Hepatic portal vein denervation impairs oral glucose tolerance but not exenatide’s effect on glycemia

Viorica Ionut,1 Ana Valeria B. Castro,1 Orison O. Woolcott,1 Darko Stefanovski,1 Malini S. Iyer,1 Josiane L. Broussard,1 Miguel Burch,2 Ram Elazary,2 Cathryn M. Kolka,1 Hasmik Mkrtchyan,1 Isaac Asare Bediako,1 and Richard N. Bergman1

1Diabetes and Obesity Research Institute and 2Department of Surgery, Cedars Sinai Medical Center, Los Angeles, California

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Isaac Asare Bediako,1 and Richard N. Bergman1

Hepatic portal vein denervation impairs oral glucose tolerance but not exenatide’s effect on glycemia. Am J Physiol Endocrinol Metab 307: E644–E652, 2014. First published August 12, 2014; doi:10.1152/ajpendo.00244.2014.—The hepatoportal area is an important glucohomeostatic metabolic sensor, sensing hypoglycemia, hyperglycemia, and hormones such as glucagon-like peptide-1 (GLP-1). We have reported previously that activation of hepatic portal sensors by intraportal infusion of glucose and GLP-1 or by subcutaneous administration of GLP-1 receptor activator exenatide and of intraportal glucose improved glycemia independent of corresponding changes in pancreatic hormones. It is not clear whether this effect is mediated via the portal vein (PV) or by direct action on the liver itself. To test whether receptors in the PV mediate exenatide’s beneficial effect on glucose tolerance, we performed (1) paired oral glucose tolerance tests (OGTT) with and without exenatide and 2) intravenous glucose tolerance tests before and after PV denervation in canines. Denervation of the portal vein affected oral glucose tolerance; post-denervation (POST-DEN) OGTT glucose and insulin AUC were 50% higher than before denervation (P = 0.01). However, portal denervation did not impair exenatide’s effect to improve oral glucose tolerance (exenatide effect: 48 ± 12 mmol·l−1·min before vs. 64 ± 26 mmol·l−1·min after, P = 0.67). There were no changes in insulin sensitivity or secretion during IVGTTs. Portal vein sensing might play a role in controlling oral glucose tolerance during physiological conditions but not in pharmacological activation of GLP-1 receptors by exenatide.

It is now recognized that the gastrointestinal tract plays an important role in controlling glucose homeostasis. The gut releases signal molecules (nutrients, hormones, metabolites) that directly, or via neural connections, alert the body to the presence of a meal and prepare an appropriate response to maintain glycemia. We and others (11, 15) have shown that the presence of glucose and of the intestinal hormone glucagon-like peptide-1 (GLP-1) in the portohepatic area triggers a series of events that result in the lowering of peripheral glycemia. The latter effect is mediated via changes in glucose uptake/output by the liver and by increased systemic glucose uptake (28). The lowering of glucose is independent of the “ileal break effect” of GLP-1 and peptide YY (PYY) and independent of corresponding changes in pancreatic hormones (insulin, glucagon). GLP-1-dependent glucose lowering could be mediated via GLP-1 receptors in the hepatoportal area (13).

Address for reprint requests and other correspondence: V. Ionut, Diabetes and Obesity Research Institute, Cedars Sinai Medical Center, 8700 Beverly Blvd., THAL 104, Los Angeles, CA 90048 (e-mail: Viorica.Ionut@cshs.org).

METHODS

Animals

Experiments were performed in male mongrel dogs (1 yr old, 28.2 ± 0.9 kg body wt) that were in the conscious relaxed state (n = 13), housed under controlled kennel conditions in the Cedars Sinai Medical Center vivarium, and fed once daily with a standard diet (27% protein, 32% fat, and 41% carbohydrate; Labdiet; PMI Nutrition International, Richmond, IN). The animals were used for experiments only if judged to be in good health as determined by body temperature, hematocrit, regularity of food intake, and direct observation. All surgical and

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Experimental procedures were approved by the Cedars Sinai Medical Center Institutional Animal Care and Use Committee.

Experimental Design

Experiments were performed in the morning after 12–16 h of fasting. In each animal, before and after portal denervation, we performed a metabolic assessment [oral glucose tolerance test (OGTT) with and without exenatide and intravenous glucose tolerance test (IVGTT)] and a hypoglycemic clamp (for physiological verification of portal vein denervation). At the end of the experimental period, the animals were euthanized and portal veins harvested for measurement of tyrosine hydroxylase by immunohistochemistry.

Surgery

Portal vein denervation was performed under general anesthesia, as described previously (10). After a midline incision, the hepatic portal vein was exposed. A myelinc-specific dye (toluidine blue 1%) was used to visualize nerve bundles along the portal vein, hepatic artery, common bile duct, and liver hilum. Only the visible nerves along the portal vein were cut, and the vein was painted with 10% phenol/alcohol vol/vol. Special care was taken to preserve the nerves running along the hepatic artery and its branches, the hepatic hilm nerves where nerve fibers enter the liver, the hepatic branch of the vasa nerve, and innervation of the common bile duct. Specifically, the portal vein was denervated distally (1–2 cm) from the liver hilum. It has been shown that, in rat, the portal vein glucose sensor is situated 1–3 cm from the liver hilum (8, 9), so this distance is likely to be even longer in larger mammals. The abdomen was then sutured closed. Animals recovered for ≥7 days after surgery before experiments were performed in the conscious state (see below).

OGTT

Basal blood samples were drawn from a peripheral vein at −20, −10, and −1 min. At t = 0, 25 g of glucose (55 ml of 50% dextrose, 454 mg/ml) was given as a bolus by oral gavage. Additional blood samples were taken at 15, 30, 45, 60, 90, 120, and 180 min. In a subset of animals (n = 9), 640 mg of acetaminophen was added to the oral gavage solution to measure gastric emptying (24). The OGTTs were performed in 12 animals.

OGTT With Exenatide

The protocol is identical to a regular OGTT, except that after basal sampling, at t = 0 an injection of exenatide (20 μg of Byetta; Amylin, San Diego, CA) was administered subcutaneously in the interscapular region. The OGTT with exenatide was performed in a subset of n = 6 animals.

IVGTT

Blood samples were drawn from a peripheral vein at −20, −10, and −1 min. Fasting values were defined as the average of the three basal samples. At t = 0, 0.3 g/kg body wt glucose (50% dextrose, 454 mg/ml; B Braun, Irvine, CA) was injected into a peripheral vein. Additional samples were taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 min. At t = 20 min, a bolus of 0.03 U/kg porcine insulin was injected into a peripheral vein, followed by sampling at 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. IVGTTs were performed in a subset of n = 6 animals.

Hypoglycemic Clamps

Intracatheters were acutely placed in peripheral veins for infusion of insulin and glucose and for blood sampling. After basal sampling (−20 to 0 min) at t = 0, insulin was infused at 30 pmol·kg⁻¹·min⁻¹ to slowly decrease glycemia to 2.5–3 mmol/l. Glucose (50% dextrose) was infused at a variable rate. The glucose infusion rate was adjusted every 10 min to achieve ~0.55 mmol/l reductions in blood glucose over 40-min periods. Thus, peripheral glucose decreased stepwise to a nadir that was reached between 120 and 160 min (steady state). Blood samples were taken every 10 min for measurements of glucose and insulin. Blood samples for measurement of catecholamines were taken at basal and at steady state. A subset of the hypoglycemic clamp data (8 out of 12) has been published previously as part of a larger study (n = 17) (10).

Blood Samples and Assay Measurements

Samples were collected into tubes coated with lithium fluoride and heparin containing EDTA (for glucose and insulin), EDTA-protamin (for glucagon), and EDTA-SMS and flash-frozen (for catecholamines). Plasma glucose concentrations were determined using a YSI 2300 autoanalyzer (YSI, Yellow Springs, OH). Insulin was measured using a human ELISA kit (Millipore, Billerica, MA) adapted in our laboratory for dog plasma (11). We used ELISA kits to measure catecholamines (Rocky Mountain Diagnostics, Colorado Springs, CO), acetaminophen (Immunalysis, Pomona, CA), and total and intact GLP-1 (Millipore). PYY was measured using RIA (Millipore).

Tyrosine Hydroxylase Immunohistochemistry

In a subset of animals, tyrosine hydroxylase immunohistochemistry was performed on portal vein tissue, as described previously (10). Briefly, the portal tissue was washed with 0.015 M PBS and dehydrated with a series of increasing concentrations of ethanol. After dehydration, the tissue was incubated in xylene [2 × 60 min and then infiltrated with paraffin, a paraplast-embedding medium (Sigma Chemical) at 60°C overnight and then embedded with fresh paraffin. Each pad was sliced across its extent at 5 μm using a rotary microtome (American Optical Instrument, Buffalo, NY). Sections were placed in a tissue-floating water bath (37°C) immediately after being sliced and were mounted on glass slides. Slides were left on a slide-warming table (37°C) to air-dry overnight. Slides were placed in a series of xylene to remove paraffin and then a series of ethanol. After a wash in tap water, the slides were placed in 3% hydrogen peroxide/methanol solution for 10 min. After a wash the slides were incubated for 25 min in citrate buffer (pH 6) at 95°C. The slides were brought to room temperature, rinsed in PBS containing 0.05% Tween-20 (PBST), incubated with anti-tyrosine hydroxylase (AB152; Millipore, Chicago, IL) at a dilution of 1:100 at 4°C overnight, and rinsed in PBST. The slides were rinsed with PBST and incubated with Dako EnVision+ System-HRP Labeled Polymer anti-rabbit (K4003; Dako) for 30 min at room temperature. After a rinse with PBST, the slides were incubated with 3,3′-diaminobenzidine for visualization. Subsequently, the slides were washed in tap water, counterstained with Harris’ hematoxylin, dehydrated in ethanol, and mounted with media. Two sections per animal were used to assess the lesion.

Calculations

IVGTT-derived indices: calculation of minimal model parameters. Insulin sensitivity (SI) and glucose effectiveness (Sg) were calculated by the application of the minimal model of glucose kinetics to the time course of glucose and insulin from the IVGTT using MINMOD Millennium software (version 6.02, 2004). The acute insulin response to glucose (AIRg) was calculated as the area under the curve (AUC) of the insulin concentrations above the average of the basal values from 0 to 10 min (2). The disposition index was calculated as the product $SI \times AIRg$ for each experiment. Kg (glucose tolerance) was calculated as the slope of the linear regression of the natural log of plasma glucose concentration vs. time from 10 to 19 min (1).

Insulin clearance. Fractional transfer rate reflecting insulin clearance was estimated using frequently sampled intravenous glucose tolerance data by fitting the insulin values after the insulin bolus to a

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single exponential insulin clearance model (26). Metabolic clearance of insulin (ml·kg\(^{-1}\)·min\(^{-1}\)) was calculated as the ratio of the dose of insulin in (μU/kg) and the area under the fitted decay curve of the single exponential insulin clearance model extended to infinity (22).

Exenatide’s effect on glycemia was calculated for the same condition (before or after denervation) as glucose AUC during OGTT minus glucose AUC during OGTT + exenatide.

Tyrosine hydroxylase quantification. Scanning and analyses were performed through the Translational Pathology Core Laboratory, Department of Pathology and Laboratory Medicine, University of California Los Angeles. The technician who performed the analyses was blind to the sample identity. Images were acquired and analyzed using Ariol SL-50 (Applied Imaging, San Jose, CA). The Ariol scanner is based on an Olympus BX61 microscope video camera (Jai CVM2CL). Ariol can perform automatic quantification of protein levels by measuring staining intensity and the pattern and location of staining in each cell. Slides were scanned at ×20 objective magnification with the DAPI and FITC filters. Optimal exposure times were determined before automated scanning. After scanning, threshold levels of the individual signals were optimized before final analysis. The readouts included area of signal (area of signal above the signal threshold) and total area analyzed and were calculated by the software. The results are reported as percent marker staining (TH fluorescence) relative to the area of tissue analyzed.

Statistical Analysis and Power Calculation

Statistical analysis. Results are presented as means ± SE unless indicated otherwise. Repeated-measurements ANOVA was used for timeline comparisons; paired t-tests with Bonferroni correction were used for time point comparisons before and after denervation. AUC was calculated using the trapezoid method. All differences were considered statistically significant when \(P < 0.05\).

Power calculation. The primary endpoint of the study was to detect a change in exenatide effect (calculated as glucose AUC during OGTT – glucose AUC during OGTT + exenatide). Exenatide effect mean ± SD, as determined from preliminary data, was 55 ± 13 mmol/min. To detect a clinically relevant 50% decrease, for a two-sided \(t\)-test, \(\alpha = 0.05\) and power = 0.9, with a sample size of \(n = 6\). The secondary end point was to detect a change in glucose AUC after denervation. Our database of OGTT in the canine indicates that glucose AUC mean ± SD = 1,902 ± 1,177; for a clinically relevant 50% change, \(\alpha = 0.05\) and power = 0.8; the sample size is 12. The post hoc power calculation for IVGTT shows that (for insulin secretion) \(m1 = 438, m2 = 300,\) and \(SD = 100; n = 6,\) power = 0.92.

RESULTS

Validation of Portal Vein Denervation

Portal vein denervation was validated via physiological and immunohistochemical methods. In a previous study, we have shown that denervation of portal vein results in a blunting of the counterregulatory response to hypoglycemia during a hyperinsulinemic hypoglycemic clamp (10). Therefore, we have performed paired hypoglycemic clamps before and after denervation and used the blunted response to hypoglycemia as a method of in vivo validation of portal vein denervation.

Hypoglycemic hyperinsulinemic clamps. Insulin infusion during the hypoglycemic clamp resulted in an increase in plasma insulin to 2,967 ± 304 (PRE-DEN) and 3,083 ± 242 pmol/l (POST-DEN), and we measured a slow decrease in plasma glucose to 2.9 ± 0.2 (PRE-DEN) and 3.0 ± 0.2 mmol/l (POST-DEN). There was a significant, 71% decrease in the epinephrine response to hypoglycemia (Fig. 1).

Tyrosine hydroxylase immunohistochemistry. After portal vein tissue was embedded in paraffin and stained for tyrosine hydroxylase, the staining was quantified using the Ariol scanner. We found a reduction in tyrosine hydroxylase content from 1.73 ± 0.52 to 0.37 ± 0.02% marker stain (\(P = 0.03\)).

Effect of Portal Vein Denervation on Body Weight and Fasting Parameters

There were no differences in body weight and fasting parameters (glucose, insulin, glucagon, intact GLP-1, PYY) before and after denervation (Table 1). However, we measured a significant reduction in total GLP-1.

Effect of Portal Vein Denervation on Oral Glucose Tolerance

To compare the effect of portal vein denervation on oral glucose tolerance, we performed OGTTs (without exenatide) before and after portal denervation.

After portal denervation, plasma glucose levels were significantly higher compared with after denervation (\(P = 0.02\) (Fig. 2A). To explore the possible mechanism of this difference in oral glucose tolerance, we measured gastric emptying via acetaminophen concentration, pancreatic hormones (insulin and glucagon) and gut hormones GLP-1 (total and intact), and PYY during the OGTT. Additionally, we performed IVGTTs to assess insulin sensitivity, insulin secretion, and insulin clearance.

Table 1. Body weight and fasted-state plasma variables before and after portal vein denervation

<table>
<thead>
<tr>
<th></th>
<th>PRE-DEN</th>
<th>POST-DEN</th>
<th>(P) Value</th>
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<tbody>
<tr>
<td>Body weight</td>
<td>28.3 ± 0.9</td>
<td>28.2 ± 0.9</td>
<td>0.88</td>
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<tr>
<td>Fasted-state variable</td>
<td></td>
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</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.3 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>53 ± 8</td>
<td>49 ± 6</td>
<td>0.61</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>82 ± 5</td>
<td>68 ± 6</td>
<td>0.08</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.83 ± 0.06</td>
<td>0.81 ± 0.07</td>
<td>0.75</td>
</tr>
<tr>
<td>Intact GLP-1, pmol/l</td>
<td>6 ± 0</td>
<td>6 ± 0</td>
<td>0.70</td>
</tr>
<tr>
<td>Total GLP-1, pmol/l</td>
<td>12 ± 1</td>
<td>9 ± 1**</td>
<td>0.002</td>
</tr>
<tr>
<td>PYY</td>
<td>161 ± 16</td>
<td>151 ± 17</td>
<td>0.48</td>
</tr>
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Values are means ± SD. PRE-DEN, before portal vein denervation; POST-DEN, after portal vein denervation; GLP-1, glucagon-like peptide-1; PYY, peptide YY.
Plasma insulin was significantly higher after denervation ($P = 0.01$; Fig. 2B), and there was a highly significant correlation between glucose and insulin levels ($P < 0.0001$). Moreover, there was a significant difference in the slope of the glucose/insulin regression line before ($65.92 \pm 6.83$) and after denervation ($92.14 \pm 8.29$, $P = 0.05$) (Fig. 3).

There was significantly higher rise to peak in acetaminophen concentration before ($62 \pm 8$ min) than after denervation ($36 \pm 6$ min, $P = 0.01$), although the total 0–180 AUC was not significantly different ($\text{AUC}_{0–180} = 298 \pm 102 \mu g \cdot ml^{-1} \cdot min$ before vs. $282 \pm 54 \mu g \cdot ml^{-1} \cdot min$, $P = 0.81$) (Fig. 4A). No statistically significant difference in intact GLP-1, total GLP-1, or PYY before and after denervation was detected (Fig. 4, B–D).

Effect of Portal Vein Denervation on Insulin Sensitivity, Insulin Secretion, and Insulin Clearance Measured by IVGTT

Hepatic portal vein denervation did not result in any changes in insulin sensitivity ($S_i$: $4.53 \pm 0.83 \mu U \cdot ml^{-1} \cdot min^{-1}$ PRE-DEN vs. $4.23 \pm 0.79 \mu U \cdot ml^{-1} \cdot min^{-1}$ POST-DEN, $P = 0.69$), insulin secretion ($\text{AIR}_g$: $438 \pm 41 \mu U \cdot ml^{-1} \cdot min^{-1}$ PRE-DEN vs. $459 \pm 44 \mu U \cdot ml^{-1} \cdot min^{-1}$ POST-DEN, $P = 0.42$), disposition index ($1,965 \pm 461$ PRE-DEN vs. $1,881 \pm 341$ POST-DEN, $P = 0.84$), intravenous glucose tolerance Kg ($3.24 \pm 0.41$ min PRE-DEN vs. $2.74 \pm 0.51$ min POST-DEN, $P = 0.29$), $S_g$ ($3.89 \pm 0.41$ min$^{-1}$/10$^{-2}$ PRE-DEN vs. $3.82 \pm 0.55$ min$^{-1}$/10$^{-2}$ POST-DEN, $P = 0.89$), or insulin clearance: (MCR$_{\text{insulin}}$: $27.44 \pm 3.68$ ml/kg PRE-DEN vs. $26.85 \pm 3.32$ ml/kg POST-DEN, $P = 0.81$) (Fig. 5).

Effect of Portal Vein Denervation on Exenatide’s Action to Improve Glucose Homeostasis

Before denervation surgery, administration of an oral glucose challenge resulted in significant increases in plasma glucose ($P < 0.05$) and insulin ($P = 0.004$) compared with basal (Fig. 6A and B).
As expected, administration of exenatide at the beginning of the OGTT resulted in significantly improved glucose tolerance, 
\( P = 0.009; \) Fig. 6A). The lower glucose was accompanied by considerable slowing of gastric emptying (as shown by the acetaminophen concentration; Fig. 6C), lower insulin (Fig. 6B), and higher glucagon suppression (OGTT-exenatide AUC\(_{0-180}\)\( = \) -2,744 ± 637 ng·l\(^{-1}\)·min\(^{-1}\) vs. OGTT-control AUC\(_{0-180}\)\( = \) -667 ± 532 ng·l\(^{-1}\)·min\(^{-1}\), 
\( P = 0.04\). Denervation of hepatic portal vein did not result in an attenuation of exenatide’s effect on glycemia.

![Fig. 4. Effect of portal denervation on plasma concentration of acetaminophen (A) and gut hormones peptide YY (PYY; B), intact glucagon-like peptide-1 (GLP-1; C), and total GLP-1 (D). Variables are presented before (•) and after portal vein denervation (○).](#)

![Fig. 5. Effect of portal denervation on intravenous glucose tolerance test-derived variables: insulin sensitivity (SI), acute insulin secretion (AIRg), disposition index (DI), glucose effectiveness (Sg), intravenous glucose tolerance (Kg), and insulin clearance (MCR).](#)
PORTAL VEIN DENERVATION AND EXENATIDE’S EFFECT

Excursion in the presence of exenatide was similar before and after surgery (fig. 6D), and there were no significant differences in insulin change, (Fig. 6E), change in acetaminophen plasma concentration reflecting gastric emptying (fig. 6F), or change in glucagon suppression (exenatide effect ΔAUC0–180: 1.654 ± 200 ng·l⁻¹·min PRE-DEN vs. 1.148 ± 200 ng·l⁻¹·min POST-DEN, P = 0.41).

Overall, denervation of the portal vein did not impact the effect of exenatide on glucose tolerance (exenatide effect ΔAUC0–180: 48 ± 12 mmol·l⁻¹·min PRE-DEN vs. 64 ± 26 mmol·l⁻¹·min POST-DEN, P = 0.67; Fig. 7).

DISCUSSION

The abdominal hepatic portal vein occupies a central locus in the architecture of the intestinal/hepatic/brain communication pathway. It is richly innervated (27) and receptive to metabolites (such as glucose and lactate) (5, 17) and hormones (such as GLP-1) (20). The central location and the ability to respond to nutrient, hormonal, and neural input has made the portal vein a true nexus of interorgan communication. However, many studies exploring the role of portal vein (and of the liver) in glucose homeostasis have been done using intraportal infusions, which inevitably results in both the liver and the portal vein being exposed to the stimulus. In a previous study, we have shown that infusion of glucose and GLP-1 in the portal vein results in a lowering of systemic glucose, an effect that 1) is independent of corresponding changes in insulin and glucagon (11), 2) is not replicated when glucose and GLP-1 are infused peripherally (12), and 3) is partially blocked by the intraportal infusion of the GLP-1 receptor antagonist exendin-(9–39) (13). In a subsequent study that used tracer to determine the site of glucose disappearance, we found that intraportal presence of glucose and GLP-1 receptor activator exenatide results in important changes in liver glucose uptake/release, with a favorable impact on glycemia (28). However, due to the experimental setup, none of these studies was able to identify whether the locus of signal initiation is the portal vein, the liver, or both.

To determine whether the portal vein glucose and GLP-1 sensor activation plays a major role in the exenatide’s role in glycemic control in the habitual subcutaneous administration, we performed OGTTs (with or without administration of exenatide) before and after portal vein denervation in a large animal model, the canine.

Confirming previous results (18), denervation of the portal vein impaired oral glucose tolerance. However, portal vein

Fig. 6. Exenatide effect on oral glucose tolerance before (PRE-DEN) and after (POST-DEN) portal denervation (n = 6). Glucose (A and D), insulin (B and E) and acetaminophen (C and F) during oral glucose tolerance test (OGTT) without (○) or with exenatide (●). *P < 0.05; **P < 0.01.
denervation did not impact exenatide’s effect on glucose homeostasis. Despite unequivocal evidence for portal denervation, and contrary to our hypothesis, we found that the effect of GLP-1 receptor activation on glucose homeostasis is not adversely affected by disconnecting the portal vein sensors from their neural connections.

There are several explanations for this finding. One is that the site of glucose and GLP-1 sensing is not the portal vein but the liver, despite GLP-1 receptor presence (as protein, but not mRNA) in the portal vein in rats (25). This would be concordant with the identification of both GLP-1 receptor mRNA and protein on primary human hepatocytes by Gupta et al. (6). The effect of GLP-1 on the liver could be direct by binding to the GLP-1 receptor and activation of pathways involved in glucose and lipid metabolism. Indeed, it has been shown that GLP-1 activates phosphoinositide 3-kinase/PKB in rat hepatocytes and stimulates the activity of glycogen synthase (23). Alternatively, the effect of GLP-1 on the liver could be mediated by binding to the GLP-1 and glucose receptors on nerve terminals in hepatic hilum area (as well as in the fibers extending into the connective tissue and hepatic lobules and ending on liver parenchymal cells in humans) (14). This in turn would initiate a neural signal to the brain and from the brain via efferent fibers to other organs (adipose tissue, muscle, liver itself) involved in a coordinated response to a glucose challenge.

Another possible explanation for our results is that the experimental design affected the magnitude of the total GLP-1 receptor activation effect, rendering a portally initiated effect undetectable. It is known that in physiological situations, a majority of the GLP-1 effect during a meal is due to the decrease in gastric emptying, with the insulinotropic effect playing a less important role (18); the insulin-sensitizing effect (decrease in glycemia in the absence of corresponding changes in pancreatic hormones) contributes an even lower proportion (~10–30%) to the glycemic decrease (28). Thus, the possible lack of effect of GLP-1 receptor activation with portal denervation might have been obscured by the strong “ileal brake” effect. Moreover, the presence of exenatide, a longer-lasting GLP-1 receptor activator, and subcutaneous administration (rather than directly into the portal vein) could have activated receptors in areas other than the portal vein and liver, with stronger effects than the hepatoportal initiated signaling. Unlike hypoglycemia, where a single signal (low plasma glucose) is detected by glucose regulation systems, an oral glucose load (or a meal) initiates multiple signals coming from the intestinal area. These signals (glucose, GLP-1, gastrin inhibitory polypeptide, PYY, etc.) are sensed by several areas: gut and afferent nerves and vasculature, wall of the portal vein, liver, and other organs in the general circulation (14). It is possible that the putative effects of exenatide or GLP-1 mediated by portal sensors were mitigated by compensatory changes such that portal denervation by comparison had a minimal impact on the overall response to a glycemic load and oral glucose tolerance.

One of the limitations of our study is that exenatide was used only acutely in a single dose. The study does not address the issue of chronic exenatide administration and does not assess whether portal denervation could impact the long-term effect of exenatide. We cannot exclude that portal denervation might have a detrimental effect on exenatide’s glucoregulatory function. Given that chronic exenatide has been shown to decrease liver steatosis (4), improve β-cell function (3), and decrease body weight in type 2 diabetic patients (16), it might be of interest to investigate in future studies whether portal GLP-1 receptors are involved in mediating these changes.

Interestingly, portal denervation appeared to worsen oral glucose tolerance, as indicated by higher glycemia and higher insulinemia after denervation. This finding is concordant with data from other investigators. Pagliassotti et al. (21) showed that hepatic denervation modified the disposition of an enteral glucose load; in their study, not only was the portal vein denervated but also the liver, the hepatic artery, and the bile duct near the hilum (in contrast, in our model portal vein alone was denervated, distal from the hepatic hilum). The investigators found a 29% increase in glucose incremental area and a 22% increase in insulin incremental area in the denervated animals. Gut hormones were not measured. In our study, the incremental increase in glucose was ~50% and in insulin ~59%. This suggests that, in fact, the worsening of glucose tolerance in the study of Pagliassotti et al. (21) was due entirely to portal vein denervation.

Higher plasma glucose and insulin postdenervation could have occurred through several mechanisms: changes in glucose absorption due to modifications in gastrointestinal motility with increased gastric emptying, leading to hyperglycemia and consequent hyperinsulinemia; hepatic and/or peripheral insulin resistance, resulting in higher levels of glucose and insulin; a combination of insulin resistance and compensatory (but inadequate) insulin secretion due to effects on the portal/hepatic-insular axis; and changes in hepatic glucose uptake due to effects on the portal/hepatic-brain axis. To explore some of these mechanisms, we quantified acetaminophen absorption (an indicator of gastric emptying) and gut hormones. In addition, we tested the correlation between glucose and insulin levels, and we investigated whether there was a significant difference in the glucose/insulin response slope, a possible indicator of changes in β-cell responsiveness to insulin. For
both before and after denervation, there was a highly significant correlation between the glucose and insulin levels ($P < 0.0001$). Moreover, there was a statistically significant difference in the slope of the glucose/insulin response, suggesting that the higher glycemia drives the insulin response and that a mechanism originating from the portohepatic/gut area contributes to higher glycemic levels. This hypothesis is supported by the finding that the higher glycemia postdenervation was associated with accelerated gastric emptying. Peak plasma acetylcholine was faster after denervation. The total acetylcholine AUC was not significantly different, but values after 60 min tend to reflect clearance as well as appearance. We can speculate that portal denervation interrupted a positive feedback loop related to enhancement of GLP-1 secretion and that reduction on GLP-1 secretion/action might have resulted in accelerated stomach emptying (19) and a corresponding increase in plasma glucose, followed by a corresponding increase in insulin secretion. Postdenervation total GLP-1 was significantly decreased in the fasting state but, it was not significantly decreased during the oral glucose tolerance test. However, the study was not powered for this outcome, and it is possible that a bigger sample size would settle this question. Clearly, further studies are required to explore this possibility.

To test whether the apparent worsening in oral glucose tolerance postdenervation was due to changes in insulin sensitivity or insulin secretion, we performed IVGTTs, which have a better ability to detect changes in insulin sensitivity or secretion, without interference from factors related to the gut (gastric emptying, absorption, intestinal hormones). We found no changes in insulin sensitivity or secretion or in liver insulin clearance. However, it is worth mentioning that IVGTT-derived insulin sensitivity reflects whole body insulin sensitivity, and not necessarily hepatic glucose uptake/release, which constitutes only a small part of it. Thus, the current working hypothesis is that the worsening of glycemia with denervation might be due to changes in gastric emptying or possibly to glucose/uptake release from the liver, phenomena that are harder to detect in the current experimental conditions.

In conclusion, we confirmed that hepatic portal vein denervation impairs glucose tolerance. However, under our current experimental conditions, hepatic portal vein denervation did not impact exenatide’s effect on glycemia. It is possible that the receptors for glucose and GLP-1 reside in the liver or in the intestine; alternatively, the pharmacological effect of exenatide on peripheral receptors might have obscured a portal effect of relatively small magnitude. Portal vein denervation appeared to negatively impact oral glucose tolerance, although the exact mechanism for this effect remains unknown.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

The authors disclose no conflicts of interest. Dr. Viorica Ionut is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

AUTHOR CONTRIBUTIONS

V.I. and R.N.B. conception and design of research; V.I., A.V.B.C., O.O.W., D.S., M.S.I., J.L.B., M.B., R.E., H.M., and I.A.B. performed experiments; V.I. and D.S. analyzed data; V.I., A.V.B.C., C.M.K., and R.N.B. interpreted results of experiments; V.I. prepared figures; V.I. drafted manuscript; V.I., O.O.W., and R.N.B. edited and revised manuscript; V.I. and R.N.B. approved final version of manuscript.

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