Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs

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Received 8 May 2001; accepted in final form 4 October 2001

Moore, Mary Courtney, Shosuke Satake, Bryan Baranowski, Po-Shuan Hsieh, Doss W. Neal, and Alan D. Cherrington. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am J Physiol Endocrinol Metab 282: E286–E296, 2002; 10.1152/ajpendo.00201.2001.—We tested the hypothesis that the loss of hepatic nerves decreases peripheral insulin sensitivity. Surgical hepatic denervation (DN) was performed in 22 dogs ~16 days before study; 7 dogs (Sham-Sal) had a sham procedure. A euglycemic hyperinsulinemic (1 mU·kg⁻¹·min⁻¹; arterial insulin 35 ± 1 μU/ml in all dogs) clamp was performed in conscious dogs. From 0 to 90 min of the clamp, all dogs received the same treatment; then the DN dogs were divided into three groups. From 90 to 180 min, DN-PoA (n = 7) and DN-PoA (n = 6) groups received acetylcholine 2.5 μg·kg⁻¹·min⁻¹ via peripheral or portal vein, respectively, and DN-Sal (n = 8) received no acetylcholine. During 150–180 min, the Sham-Sal, DN-Sal, DN-PoA, and DN-PoA groups exhibited glucose infusion rates of 12.4 ± 0.8, 9.3 ± 0.8 (P < 0.05 vs. Sham-Sal), 9.1 ± 0.1 (P < 0.05 vs. Sham-Sal), and 12.7 ± 1.6 mg·kg⁻¹·min⁻¹; nonhepatic glucose uptakes of 11.5 ± 0.9, 8.9 ± 0.7 (P < 0.05 vs. Sham-Sal), 8.6 ± 0.9 (P < 0.05 vs. Sham-Sal), and 11.9 ± 1.7 mg·kg⁻¹·min⁻¹; net hindlimb glucose uptakes of 18.4 ± 2.1, 13.7 ± 1.1 (P < 0.05 vs. Sham-Sal), 17.5 ± 1.9, and 16.7 ± 3.2 mg/min; and glucose utilization rates of 14.4 ± 1.4, 10.4 ± 0.8 (P < 0.05 vs. Sham-Sal), 9.8 ± 0.9 (P < 0.05 vs. Sham-Sal), and 13.6 ± 1.8 mg·kg⁻¹·min⁻¹, respectively. DN caused peripheral insulin resistance, and intraportal but not peripheral acetylcholine restored insulin sensitivity.

Hepatic nerves; acetylcholine

Hepatic denervation has been reported to result in peripheral insulin resistance in both rats and cats (49–51). The insulin resistance, which can be localized primarily to skeletal muscle (50), is reversible by intraportal, but not peripheral, infusion of acetylcholine (49) or a nitric oxide donor (35). This has led Lautt (19) to propose that insulin stimulates a hepatic parasympathetic reflex. According to this hypothesis, the reflex involves the release of acetylcholine, which binds to muscarinic receptors and stimulates the production of nitric oxide within the liver. As a result, the liver releases a humoral factor termed the hepatic insulinsensitizing substance, or HISS, that sensitizes skeletal muscle to insulin or has a direct insulin-like action (19). Although this is a very intriguing suggestion, the studies upon which it is based were performed in acutely denervated anesthetized animals under nonsteady-state conditions with the use of bolus injections of insulin. Therefore, the relevance of these findings to stable, conscious animals receiving physiological amounts of insulin remains to be established.

We designed the current studies to test the hypothesis that loss of the hepatic nerves would impair peripheral glucose disposal under carefully controlled hormonal conditions in a physiological model. Specifically, we examined the response of conscious dogs ~16 days after hepatic denervation, utilizing established methods for evaluating insulin sensitivity (36) and a physiologically relevant hyperinsulinemia.

MATERIALS AND METHODS

Animals, Diets, and Experimental Preparation

Studies were carried out on 29 conscious 18-h-fasted adult dogs of either sex with a mean wt of 24 ± 2 kg. The protocol was approved by the Vanderbilt University Medical Center Animal Care Subcommittee. The animals were housed according to American Association for the Accreditation of Laboratory Animal Care International guidelines and fed a diet of meat (Kal-Kan, Vernon, CA) and chow (Purina Lab Canine Diet 5006, Purina Mills, St. Louis, MO) composed of 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight. Approximately 16 days before study, all dogs underwent a laparotomy under general anesthesia. Twenty-two of the dogs underwent complete surgical denervation of the liver, and the other seven dogs underwent a sham procedure, with the hepatic nerves left intact (28). Silicone rubber catheters (Dow Corning, Midland, MI) were inserted in the portal and left common hepatic veins, a splenic and a jejunal vein, and the femoral artery, as previously described (28). Starting at the junction of the left renal vein and the inferior vena cava (IVC), a silicone rubber catheter (0.03 in. ID) was introduced via a trocar and advanced ~4 cm into the renal vein, so that the distal tip was positioned ~1 cm from the hilus. The catheter was secured by...
HEPATIC DENERVATION AND INSULIN SENSITIVITY

Experimental Design

Each experiment consisted of a 100-min equilibration period (−140 to −40 min) and a 40-min basal period (−40 to 0 min), followed by two 90-min experimental periods (0–90 and 90–180 min). At −140 min, a continuous peripheral infusion of indocyanine green dye (ICG; Sigma, St. Louis, MO; 4 μg·kg⁻¹·min⁻¹) was begun to provide confirmation of hepatic vein catheter placement and a second measurement of hepatic blood flow. In addition, a peripheral infusion of somatostatin (0.8 μg·kg⁻¹·min⁻¹; Bachem, Torrance, CA) was begun to suppress endogenous insulin and glucagon secretion. Basal intraportal infusions of insulin (300 μU·kg⁻¹·min⁻¹) and glucagon (0.5 ng·kg⁻¹·min⁻¹) were also begun (both hormones obtained from Eli Lilly, Indianapolis, IN). Plasma glucose concentrations were monitored every 5 min, and the insulin infusion rate was adjusted as necessary to maintain euglycemia. The final alteration in the insulin infusion rate was made ≥30 min before the start of the basal period, and the rate remained unchanged from that point until 0 min. The final insulin infusion rate was significantly lower in the 7 hepatic-denervated dogs than in the 22 hepatic-denervated dogs (227 ± 18 and 283 ± 20 μU·kg⁻¹·min⁻¹, respectively; P < 0.05). At 0 min, the intraportal infusion of insulin was discontinued, and insulin was then infused continuously at 1 μU·kg⁻¹·min⁻¹ via the left lateral saphenous vein. Plasma glucose concentrations were analyzed every 5 min, and glucose was infused via the right cephalic vein as necessary to maintain euglycemia. In addition, 14 of the hepatic-denervated animals received an infusion of acetylcholine at 2.5 μg·kg⁻¹·min⁻¹ between 90 and 180 min, with 7 of the dogs receiving the infusion via peripheral vein (DN-PeA group) and 7 receiving it via the portal vein (DN-PoA). The hepatic-denervated dogs (Sham-Sal group, n = 7) and 8 of the hepatic-denervated animals (DN-Sal group) received saline instead of acetylcholine.

Blood samples (4–9 ml) were obtained from the artery every 10–15 min during the basal and experimental periods, and samples were also taken from the portal, hepatic, iliac, and renal veins every 20 min during the basal period and every 15–30 min during the experimental period. The collection, processing, and analysis of blood samples have been described in detail elsewhere (27). At 180 min, the dogs were killed with an overdose of pentobarbital sodium, and tissue was immediately taken from each liver lobe and freeze-clamped in liquid nitrogen for analysis of norepinephrine concentration (25). The liver norepinephrine concentrations in the hepatic-denervated dogs were <3% of those in normally innervated dogs, indicating virtually complete hepatic denervation. A necropsy was performed to confirm the proper placement of the sampling and infusion catheters.

Calculations

Hepatic blood flow (HBF) was calculated by two methods, ultrasonic flow probes and dye extraction (27). The results obtained with ultrasonic flow probes and ICG were not significantly different. Because the flow probes make it possible to determine the relative proportions of the HBF provided by the hepatic artery and the portal vein, calculations reported in this paper utilize HBF obtained from the flow probes.

Net hepatic balance (NHB) was calculated by the formula NHB = [S]I × HBF − ([S]H × ABF + [S]P × PBF), where [S] is substrate concentration; H, A, and P refer to the hepatic, femoral, and portal vein substrate concentrations, respectively; and HBF, ABF, and PBF refer to total hepatic blood flow, hepatic arterial flow, and portal vein flow, respectively. A negative value indicates net uptake. Net hindlimb balance was calculated as ([S]H−[S]A)×IBF, where [S]H refers to the substrate concentration in the iliac vein and IBF to iliac vein blood flow. Net fractional extraction of glucose by the hindlimb was net hindlimb balance ÷ [G]A × IBF, where [G]A is the glucose concentration in the femoral artery.

Tracer-determined rates of glucose appearance (Ra) and glucose utilization (Ru) were determined by use of a primed, constant infusion of [3-3H]glucose. Data were calculated using a two-compartment model described by Mari (21) with canine parameters as reported by Dobbins et al. (8). Tracer-determined glucose production (endogenous Ru) was calculated as the difference between total Ra and the exogenous glucose infusion rate. The renal balance of [3H]glucose was calculated in a manner similar to the calculation of cold renal glucose balance, by use of plasma [3H]glucose values (dpm/ml) and renal plasma flow. The results were divided by the femoral artery [3H]glucose specific activity (dpm/mg glucose) to convert them to milligrams per kilogram per minute. Renal glucose production was calculated as the sum of the tracer-determined renal glucose uptake and net renal glucose balance.

Data are presented as means ± SE. The trapezoidal rule was used for determination of area under the curve (AUC). SPSS for Windows 10.0 (SPSS, Chicago, IL) was used for statistical analysis. Time course data were analyzed with repeated-measures ANOVA with post hoc analysis by univariate F-tests. ANOVA was used for comparison of AUC values. Results were considered statistically significant at P < 0.05.

RESULTS

This investigation addressed two questions. I Does hepatic denervation result in loss of insulin sensitivity?
in nonhepatic tissues? If so, is skeletal muscle the primary tissue affected? (This is answered by a comparison of Sham-Sal and DN-Sal). 2) If loss of insulin sensitivity occurs with hepatic denervation, can it be corrected by intraportal but not peripheral infusion of acetylcholine? (This is a comparison of DN-PeA and DN-PoA with each other and with the other two groups). The data related to these two questions are presented separately.

**Hormone concentrations.** There were no significant differences in plasma insulin concentrations between Sham-Sal and DN-Sal (Fig. 1). During the experimental periods, the arterial plasma insulin concentrations were 5- to 6-fold basal, and the hepatic sinusoidal plasma concentrations were ~2-fold basal. The basal arterial glucagon concentrations were similar in the two groups, with the concentrations declining ~15% over the course of the experiment.

**Blood flow.** Hepatic ABF during the basal and experimental periods tended to be greater in DN-Sal than in Sham-Sal (7.0 ± 1.2 vs. 5.2 ± 0.5 ml·kg⁻¹·min⁻¹, basal, and 8.2 ± 1.1 vs. 6.6 ± 0.8 ml·kg⁻¹·min⁻¹, experimental, \( P = 0.2 \) for both; data not shown). However, neither PBF nor total HBF was altered by hepatic denervation. PBF averaged 23.4 ± 1.5 and 22.5 ± 1.3 ml·kg⁻¹·min⁻¹ (basal) and 24.2 ± 1.2 and 20.8 ± 1.3 ml·kg⁻¹·min⁻¹ (experimental) in Sham-Sal and DN-Sal, respectively. Total HBF averaged 28.6 ± 1.9 and 29.5 ± 2.2 ml·kg⁻¹·min⁻¹ (basal) and 30.8 ± 1.7 and 29.0 ± 1.9 ml·kg⁻¹·min⁻¹ (experimental) in Sham-Sal and DN-Sal, respectively.

RBF appeared to increase in response to hyperinsulinemia, as demonstrated by an increase (\( P < 0.05 \)) in RBF over basal rates during the experimental period in both groups (10.3 ± 0.9 and 12.2 ± 1.5 ml·kg⁻¹·min⁻¹, basal, and 11.9 ± 0.9 and 13.2 ± 1.7 ml·kg⁻¹·min⁻¹, experimental, in Sham-Sal and DN-Sal, respectively). RBF was not significantly different between the groups at any time. Hindlimb blood flow did not appear to be altered by hepatic denervation or infusion of insulin (22 ± 21 and 223 ± 47 ml/min, basal, and 209 ± 22 and 202 ± 45 ml/min, experimental, in Sham-Sal and DN-Sal, respectively; no significant differences between groups or change over time).

**Glucose metabolism.** Arterial blood glucose concentrations remained basal throughout the experimental period in Sham-Sal and DN-Sal (Fig. 2). Net hepatic glucose output was similar in these groups during the control period (~1.8 mg·kg⁻¹·min⁻¹), and a low rate of net hepatic glucose uptake was evident by the end of the experimental period. There were no significant differences in net hepatic glucose balance (NHGB) between the groups. During the last 30 min of study, the glucose infusion rates averaged 12.4 ± 0.8 and 9.3 ± 0.8 mg·kg⁻¹·min⁻¹, the nonhepatic glucose uptake rates were 11.5 ± 0.9 and 8.9 ± 0.7 mg·kg⁻¹·min⁻¹, and the net hindlimb glucose uptakes were 18.9 ± 2.2 and 13.7 ± 1.1 mg/min in Sham-Sal and DN-Sal, respectively (\( P < 0.05 \) for all parameters, Fig. 3). The net fractional extraction of glucose by the hindlimb had a tendency (\( P = 0.07 \)) to be greater in Sham-Sal than in DN-Sal (0.15 ± 0.03 vs. 0.11 ± 0.01, data not shown).

Endogenous glucose \( R_d \) in both Sham-Sal and DN-Sal declined during the experimental period, reaching rates not different from zero by the end of the study (Table 1). There were no significant differences in glucose \( R_d \) between these two groups at any time, and the change in \( R_d \) between the basal period and the final sampling time did not differ significantly between the groups. Glucose \( R_d \) did not differ significantly between Sham-Sal and DN-Sal either during the basal period or during the first 90 min of the experimental period (Table 1). The two groups differed from 90 min onward, and during the last 30 min, the mean \( R_d \) for the Sham-Sal group was significantly greater than that.
The kidneys did not exhibit significant glucose production at any time in either group. Renal glucose uptake did not differ between Sham-Sal and DN-Sal, and it did not change over time within the groups (data not shown).

**Gluconeogenic precursor and nonesterified fatty acid concentrations and balance data.** The arterial blood lactate concentrations were not significantly different between Sham-Sal and DN-Sal during the basal period, and they did not change significantly in either group as the study progressed (Table 2). Net hepatic lactate output was not significantly different in the two groups during the basal period. Early in the experimental period, it increased by approximately the same amount in both groups, after which it returned to rates no different from basal. Net hindlimb lactate uptake was evident at baseline in both the Sham-Sal and DN-Sal groups, but by the end of the experiment, the two groups exhibited similar rates of net hindlimb lactate release (Table 2). Both Sham-Sal and DN-Sal exhibited net renal uptake of lactate throughout the study, and it did not change significantly over time.

The arterial blood glycerol concentrations declined by ~45% in both Sham-Sal and DN-Sal over the course of the experimental period, and the net hepatic uptake of glycerol fell by a similar percentage (Table 3). Neither parameter differed between the groups at any time. The net hindlimb release of glycerol declined 48% from basal in Sham-Sal and 67% in DN-Sal, but the variability in the measurement precluded achieving statistical significance for the change in either group. Both groups exhibited slight net renal uptake of glycerol that did not change significantly over time in either group.

Arterial plasma nonesterified fatty acid (NEFA) concentrations and net hepatic NEFA uptake were not significantly different between Sham-Sal and DN-Sal during the basal period or throughout the experimental period (Table 4). NEFA concentrations declined
Table 1. Tracer-determined endogenous glucose $R_a$ and $R_d$ in conscious 18-h-fasted dogs with (DN-Sal) or without (Sham-Sal) hepatic denervation

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
<th>120</th>
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<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Endogenous glucose $R_a$, mg·kg$^{-1}$·min$^{-1}$</td>
<td>3.0±0.4</td>
<td>1.2±0.7</td>
<td>1.7±0.3</td>
<td>2.0±0.5</td>
<td>1.1±0.7</td>
<td>0.8±0.7</td>
<td>0.4±0.8</td>
<td>0.1±1.0</td>
<td>0.3±0.9</td>
<td>-0.1±0.8</td>
<td>-0.4±0.5</td>
<td>0.0±0.8</td>
</tr>
<tr>
<td>Sham-Sal</td>
<td>3.0±0.2</td>
<td>2.1±0.2</td>
<td>1.8±0.3</td>
<td>1.4±0.3</td>
<td>1.7±0.2</td>
<td>1.5±0.3</td>
<td>1.3±0.3</td>
<td>1.1±0.3</td>
<td>0.9±0.4</td>
<td>0.3±0.3</td>
<td>0.5±0.3</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>3.0±0.4</td>
<td>6.0±0.8</td>
<td>7.4±0.8</td>
<td>8.8±0.8</td>
<td>10.4±0.8</td>
<td>11.2±0.7</td>
<td>11.9±0.8</td>
<td>12.7±1.2</td>
<td>12.7±1.1</td>
<td>12.7±0.9</td>
<td>13.8±1.2</td>
<td>15.1±1.6</td>
</tr>
<tr>
<td>Glucose $R_d$, mg·kg$^{-1}$·min$^{-1}$</td>
<td>3.0±0.2</td>
<td>5.9±0.8</td>
<td>7.1±0.9</td>
<td>8.0±1.0</td>
<td>8.7±1.0</td>
<td>9.2±0.9*</td>
<td>9.3±0.9*</td>
<td>9.4±0.8*</td>
<td>9.5±0.8*</td>
<td>9.7±0.3*</td>
<td>10.1±0.8*</td>
<td>10.7±0.8*</td>
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</tbody>
</table>

Values are means ± SE. The basal period includes data between −40 and 0 min; $n = 7$ and 8 in hepatic innervated dogs (Sham-Sal) and denervated dogs given no acetylcholine (DN-Sal), respectively. $R_a$ and $R_d$, production and utilization, respectively. There are no significant differences in $R_a$ between groups. All rates of $R_a$ and $R_d$ during the experimental period are significantly ($P < 0.05$) different from corresponding basal rates within the same group. *$P < 0.05$ vs. Sham-Sal.

~80% in both groups, and net hepatic NEFA uptake decreased similarly ($P < 0.05$ for change from basal in both parameters for both groups).

**Does Portal But Not Peripheral Infusion of Acetylcholine Restore Insulin Sensitivity in Hepatic-Denervated Dogs?**

**Hormone concentrations and blood flow.** The arterial and hepatic sinusoidal plasma insulin concentrations and arterial plasma glucagon concentrations in DN-PeA and DN-PoA (Table 5) did not differ from those in Sham-Sal and DN-Sal in any respect. Acetylcholine infusion, particularly via the portal vein, significantly increased hepatic ABF (Δ4.1 ± 1.4 and 8.9 ± 2.4 ml·kg$^{-1}$·min$^{-1}$ during 90–180 min in DN-PeA and DN-PoA, respectively; $P < 0.05$ vs. rates in the same groups during basal period, $P < 0.05$ vs. the 90- to 180-min rates in Sham-Sal and DN-Sal). PBF was unaffected by acetylcholine infusion. RBF increased in response to hyperinsulinemia in DN-PeA and DN-PoA, as it did in Sham-Sal and DN-Sal, but it was not significantly altered by acetylcholine infusion. Hindlimb blood flow did not change with acetylcholine infusion, but it tended to be higher in DN-PeA and DN-PoA than in the other two groups throughout the study.

**Glucose metabolism.** The arterial blood glucose concentrations remained at basal level in DN-PeA and DN-PoA throughout the basal and experimental periods (Table 5), and they did not differ from the concentrations in Sham-Sal and DN-Sal (data not shown). There were no significant differences in NHGB between DN-PeA and DN-PoA during either the basal or the experimental period, and NHGB in the two groups receiving acetylcholine did not differ from NHGB in the Sham-Sal and DN-Sal groups at any time. During the last 30 min of study, the glucose infusion rates averaged 9.1 ± 1.0 and 12.7 ± 1.6 mg·kg$^{-1}$·min$^{-1}$ in DN-PeA and DN-PoA, respectively ($P < 0.05$ for DN-

Table 2. Arterial blood concentrations and net hepatic, hindlimb, and renal balance of lactate in conscious 18-h-fasted dogs with (DN-Sal) or without (Sham-Sal) hepatic denervation

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th>30</th>
<th>60</th>
<th>75</th>
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<th>120</th>
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<th>165</th>
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<tr>
<td>Arterial blood lactate, μmol/l</td>
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<tr>
<td>Sham-Sal</td>
<td>723±129</td>
<td>877±129</td>
<td>899±121</td>
<td>891±111</td>
<td>842±114</td>
<td>848±111</td>
<td>816±105</td>
<td>802±85</td>
<td>876±104</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>757±146</td>
<td>928±106</td>
<td>891±83</td>
<td>871±93</td>
<td>842±88</td>
<td>760±102</td>
<td>723±107</td>
<td>698±100</td>
<td>785±107</td>
</tr>
<tr>
<td>Net hepatic lactate balance, μmol·kg$^{-1}$·min$^{-1}$</td>
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<tr>
<td>Sham-Sal</td>
<td>8.2±5.8</td>
<td>15.9±3.7*</td>
<td>12.1±3.2</td>
<td>11.6±3.7</td>
<td>10.4±3.7</td>
<td>4.3±3.8</td>
<td>5.4±2.9</td>
<td>4.2±3.5</td>
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<tr>
<td>DN-Sal</td>
<td>6.9±3.4</td>
<td>13.0±2.3*</td>
<td>13.0±2.5</td>
<td>10.9±2.0</td>
<td>10.8±2.3</td>
<td>8.5±1.9</td>
<td>7.0±2.0</td>
<td>7.5±2.3</td>
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<tr>
<td>Net hindlimb lactate balance, μmol/min</td>
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<tr>
<td>Sham-Sal</td>
<td>-14.6±10.3</td>
<td>-14.3±9.7</td>
<td>-20.5±8.9</td>
<td>-12.9±7.4</td>
<td>-11.0±10.5</td>
<td>-3.9±5.2</td>
<td>-3.8±6.1</td>
<td>5.7±8.5*</td>
<td>8.1±7.7</td>
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<tr>
<td>DN-Sal</td>
<td>-12.0±7.7</td>
<td>-8.4±7.0</td>
<td>-17.3±3.7</td>
<td>-10.4±4.2</td>
<td>-14.9±4.3</td>
<td>-2.6±2.9</td>
<td>-0.7±3.1*</td>
<td>6.6±2.6*</td>
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<tr>
<td>Net renal lactate balance, μmol·kg$^{-1}$·min$^{-1}$</td>
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<tr>
<td>Sham-Sal</td>
<td>-1.0±0.4</td>
<td>-1.3±0.7</td>
<td>-1.9±0.3</td>
<td>-2.0±0.4</td>
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<tr>
<td>DN-Sal</td>
<td>-1.8±0.5</td>
<td>-1.2±0.4</td>
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<td>-2.5±0.8</td>
<td>-1.3±0.7</td>
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</table>

Values are means ± SE; $n = 7$ and 8 in Sham-Sal and DN-Sal, respectively. Negative values indicate net uptake. There are no significant differences among groups in any parameter. *$P < 0.05$ vs. basal period in the same group.
Table 3. Arterial blood concentrations, net hepatic and renal uptake, and net hindlimb output of glycerol in conscious 18-h-fasted dogs with (DN-Sal) or without (Sham-Sal) hepatic denervation

<table>
<thead>
<tr>
<th></th>
<th>Basal Period</th>
<th>Experimental Period, min</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Arterial blood glycerol, μmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-Sal</td>
<td>69 ± 8</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>67 ± 13</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>Net hepatic glycerol uptake, μmol·kg⁻¹·min⁻¹</td>
<td>1.2 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Sham-Sal</td>
<td>1.3 ± 0.2</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>2.0 ± 1.0</td>
<td>0.3 ± 0.6</td>
</tr>
<tr>
<td>Net hindlimb glycerol release, μmol/min</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Sham-Sal</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>2.1 ± 0.6</td>
<td>0.6 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 and 8 in Sham-Sal and DN-Sal, respectively. There are no significant differences between groups in any parameter. *P < 0.05 vs. basal period in the same group.

PeA vs. Sham-Sal and DN-PoA; not significant (NS) for DN-PoA vs. Sham-Sal; Fig. 4. Nonhepatic glucose uptake averaged 8.6 ± 0.9 and 11.9 ± 1.7 mg·kg⁻¹·min⁻¹ in DN-PeA and DN-PoA, respectively (P < 0.05 for DN-PeA vs. Sham-Sal and DN-PoA; NS for DN-PoA vs. Sham-Sal). Net hindlimb glucose uptake was 17.5 ± 1.9 and 16.7 ± 3.2 mg·min⁻¹ (NS) in DN-PeA and DN-PoA, respectively (NS between these groups and Sham-Sal and DN-Sal; Fig. 4). The net fractional glucose extraction by the hindlimb was 0.10 ± 0.02 and 0.11 ± 0.02 (P = 0.8) in DN-PeA and DN-PoA, respectively (P = 0.07 for DN-PeA vs. Sham-Sal; P = 0.26 for DN-PoA vs. Sham-Sal).

Endogenous glucose R₄ declined similarly in DN-PeA and DN-PoA during the experimental period, and it did not differ from Sham-Sal or DN-Sal at any time. The glucose R₄ was lower (P = 0.05) in DN-PeA than in DN-PoA (9.8 ± 0.9 vs. 13.6 ± 1.8 mg·kg⁻¹·min⁻¹ during the last 30 min of the experiment; P < 0.05 for DN-PeA vs. Sham-Sal; NS for DN-PoA vs. Sham-Sal).

Glucogenic precursor and NEFA concentrations and balance data. The arterial blood concentrations and organ balances of lactate, glycerol, and NEFA did not differ between DN-PeA and DN-PoA at any time, and these groups did not differ from Sham-Sal and DN-Sal in any parameter (data not shown).

DISCUSSION

Hepatic Denervation and Peripheral Insulin Sensitivity

Insulin sensitivity was evaluated by comparing four measures of glucose disposal in sham- and hepatic-denervated dogs during a hyperinsulinemic euglycemic clamp. Between 150 and 180 min, when steady-state conditions existed, hepatic denervation was associated with a 25% reduction in the glucose infusion rate, a 23% reduction in nonhepatic glucose uptake, a 28% reduction in hindlimb glucose uptake, and a 28% reduction in glucose R₄ (P < 0.05 for all parameters). The four methods for evaluating peripheral glucose disposal provide different ways of evaluating insulin sensitivity. The rate of glucose infusion and the glucose R₄ during the clamp reflect whole body glucose utilization. Under these euglycemic conditions and in the presence of only a modest increase in liver sinusoidal insulin, net hepatic glucose uptake was very low, and for the most part whole body glucose uptake was attributable

Table 4. Arterial plasma NEFA and net hepatic NEFA uptake in conscious 18-h-fasted dogs with (DN-Sal) or without (Sham-Sal) hepatic denervation

<table>
<thead>
<tr>
<th></th>
<th>Basal Period</th>
<th>Experimental Period, min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Arterial plasma NEFA, μmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-Sal</td>
<td>575 ± 63</td>
<td>191 ± 50</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>598 ± 88</td>
<td>124 ± 24</td>
</tr>
<tr>
<td>Net hepatic NEFA uptake, μmol·kg⁻¹·min⁻¹</td>
<td>2.2 ± 0.4</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Sham-Sal</td>
<td>1.7 ± 0.4</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>DN-Sal</td>
<td>1.6 ± 0.6</td>
<td>0.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 and 8 in Sham-Sal and DN-Sal, respectively. NEFA, nonesterified fatty acids. Basal values are the means of sampling points between −40 and 0 min. There are no significant differences between groups. All concentrations and uptake rates during the experimental period are significantly (P < 0.05) less than the corresponding values for the same group during the basal period.
Table 5. Arterial and hepatic sinusoidal plasma insulin concentrations, arterial plasma glucagon concentrations, arterial blood glucose concentrations, and net hepatic glucose balance in conscious 18-h-fasted hepatic-denervated dogs receiving acetylcholine infusion via peripheral (DN-PeA) or portal (DN-PoA) vein

<table>
<thead>
<tr>
<th></th>
<th>Basal Period</th>
<th>Experimental Period</th>
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<tbody>
<tr>
<td></td>
<td>−40 to 0 min</td>
<td>0–90 min</td>
</tr>
<tr>
<td>Arterial plasma insulin, μU/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN-PeA</td>
<td>6 ± 1</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>DN-PoA</td>
<td>6 ± 1</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Hepatic sinusoidal plasma insulin, μU/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN-PeA</td>
<td>16 ± 3</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>DN-PoA</td>
<td>16 ± 2</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Arterial plasma glucagon, pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN-PeA</td>
<td>49 ± 8</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>DN-PoA</td>
<td>40 ± 4</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>Arterial blood glucose, mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN-PeA</td>
<td>80 ± 3</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>DN-PoA</td>
<td>81 ± 2</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>Net hepatic glucose balance, mg·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN-PeA</td>
<td>1.6 ± 0.2</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>DN-PoA</td>
<td>1.5 ± 0.4</td>
<td>−0.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7/group. Negative values indicate net uptake. There are no significant differences between these 2 groups, and there are no significant differences for the parameters in this table between these groups and Sham-Sal or DN-Sal.

Fig. 4. Mean rates of glucose infusion (A), net nonhepatic glucose uptake (B), net hindlimb glucose uptake (C), and glucose utilization (Rd, D) during 150–180 min in 18-h-fasted dogs with sham hepatic denervation (Sham-Sal, n = 7) and in 3 groups of hepatic-denervated dogs: one group receiving no acetylcholine (DN-Sal, n = 8), 1 group receiving a peripheral infusion of acetylcholine (DN-PeA, n = 7) from 90 to 180 min, and 1 group receiving a portal infusion of acetylcholine (DN-PoA, n = 7) from 90 to 180 min. Groups marked with different letters are significantly different from one another (P < 0.05).
able [34 ± 2 vs. 35 ± 2 μU/ml in Sham-Sal and DN-Sal, respectively, during the experimental period (Fig. 1)]. This suggests that the difference in the increment in insulin infusion was simply not great enough to result in differing insulin levels, given the normal variation in insulin clearance.

The reduction in insulin-stimulated glucose disposal in the current studies is more subtle than that reported by Xie and Lautt (49, 50) and Xie et al. (51) in anesthetized rats (49–71% reduction in insulin effectiveness in response to intraperitoneal atropine injection or acute hepatic denervation), although it is close to that seen in anesthetized cats (~35% reduction in insulin effectiveness in response to acute hepatic denervation). The differences in the magnitude of the response in our findings and those in rats are probably explained by differences in study conditions (see next few paragraphs) or in the species used.

One difference between the current studies and those of Xie and Lautt (49, 50) was the procedure used to assess insulin sensitivity. The rapid insulin sensitivity test (RIST) employed by Lautt (19) involves the injection of a bolus dose of insulin, resulting in insulin concentrations that are initially supraphysiological, with the amount of glucose required to maintain euglycemia serving as an index of insulin sensitivity. In contrast, we employed the hyperinsulinemic euglycemic clamp, which resulted in only mild-to-moderate levels of hyperinsulinemia at all times. The RIST is not as well established as the more widely accepted hyperinsulinemic euglycemic clamp (36). The initially high levels of insulin could potentially affect the results by producing effects other than those seen with physiological insulin levels, e.g., a central nervous system effect (7, 48) or hemodynamic changes (43).

A second difference in the study design was that Lautt (19) performed his studies in animals that had undergone acute hepatic denervation, whereas our dogs were allowed ~16 days to recover from surgery and were studied only if they appeared to be fully healthy. Uncomplicated elective surgery is associated with insulin resistance in the intraoperative and early postoperative periods in human patients and animal models, with the magnitude of the insulin resistance being determined by the extent of the surgical procedures (2, 39, 41, 44–46). Thus the acute surgical insult could have exacerbated any insulin resistance resulting from the loss of liver nerves. However, Lautt also studied animals that received intraportal injection of atropine, rather than surgical denervation, and found that the degree of insulin resistance was similar in the two treatments (49, 50). Hepatic denervation of the animals after atropine treatment did not produce any further impairment in insulin effectiveness (49, 50). Therefore, the magnitude of the insulin resistance observed in the studies of Lautt and colleagues cannot have resulted only from the stress of the surgical procedure.

A third difference in study procedures was that Xie and Lautt (49, 50) studied pentobarbital-anesthetized rats and cats, whereas we studied conscious dogs. Numerous investigations have found that pentobarbital anesthesia is associated with alterations in glucose metabolism (5, 15, 31). Glucose turnover was decreased 30% in pentobarbital-anesthetized rats compared with conscious rats, with glucose utilization by the soleus and adductor longus muscles being reduced ~75–80% and brain glucose uptake reduced 50% (31). Lang et al. (15) observed a 30–50% reduction in the glucose Rₐ, glucose recycling, and metabolic clearance in pentobarbital-anesthetized rats, but they found these reductions only in animals allowed to become hypothermic during anesthesia. However, Clark et al. (5) noted changes in glucose kinetics in animals under pentobarbital anesthesia even when the animals were maintained euthermic. These changes included a low basal glucose Rₐ, a blunting of the insulin-stimulated fall in Rₐ, and a 33% reduction in whole body glucose utilization. Consequently, the anesthesia may have accentuated the insulin resistance observed by Xie and colleagues in hepatic-denervated animal models (49–51).

A fourth difference between our study conditions and those of Xie and colleagues (49–51), which may have impacted on the magnitude of the response, is our use of somatostatin to suppress pancreatic hormone secretion. This allowed us to be sure that there would be no subtle differences in insulin and glucagon among the groups that would confound our results. Xie and colleagues (49–51) did not measure the plasma glucagon or insulin response in most studies. During a study in cats, they did measure insulin, once before and once after insulin injection, and the insulin concentration appeared to increase less in the denervated cats than in the control animals (50). Differences in insulin clearance, which would alter insulin levels, would confound data interpretation. Thus the use of somatostatin in our studies was necessary to prevent any differences in pancreatic hormones among the groups. Both in vitro (3) and in vivo (1, 3, 4, 26) data indicate that somatostatin should not alter glucose utilization in the overnight-fasted, unstressed dogs used in this investigation. Moreover, any potential effect of somatostatin should have been the same in all groups, because all of the animals received it.

Finally, work from the laboratory of Lautt and associates suggests that fasting decreases the response to HISS. Overnight-fasted rats required significantly less glucose to maintain euglycemia during the RIST than overnight-fasted rats that had been allowed to feed ad libitum for 1 h before study (20). Insulin sensitivity, as determined by the RIST, decreased in rats over the course of a 6-h fast (18). This contrasts with earlier work that demonstrated no difference in whole body insulin sensitivity among rats fasted for 6, 24, or 48 h (14). It is noteworthy that the studies of the effect of fasting on the response to HISS were performed on overnight-fasted rats (20), which are very different in metabolic status from overnight (18 h)-fasted dogs. The dogs, which are accustomed to being fed one meal daily, require nearly 18 h for absorption of the chow and meat diet (6). In the dog, hepatic glycogen stores are high after 18 h of fasting (11), and the liver is a net
present in liver can improve glucose tolerance (23). In contrast, hepatic glycogen reserves in the rat are markedly depleted after an overnight fast, and the liver is a net consumer of lactate (9, 17, 30, 42). Thus an overnight fast in the dog is unlikely to blunt the HISS response nearly as much as it would in the rat.

The insulin resistance in our model appears to be linked to the lack of hepatic nerves per se, rather than to some secondary effect of the denervation procedure. Clearly, the insulin resistance observed was not a consequence of increased stress in the hepatic-denervated dogs. Plasma cortisol concentrations, a sensitive indicator of stress, were not different between hepatic-denervated and sham-denervated dogs (47). In the current investigation, arterial plasma norepinephrine concentrations were $132 \pm 30$ and $185 \pm 12$ pg/ml, and arterial plasma epinephrine levels were $80 \pm 20$ and $131 \pm 21$ pg/ml in hepatic- and sham-denervated dogs, respectively (NS for both parameters, data not shown).

The presence of a liver factor that improves peripheral glucose uptake has long been suspected. In 1954, Lang et al. (16) examined glucose disposal in intact and eviscerated dogs infused with insulin (10 U·kg$^{-1}$·h$^{-1}$) and varying dosages of glucose. Hindlimb glucose uptake was reduced 60% in eviscerated dogs, and cross-perfusion of an eviscerated dog with blood from an intact dog reversed the suppression of glucose uptake. The investigators suggested that a humoral factor released by the liver was necessary for maximal rates of glucose uptake, and insulin (2 U·kg$^{-1}$·h$^{-1}$) were simultaneously infused with either saline or an extract derived from the supernatant of homogenized liver from normal rats. The rats receiving saline exhibited a steady rise in blood glucose over the 4 h of infusion, whereas those receiving the liver extract exhibited no increase in blood glucose (23). Glucose uptake in an isolated perfused rat hindquarter preparation was increased 48–60% by joint perfusion with an isolated liver, and addition of crude liver extract or deproteinized liver or kidney extract to the perfusate enhanced glucose uptake in a similar fashion (33). Thus evidence supports a relationship between the liver (and/or other visceral organs) and peripheral insulin sensitivity.

The role of liver nerves in peripheral insulin sensitivity has been less well established. In humans, cirrhosis of the liver, a disorder associated with a high incidence of autonomic (particularly parasympathetic) dysfunction (12), is also associated with peripheral insulin resistance (22, 29, 32, 37, 38). This peripheral insulin resistance is related to a defect in nonoxidative glucose disposal, i.e., muscle glycogen storage (22, 29, 37, 38). On the other hand, insulin sensitivity was restored to normal in nondiabetic cirrhotic individuals that underwent hepatic transplantation and thus had little or no extrinsic liver innervation (22, 32). These findings imply that the extrinsic hepatic innervation is not essential for an effect of the liver on insulin-stimulated glucose uptake in nonhepatic tissues. Nevertheless, the current data, in conjunction with those from Lautt’s laboratory (49–51), strongly suggest that the liver nerves are required for full insulin sensitivity. A decrease in muscle insulin sensitivity is observed in animal models of hepatic denervation, even though there may not be agreement about the magnitude of the defect.

Net hindlimb uptake of glucose was significantly greater in Sham-Sal than in DN-Sal, consistent with a suppression of insulin-stimulated skeletal muscle glucose uptake in the hepatic-denervated dog, as previously observed in the cat (50). Lipolysis and reesterification of lipids did not appear to be altered in the hepatic-denervated dogs. Arterial concentrations of glycerol and NEFA and their net hepatic uptakes did not differ significantly between Sham-Sal and DN-Sal. Likewise, net hindlimb glycerol release was not different in the two groups. Therefore, adipose tissue did not appear to be a site of insulin resistance. Similarly, the change from basal in NHGB and the decline in glucose $R_{d}$ over the course of the experiment were very similar between the groups, suggesting that the liver, like adipose tissue, was not a major site of insulin resistance in the hepatic-denervated dogs. Thus there was no evidence that tissues other than skeletal muscle were responsible for the reduction in insulin sensitivity accompanying hepatic denervation.

**Effect of Acetylcholine Infusion on Insulin-Mediated Glucose Disposal**

The second major question addressed by these investigations was whether acetylcholine infusion could restore insulin sensitivity to the peripheral tissues in the hepatic-denervated dog. Clearly, intraportal infusion of acetylcholine in the hepatic-denervated dogs restored the glucose infusion rate, nonhepatic glucose uptake, hindlimb glucose uptake, and glucose $R_{d}$ to values no different from those in Sham-Sal (Fig. 4). On the other hand, peripheral infusion of acetylcholine resulted in rates of glucose infusion, nonhepatic glucose uptake, and glucose $R_{d}$ that were not significantly different from those evident in DN-Sal. Net hindlimb glucose uptake in DN-PEa was not significantly different from that in either DN-Sal or Sham-Sal. The apparent similarity in hindlimb glucose uptake between Sham-Sal and DN-PEa was probably a consequence of an increased glucose load to the hindlimb in DN-PEa vs. Sham-Sal ($184 \pm 19$ vs. $143 \pm 24$ mg/min, $P = 0.2$) resulting from the higher hindlimb blood flow in that group. Net hindlimb fractional glucose extraction during the last 30 min of study was 32% greater in Sham-Sal than in DN-PEa ($0.15 \pm 0.03$ vs. $0.10 \pm 0.02$, $P = 0.076$).

In conclusion, chronic hepatic denervation was associated with a modest decrease in insulin sensitivity in conscious dogs during a hyperinsulinemic euglycemic clamp. Intraportal, but not peripheral, infusion of acetylcholine restored insulin sensitivity in hepatic-denervated dogs.
erved dogs. In agreement with the findings of Xie and Lautt (50), the skeletal muscle appeared to be the tissue most responsible for the loss of insulin sensitivity. Although the loss of insulin sensitivity with hepatic denervation appears to be modest in the presence of physiological hyperinsulinemia, it may help to explain the presence of peripheral insulin resistance in human disease states, such as cirrhosis and diabetes, that are associated with autonomic dysfunction.

The authors appreciate the support of Wanda Sneed, Angelina Penaloza, and Eric Allen in the Hormone Core Laboratory of Vanderbilt Diabetes Research and Training Center, as well as the assistance of Margaret Converse and Jon Hastings.

These studies were supported by the National Institute of Diabe-
tes and Digestive and Kidney Diseases Grants DK-18243 and DK-
20593 (Diabetes Research and Training Center).

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