulin gradient may be related to basal suppression of GP or to other aspects of liver metabolism.

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