Insulin inhibits glucose production by a direct effect in diabetic depancreatized dogs during euglycemia

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Gupta, Nechar, Harmanjit Sandhu, Tracy Goh, Keyur Shah, Stephanie R. Wiesenthal, Hidenori Yoshii, Victor Chong, Tony K. T. Lam, C. Andrew Haber, Wendy Williams, Vaja Tchipashvili, and Adria Giacca. Insulin inhibits glucose production by a direct effect in diabetic depancreatized dogs during euglycemia. Am J Physiol Endocrinol Metab 283:E1002–E1007, 2002; 10.1152/ajpendo.00091.2002.—In our previous studies in nondiabetic dogs and humans, insulin suppressed glucose production (GP) by both an indirect extrahepatic and a direct hepatic effect. However, insulin had no direct effect on GP in diabetic depancreatized dogs under conditions of moderate hyperglycemia. The present study was designed to investigate whether insulin can inhibit GP by a direct effect in this model under conditions of euglycemia. Depancreatized dogs were made euglycemic (~6 mmol/l), rather than moderately hyperglycemic (~10 mmol/l) as in our previous studies, by basal portal insulin infusion. After ~100 min of euglycemia, a hyperinsulinemic euglycemic clamp was performed by giving an additional infusion of insulin either portally (POR) or peripherally at about one-half the rate (½ PER) to match the peripheral venous insulin concentrations. The greater hepatic insulin load in POR resulted in greater suppression of GP (from 16.5 ± 1.8 to 12.2 ± 1.6 μmol·kg⁻¹·min⁻¹) than ½ PER (from 17.8 ± 1.9 to 15.6 ± 2.0 μmol·kg⁻¹·min⁻¹, P < 0.001 vs. POR), consistent with insulin having a direct hepatic effect in suppressing GP. We conclude that the direct effect of insulin to inhibit GP is present in diabetic depancreatized dogs under conditions of acutely induced euglycemia. These results suggest that, in diabetes, the prevailing glycemic level is a determinant of the balance between insulin’s direct and indirect effects on GP.

Peripheral and hepatic effects of insulin; direct and indirect effects of insulin; hyperglycemia; free fatty acids

Insulin has a strong inhibitory effect on glucose production (GP). This inhibition is in part direct, i.e., due to hepatic sinusoidal insulin’s interaction with the hepatocyte insulin receptor (18, 19, 28, 29, 31, 32), and in part indirect, due to peripheral insulin’s actions on extrahepatic tissues (1, 18, 19, 23, 28, 29, 31, 32). These actions consist mainly of the antilipolytic effect of insulin in the adipose tissue (16, 17, 24, 25, 30). In nondiabetic animals and humans, the importance of either the direct or the indirect regulation of GP by insulin has been differently emphasized (2, 4). Undoubtedly, however, under normal physiological conditions, the impact on GP of even a small direct effect of insulin is magnified by the greater hepatic than peripheral insulinization.

Individuals with type 1 diabetes are treated with subcutaneous injections of insulin, which result in peripheral absorption of insulin and thus a level of hepatic insulinization that is not greater than peripheral insulinization. To the extent that the direct effect of insulin plays a role in the suppression of GP, peripheral hyperinsulinemia should be required to elevate the hepatic sinusoidal levels to adequately suppress GP. Because hyperinsulinemia has been associated with atherosclerosis (27) and recently also with some types of cancer (11), it is important to determine whether the nonphysiological route of insulin administration can contribute to hyperinsulinemia in diabetes.

However, GP may be differently regulated by hepatic and peripheral insulin effects in nondiabetic vs. diabetic individuals. In moderately hyperglycemic depancreatized dogs, we could not detect any direct effect of insulin, as we found that suppression of GP was proportional to peripheral, but not hepatic, insulin load at both high physiological and low insulin levels (9, 10). In humans with type 2 diabetes and moderate hyperglycemia, we found that the direct effect of insulin on steady-state GP was also undetectable (14).

The lack of a direct effect of insulin on suppression of GP in diabetic dogs and humans may be due to hyperglycemia and/or to chronic effects of the diabetic state. The aim of the present study was to determine whether, with correction of hyperglycemia—

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mia, we would be able to detect a direct effect of insulin on GP in depancreatized dogs, similar to nondiabetic dogs.

We studied depancreatized dogs in which euglycemia (~6 mmol/l) was acutely induced by basal portal insulin replacement, and we performed a hyperinsulinemic euglycemic clamp by giving an additional infusion of insulin either portally (POR) or peripherally at approximately one-half the rate (½ PER) to match the peripheral insulin levels, assuming that hepatic insulin extraction was ~50%. Our hypothesis was that POR would have suppressed GP to a greater extent than ½ PER.

**MATERIALS AND METHODS**

**Experimental animals and preparation.** The animal model and preparation were the same as in our previous studies (9, 10). All procedures were in accordance with the Canadian Council of Animal Care Standards and were approved by the Animal Care Committee of the University of Toronto. The studies were performed on six postabsorptive, depancreatized male dogs (model of type 1 diabetes mellitus) weighing 25–35 kg. Before surgery, atropine (0.02 mg/kg) and acepromazine (0.1 mg/kg) were administered to prevent throat secretions and to sedate the animal, respectively. After general anesthesia was induced by an intravenous dose of sodium thiopental (25 mg/kg), the dogs were intubated for assisted ventilation. Anesthesia was maintained through 0.5% halothane in carrier gas consisting of 60% nitric oxide (Canox, Toronto, ON, Canada) and 40% oxygen (Canox).

The abdominal cavity was opened with a midline laparotomy. The pancreas was completely removed, and care was taken to preserve duodenal vascularization through the pancreatoduodenal vessels.

In all dogs, a Silastic cannula (0.05 in. ID, Baxter Healthcare, McGaw Park, IL) was inserted into the portal vein through a branch of the splenic vein and advanced until the tip was ~1.0 cm beyond the point of confluence of the splenic vein with the portal vein, i.e., ~5 cm from the branching point of the portal vein into its left and right bifurcations to the liver. Three Silastic cannulas (one 0.05 in. ID and two 0.03 in. ID) were inserted into the jugular vein and advanced into the superior vena cava. In addition, a Silastic cannula (0.05 in. ID) was inserted into the 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, Ontario, Canada) and bandaged around the dog’s neck. An analgesic (buprenorphine) was administered intravenously during the surgery and intramuscularly after surgery (0.05 mg/kg). In addition, a small dose of NPH insulin (Iletin II NPH insulin; Eli Lilly, Indianapolis, IN) was injected subcutaneously to regulate glycemia. The cannulas were regularly flushed (every 3–4 days) with saline and filled with heparin to maintain patency.

The dogs received a mixture of dry chow mixed with canned meat once a day. The food was supplemented with folic acid and iron. In addition, pancreatic enzymes (Cotazym; Organon Canada, Toronto, ON, Canada) were given orally to replace the lost exocrine function of the pancreas. Regular porcine (Iletin II regular insulin; Eli Lilly) and NPH porcine insulin were injected subcutaneously. The insulin dose was adjusted to maintain glyceremia <1%, as in our previous studies (9, 10). Body weight, body temperature, hematocrit, stools, and food intake were monitored regularly.

Paired experiments were carried out in random order ≥2 wk after surgery, and ≥10 days were allowed between each experiment. Only dogs that were healthy, with a hematocrit >35% and with glucosuria <1% were allowed to undergo experiments. The dogs received the normal amount of food the day before the experiment. The regular insulin dose was unaffected, and the NPH insulin was reduced to one-half or one-third of the previous day’s dose. The experiments were performed after an overnight fast of ≥18 h.

**Experimental protocol.** On the morning of the experiment (Fig. 1), the dogs were hyperglycemic (>20 mmol/l) due to their reduced NPH insulin dose the day before. At the onset of the experiment, regular porcine insulin was initially infused intraorally at a high dose. The dose was then gradually reduced to basal levels with the goal of obtaining constant euglycemia (5–7 mmol/l in dogs). When glucose levels decreased below 16 mmol/l (i.e., ~80 min after the insulin infusion was started), a bolus of tracer (7.77 × 10⁵ dpm of [3-³H]glucose; New England Nuclear, Boston, MA) followed by a continuous tracer infusion (5.55 × 10⁶ dpm/min) was given to enable the measurements of GP and glucose utilization (GU).

Approximately 120 min were required to reach euglycemia after the tracer infusion was initiated and 60 additional minutes of euglycemia elapsed before the first basal sample was taken at ~40 min. At that time, the insulin infusion (portal insulin replacement) had been kept fixed at a basal rate for ≥30 min. Both the portal insulin replacement and the tracer infusions were continued throughout the experiment. Basal samples were taken every 10 min for 40 min. At time 0, an additional infusion of insulin was given either...
portally (POR) or at approximately one-half the dose peripherally (½ PER) to match the peripheral insulin levels obtained with the POR treatment (due to ~50% insulin extraction by the liver) and also to obtain a large difference in the estimated hepatic insulin levels. The dose that matched the peripheral insulin levels in our dogs was slightly less than 50% (2.34 pmol·kg\(^{-1}\)·min\(^{-1}\)) of the dose used in POR (5.4 pmol·kg\(^{-1}\)·min\(^{-1}\)). This dose was determined experimentally on the basis of the results of the insulin levels in the first two dogs. All insulin infusions were prepared in saline containing ~4% (wt/vol) of the dog’s own plasma.

Plasma glucose was clumped at the initial preclamp glycemic level for 3 h with a variable exogenous dextrose infusion (50% dextrose, Abbott Laboratories, Montreal, QC, Canada), which was adjusted according to plasma glucose concentrations determined every 5 min. The dextrose infusion was spiked with [3-\(^3\)H]glucose tracer (hot glucose) according to Finegood et al. (6, 7) to prevent the decline in the glucose specific activity during the glucose clamp and thus minimize errors that are associated with the use of a one-compartment, fixed-volume model method for calculations of GP (5). The following equation by Finegood et al. (7), as modified by Giacca et al. (9) to account for partial suppression of GP, was used to calculate specific activity of the dextrose infusion

\[
SA_{GINF} = \frac{I \times ([GINF(ss)/Ra(b)] - F)}{GINF(ss) \times BW} \times 1,000
\]

where \(SA_{GINF}\) is the specific activity of the glucose infused (dpm/µmol), \(I\) is the constant tracer infusion (dpm/min), \(GINF(ss)\) is the steady-state glucose infusion rate (µmol·kg\(^{-1}\)·min\(^{-1}\)), \(Ra(b)\) is the basal GP (µmol·kg\(^{-1}\)·min\(^{-1}\)), \(F\) is the steady-state suppression of GP, \(F = [Ra(b) - Ra(ss)]/Ra(b)\), \(Ra(ss)\) is the steady-state GP (µmol·kg\(^{-1}\)·min\(^{-1}\)), and \(BW\) is the weight of the dog (kg). The initial estimates were derived from the values obtained in our previous study (9).

Arterial samples for glucose specific activity, insulin, glucagon, and free fatty acid (FFA) levels were taken every 10 min in the 1st and 3rd h and every 15 min in the 2nd h of the hyperinsulinemic clamp.

**Laboratory methods.** Plasma glucose concentrations were measured by the glucose oxidase method (13) on a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). The radioimmunoassays for insulin and glucagon were performed using kits from Pharmacia and Diagnostic Products, respectively. The coefficients of variation of the assays are <7 and <16%, respectively. The FFA concentrations were determined with the fluorometric method of Miles et al. (20). For determination of [3-\(^3\)H]glucose radioactivity, plasma was deproteinized in equal volumes of 5% (wt/vol) zinc sulfate and 0.3 N barium hydroxide (BDH, Sigma Diagnostics, St. Louis, MO). An aliquot of the supernatant was evaporated to dryness to eliminate tritiated water. After addition of 1 ml of double-distilled water, 10 ml of liquid scintillation solution (Ready Safe; Beckman, Fullerton, CA) were added, the tubes were vortexed, and radioactivity from [3-\(^3\)H]glucose was measured in a β-scintillation counter (Camber 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.

**Calculations.** GP was calculated as the endogenous rate of appearance measured with [3-\(^3\)H]glucose. A modified one-compartment model of Steele, which accounted for the mixture of labeled and unlabeled glucose infused during the euglycemic clamp, was used to calculate GP and GU (6). Data were smoothed with the optimal segments routine using the optimal error algorithm (3).

We calculated the first-pass hepatic insulin extraction (HIE) of portally delivered insulin using the formula

\[
HIE (\%) = \frac{\Delta INF_{POR} - (\Delta [Ins]_{PE-POR} \times INF_{1/2, PER} / [Ins]_{PE-1/2, PER}) \times 100}{\Delta INF_{POR}}
\]

where \(\Delta INF_{POR}\) is the additional portal insulin infusion during the POR treatment, \(\Delta [Ins]_{PE-POR}\) is the increment in the peripheral insulin level due to \(INF_{POR}\), \(INF_{1/2, PER}\) is the ½ PER insulin infusion in the paired ½ PER treatment in the same dog, and \(\Delta [Ins]_{PE-1/2, PER}\) is the increment in peripheral insulin due to \(INF_{1/2, PER}\). INF\(_{1/2, PER}\)/[Ins]\(_{PE-1/2, PER}\) represents plasma insulin clearance (PCR), and PCR \(\times [Ins]_{PE-POR}\) is the systemic appearance of portally delivered insulin.

**Statistical analysis.** The data were expressed as means ± SE. Two-way analysis of variance (ANOVA) was carried out for differences between experimental groups, as all experiments were paired. Data were also analyzed within each group for differences between the experimental periods (basal: from ~40 to 0 min; clamp: from 0 to 180 min). Calculations were performed with SAS software (SAS Statistical Analysis System, Cary, NC) using “group” and “dog” and “period” and “dog” as independent variables in the two-way ANOVA models. Significance was accepted at \(P < 0.05\).

**RESULTS**

The following results are based on an \(n = 6\) for POR and ½ PER. The portal insulin dose required to maintain euglycemia was 4.1 ± 1.0 (POR) and 4.2 ± 1.1 pmol·kg\(^{-1}\)·min\(^{-1}\) (½ PER) \([P = \text{not significant (NS)}]\), and the basal peripheral insulin levels were ~60 pmol/l (also \(P = \text{NS}\); Fig. 2A). The additional portal insulin infusion in the POR treatment raised the peripheral insulin levels to ~150 pmol/l, which was not different from the rise in insulin levels observed with the additional peripheral insulin infusion in ½ PER. The HIE was calculated to be 55.7%.

The plasma glucose levels were maintained constant (~6 mmol/l) at basal euglycemia (5.7 mmol/l in dogs) (Fig. 2B). Plasma glucose specific activity was also maintained close to the basal levels (Fig. 2C).

The GINF necessary to maintain glycemia constant during the hyperinsulinemic clamp was significantly greater in POR (\(P < 0.001\)) than in ½ PER (Fig. 3A). As expected at matched peripheral insulin levels, GU was similar in the basal state and increased to a similar extent in response to insulin (Fig. 3B).

Basal endogenous GP was 16.5 ± 1.8 and 17.8 ± 1.9 µmol·kg\(^{-1}\)·min\(^{-1}\) \((P = \text{NS})\) in the POR and ½ PER treatments, respectively. When the additional insulin was given, GP was suppressed to a level that was much lower (12.2 ± 1.6 µmol·kg\(^{-1}\)·min\(^{-1}\)) in POR than in ½ PER (15.6 ± 2.0 µmol·kg\(^{-1}\)·min\(^{-1}\), \(P < 0.001\) vs. POR; Fig. 4). In fact, with the ½ PER treatment, the GP suppression barely reached significance \((P < 0.05)\).

The basal FFA levels were similar in the POR and ½ PER groups. During the hyperinsulinemic clamps, the FFA levels were suppressed to a similar extent in both treatments (Table 1). The basal glucagon levels were
also similar in the basal state and during the clamps in both treatments (Table 1).

DISCUSSION

In this study in euglycemic depancreatized dogs, at matched peripheral insulin levels, portal insulin infusion (POR treatment) was more effective than half-rate peripheral insulin infusion (½ PER treatment) in suppressing GP during a hyperinsulinemic clamp, consistent with our previous findings in normal dogs (19) and humans (18). The ½ PER treatment, which only marginally suppressed GP, resulted in peripheral insulin levels equal to those of the POR treatment, but the hepatic sinusoidal insulin levels were presumably much lower than those in the POR treatment (assuming a portal-peripheral gradient of 2.5 (10) and 72% contribution of the portal flow to hepatic blood flow (12)).

Unlike our previous studies in depancreatized dogs at the same insulin dose (9) but under conditions of moderate hyperglycemia, the GINF rate was significantly greater in the POR treatment compared with the ½ PER treatment. GINF is dependent on both GU

Fig. 2. Plasma insulin (A), glucose (B), and glucose specific activity (C) during the experimental protocol (described in Fig. 1).

Fig. 3. Glucose infusion rate (A) and glucose utilization (B) during the experimental protocol (described in Fig. 1). a, Glucose infusion rate significantly greater in POR than in ½ PER (P < 0.001, 2-way ANOVA).

Fig. 4. Glucose production during the experimental protocol (described in Fig. 1). a, Glucose production lower during the hyperinsulinemic clamp in POR than in ½ PER (P < 0.001, 2-way ANOVA).
and GP, and with matched GU this observation indicated that GP was suppressed to a greater extent with POR than with 1/2 PER.

Indeed, unlike the previous study in hyperglycemic dogs, the suppression of GP in the euglycemic dogs was greater with POR than with 1/2 PER. The fact that a greater suppression of GP was found with POR leads to the conclusion that the greater hepatic insulin load in this treatment suppressed GP by a direct effect under conditions of euglycemia.

Because in the present study euglycemia was achieved by increasing the rate of the basal insulin infusion compared with the previous studies in depancreatized dogs when moderate hyperglycemia was achieved (9,10), we cannot exclude that the higher insulin level itself or some other metabolic parameter that was improved by basal insulin replacement allowed the direct effect of insulin to become manifest. However, in our studies in nondiabetic dogs, the direct effect of insulin was, if anything, less evident at high rather than low insulin levels (19). Also, during the clamp period, glucose was the only parameter that was markedly different from what it was in the previous study when the same additional insulin dose was infused portally or peripherally (9). Basal and clamp immunoreactive glucagon levels were lower in the present than in the previous study, in part because of the more specific antibody used in the radioimmunoassay; however, even if “true” 3,500-MW glucagon was lower in the present study, this should have diminished rather than potentiated the direct effect of insulin, as explained below.

Glucose is known to have acute and profound effects on glycogen metabolism by suppressing glycogen phosphorylase and enhancing glycogen synthase activities (22). In in vivo studies by Rossetti et al. (26), hyperglycemia caused a marked inhibition of GP mainly through the suppression of glycogenolysis with no apparent changes in gluconeogenesis. Regarding insulin’s effect on GP, Sindelar et al. (28) showed that, acutely, a selective elevation of hepatic sinusoidal insulin also inhibits GP through suppression of glycogenolysis but leaving gluconeogenesis intact. On the basis of the results of the present study, we hypothesize that, with glycogenolysis already maximally suppressed by hyperglycemia, hepatic insulin may not further suppress GP, whereas, with correction of hyperglycemia, glycogenolysis would be restored, allowing for the direct effect of insulin on glycogenolysis to become manifest. This hypothesis is also supported by the results of our studies (8,15) and those of other authors (21) showing that glucagon, which is a stimulator of glycogenolysis, potentiates the direct effect of insulin.

In the present study, the direct effect of insulin was tested after only 100 min of achievement of euglycemia. The results are therefore consistent with an acute effect of the prevailing glucose level on insulin’s direct effect rather than a chronic effect of improved control. An interesting question is whether there is also a chronic effect of the glucose level or of the diabetic state itself on insulin’s direct effect in suppressing GP. It is possible that, with tighter glycemic control, hepatic sensitivity to insulin is improved, which could enhance insulin’s direct effect on GP. To address this question, the results of the present study should be compared with those from dogs maintained under tighter glycemic control throughout the treatment period. However, in the present study, the difference in GP suppression was comparable to that seen in our previous studies in nondiabetic dogs (19), suggesting that the acute effect of euglycemia may be sufficient to restore insulin’s direct effect on GP.

Other interesting questions are whether euglycemia restores insulin’s direct effect on GP in type 2 diabetic humans, which is the threshold for hyperglycemia to diminish insulin’s direct effect on GP, and whether diabetic and nondiabetic subjects rely mainly on insulin’s indirect effect to inhibit GP when glucose levels are in the postprandial range. In our studies in type 2 diabetic humans maintained at their basal glucose levels (~9 mmol/l), the direct effect of insulin on steady-state suppression of GP was abolished; however, insulin still had a detectable direct suppressive effect on GP in the early periods of the clamp (14). The latter discrepancy with the dog data may be related to the 1 mmol/l difference in glycemia, to species difference, and/or to different experimental conditions (tolbutamide stimulation of endogenous insulin secretion in humans).

In summary, the present study shows that, in depancreatized dogs under conditions of basal euglycemia, at matched peripheral insulin levels, portal insulin infusion is more effective than half-rate peripheral insulin infusion in suppressing GP during a euglycemic clamp. This clearly indicates that the greater hepatic insulinization with portal insulin delivery has a direct effect of inhibiting GP. These results were obtained after only 100 min of euglycemia, which suggests that acute insulin-induced normalization of the prevailing plasma glucose level is capable of restoring insulin’s direct effect on GP and therefore that hyperglycemia, rather than chronic effects of diabetic state, abolishes insulin’s direct effect on GP in diabetic depancreatized dogs.

In conclusion, we have shown for the first time that the direct effect of insulin in suppressing GP is present...
in a model of type 1 diabetes under conditions of acutely induced euglycemia. These results have clinical implications, because if insulin had no direct effect on GP under any conditions in diabetes, peripheral insulin treatment of diabetic individuals would not result in peripheral hyperinsulinemia. Instead, our studies are consistent with the notion that deficiency of this direct effect due to hepatic hypoinsulinaemia during peripheral insulin delivery can account, in part, for the peripheral hyperinsulinemia of insulin-treated diabetes (hyperinsulinemia in this condition may also be due to insulin resistance). Because hyperinsulinemia has been associated with atherosclerosis (27) and recently also with some types of cancer (11) and because portal insulin delivery has the potential of normalizing plasma glucose levels while minimizing hyperinsulinemia, further investigation aimed at producing safe and relatively noninvasive portal insulin delivery systems appears to be justified.

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