Chronic hepatic artery ligation does not prevent liver from differentiating portal vs. peripheral glucose delivery

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Chronic hepatic artery ligation does not prevent liver from differentiating portal vs. peripheral glucose delivery. Am J Physiol Endocrinol Metab 285: E845–E853, 2003. First published May 28, 2003; 10.1152/ajpendo.00130.2003. —Infusion of glucose into the hepatic artery blocks the stimulatory effect of the "portal signal" on net hepatic glucose uptake (NHGU) during portal glucose delivery. We hypothesized that hepatic artery ligation (HAL) would result in enhanced NHGU during portal glucose infusion because the arterial glucose concentration would be perceived as lower than that in the portal vein. Fourteen dogs underwent HAL ~16 days before study. Conscious 42-h-fasted dogs received somatostatin, intraportal insulin, and glucagon infusions at fourfold basal and at basal rates, respectively, and peripheral glucose infusion to create hyperglycemia. After 90 min (period 1), seven dogs (HALpo) received intraportal glucose (3.8 mg·kg⁻¹·min⁻¹) and seven (HALpu) continued to receive only peripheral glucose for 90 min (period 2). These two groups were compared with nine non-HAL control dogs (control) treated as were HALpo. During period 2, the arterial plasma insulin concentrations (24 ± 3, 20 ± 1, and 24 ± 2 μU/ml) and hepatic glucose loads (39.1 ± 2.5, 43.8 ± 2.9, and 37.7 ± 3.7 mg·kg⁻¹·min⁻¹) were not different in HALpo, HALpu, and control, respectively. HALpo exhibited greater (P < 0.05) NHGU than HALpu and control (3.1 ± 0.3, 2.0 ± 0.4, and 2.0 ± 0.1 mg·kg⁻¹·min⁻¹, respectively). Net hepatic carbon retention was approximately twofold greater (P < 0.05) in HALpo than in HALpu and control. NHGU and net hepatic glycogen synthesis during peripheral glucose infusion were not enhanced by HAL. Even though there exists an intrahepatic arterial reference site for the portal vein glucose concentration, the failure of HAL to result in enhanced NHGU during peripheral glucose infusion suggests the existence of one or more comparison sites outside the liver.

NET HEPATIC GLUCOSE UPTAKE (NHGU) is enhanced approximately twofold when glucose is delivered via the hepatic portal vein vs. a peripheral vein (29, 34). This “portal signal” appears to be neurally mediated (1). When glucose is delivered via the portal vein, the portal venous glucose concentration is greater than the glucose concentration in the arterial circulation, i.e., a negative arterial-portal (A-P) gradient exists. The rate of NHGU is related not to the portal vein glucose level per se (29) but instead to the magnitude of the negative A-P glucose gradient, up to approximately −18 mg/dl (35). This implies that the portal vein glucose concentration is compared with the arterial concentration at some site, triggering a neural response that includes enhancement of NHGU. The most likely reference sites for comparison with the portal vein glucose concentration would appear to be arteries perfusing the brain (specifically the hypothalamus; see Refs. 33 and 38), the carotid bodies (6, 7, 36), and the liver itself (14, 44).

Hsieh et al. (17, 18) conducted two studies in an attempt to identify the arterial reference site for the portal signal. In the first, they infused glucose in the portal vein to create the portal signal while simultaneously infusing either saline or glucose bilaterally in the vertebral and carotid arteries. Glucose was infused in the head at a rate calculated to prevent any negative A-P glucose gradient from existing between the brain and the portal vein. NHGU was no different during head infusions of saline and glucose (which would ablate any negative A-P glucose gradient between the portal vein and the brain), indicating that the head arterial glucose concentration was not the primary reference site for the sensing of the portal signal (17). Moreover, the carotid bodies are perfused by branches from the vertebral and carotid arteries (12) so that in all likelihood there would have been no gradient between the glucose concentrations in the portal vein and carotid bodies during glucose infusion in the head. Thus the carotid bodies are unlikely to be the reference site for comparison of the portal vein glucose concentration.

On the other hand, when glucose was infused simultaneously in the portal vein and the hepatic artery so that there was no negative intrahepatic A-P glucose gradient, there was no enhancement of NHGU, relative to the rate observed during peripheral glucose infusion (18). These data support a primary role for the hepatic artery as the reference site for the portal signal. We hypothesized that, if this is the case, ligation of the hepatic artery would result in enhancement of

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undergoing hepatic artery ligation before surgery and 12 days postoperatively in dogs.

MATERIALS AND METHODS

Animals and surgical procedures. Studies were carried out on 14 conscious 42-h-fasted mongrel dogs of either sex with a mean weight of 24 ± 1 kg. Diet and housing were as previously described (34), and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

Approximately 16 days before study, each dog underwent a laparotomy for insertion of sampling catheters in the left common hepatic vein, the portal vein, and a femoral artery (E846 HEPATIC ARTERY LIGATION AND GLUCOSE UPTAKE). After completion of the experiment, microsphere injection was carried out with HAL (until it was established that the technique resulted in complete obliteration of the arterial blood supply to the liver, with no revascularization). In addition, in two of the HAL dogs, 5 ml blood were drawn from the portal vein and the hepatic vein sampling catheters immediately before the experimental period to monitor the glucose level. Arterial, portal vein, and hepatic vein blood gases were drawn to evaluate hepatic oxygen delivery and extraction; the samples were immediately placed on ice and were analyzed promptly using a blood gas analyzer (Radiometer, Copenhagen, Denmark). The total amount of blood withdrawn did not exceed 15% of the animal’s blood volume, and two volumes of normal saline were infused for each volume of blood withdrawn.

After completion of the experiment, microsphere injection and analysis were carried out with a modified version of the procedure of Simonson et al. (43). The animal was anesthe- sized with pentobarbital sodium, and the abdominal cavity was opened. The left atrium was accessed via a 3-cm incision between the third and fourth ribs, and green microspheres (15.5 ± 0.4 μm diameter; E-Z Trac Ultraspheres; Interactive Medical Technology, North Hollywood, CA) suspended in paraformaldehyde and saline solution (total volume 4 ml) were injected into the left atrium, followed by 2 ml of saline. Later (2 min), two 2 × 2-cm tissue samples were taken from each of three liver lobes (one dorsal and one ventral sample from each). The animal was then killed with an overdose of pentobarbital sodium. Hepatic tissue was taken in five dogs with HAL (until it was established that the technique resulted in complete obliteration of the arterial blood supply to the liver, with no revascularization). In addition, in two of the HAL dogs, 5 ml blood were drawn from the portal vein and the hepatic vein sampling catheters immediately before the liver samples were removed to allow examination of the blood for microspheres. Two dogs with intact hepatic arteries under- went the microsphere procedures in a manner identical to the dogs with HAL. To further validate that there had been no revascularization of the liver, we carefully skeletonized the liver of three HAL dogs at necropsy and determined that there was no apparent blood supply other than the portal vein.

Table 1. Serum values for indicators of liver function before surgery and 12 days postoperatively in dogs undergoing hepatic artery ligation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Range</th>
<th>Preoperative</th>
<th>Postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin, g/dl</td>
<td>2.7–4.4</td>
<td>3.1 ± 0.1</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Bilirubin, mg/dl</td>
<td>0.10–0.30</td>
<td>0.20 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>AP, U/l</td>
<td>5–121</td>
<td>39 ± 7</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>12–118</td>
<td>41 ± 4</td>
<td>58 ± 15</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>15–66</td>
<td>22 ± 1</td>
<td>23 ± 2</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 12 dogs for pre- and postoperative values. Bilirubin, total bilirubin; AP, alkaline phosphatase; ALT, alanine-leucyl transferase; AST, aspartate-serine transferase.
Processing and analysis of samples. Plasma glucose, hematocrit, blood lactate, and plasma insulin and glucagon were determined as described previously (34) on the samples taken throughout the studies.

The postmicrosphere blood samples were placed in tubes containing sodium EDTA and centrifuged, and the plasma was discarded. The liver tissue samples were minced finely. All reagents used in microsphere analysis were purchased as concentrates from Interactive Medical Technology and were diluted as directed by the manufacturer. The samples were processed and analyzed as described by Hale et al. (15). Briefly, the blood cells and weighed tissues were digested to homogeneity, the homogenate was centrifuged, and the pellet was washed and resuspended three times. An aliquot of the final solution was placed in a hemocytometer (Improved Neubauer, Hauser Scientific, Horsham, PA), and microspheres in the sample were counted.

In three HAL dogs and three control dogs, norepinephrine was determined on tissues removed from all seven liver lobes, as previously described (27), to assess the extent of inadvertent denervation of liver tissue during the HAL. Liver weights at necropsy were indistinguishable between control and hepatic artery-ligated dogs (24.4 ± 1.0 and 24.9 ± 2.0 g/kg body wt, respectively).

Calculations and data analysis. Hepatic blood flow (HBF) was measured by an ultrasonic flowmeter and by use of ICG extraction. The results obtained with Transonic flow probes (when functional) and ICG were not significantly different, but all data reported here were calculated by using the ICG-determined flows because failure of 7 of the 14 Transonic probes in the HAL dogs. It should be noted that the use of ICG did not require an assumption about the distribution of flow between the hepatic artery and portal vein, since the hepatic artery was ligated. In control dogs, ICG-derived flows were used for data analysis to be consistent with the other groups. In that group, the hepatic artery was assumed to supply 20% of HBF in the basal period and 25% in the experimental period, based on the distribution determined by the flow probes in the same dogs.

The recovery of PAH across the liver was measured as reported previously (29). An experiment was defined as having poor mixing (and was excluded from the database) if poor mixing was observed in more than two of the five timed points in the experimental period. Twenty dogs were studied (8 in the HALpo group and 12 in the HALle group). One was not included in the database because of a failure of sampling catheters, one was excluded because of a poor glucose clamp, and four were omitted because of poor mixing of the portal infused with portal blood. In the animals included in the database, the ratio of recovered to infused PAH was 1.1 ± 0.1 in both the portal and the hepatic veins (with a ratio of 1.0 representing ideal mixing).

The rate of substrate delivery to the liver, or hepatic substrate load, was calculated by a direct (D) method as Loadin, (D) = (|S|P × PBF), where |S| is the substrate concentration, P denotes the portal vein, and BF refers to blood flow. For control dogs, the calculation was similar: |S|A × ABF = |S|P × PBF, where A refers to the artery. To minimize any potential errors arising from incomplete mixing of infused substrate with the blood, hepatic glucose load during intraportal glucose infusion was also calculated by an indirect (I) method: Loadin, (I) = (|G|P × PBF) + GIRpv − GUG, where |G| is the blood glucose concentration, GIRpv is the intraportal glucose infusion rate, and GUG is the uptake of glucose by the gastrointestinal tract during the period of peripheral glucose infusion (29). The load of a substrate exiting the liver was calculated as Loadout = (|S|H × HBF), where H refers to the hepatic vein.

Direct and indirect methods were used in calculation of net hepatic balance (NHB) of glucose. The direct calculation was NHB(D) = Loadin, (D) − Loadout, (D), and the indirect calculation was NHB(I) = Loadin, (I) − Loadout, (I). A negative value indicates net uptake by the liver. The results did not differ significantly between the two methods. To minimize any impact of incomplete mixing of the glucose infused with the portal vein blood, the results shown in this report were based on the indirect calculation. Only the direct calculation was employed for substrates other than glucose. Nonhepatic glucose uptake was calculated as the glucose infusion rate minus NHB of glucose. Net fractional substrate extraction by the liver was calculated directly and indirectly as the ratio of NHB to Loadin. Net hepatic carbon retention, an indicator of the carbon available for hepatic glycogen synthesis, was the difference between NHGU and net hepatic lactate output. This ignores the contribution arising from hepatic uptake of gluconeogenic amino acids, as well as the need to supply glucose for hepatic oxidation. However, these two rates are quantitatively similar and offsetting (39).

Hepatic oxygen delivery (DO2) was calculated as SaO2 Loadin × 1.34 × (0.33 × hematocrit), where SaO2 refers to the oxygen saturation (11). Hepatic oxygen extraction was calculated as for fractional extraction of substrates.

For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using correction factors (the mean of the ratio of the blood to the plasma concentration) previously established in our laboratory for the dog (18, 19, 34).

Statistical analysis. All data are presented as means ± SE. Time course data were analyzed with repeated-measures ANOVA, and univariate F-tests were used for post hoc comparisons. Statistical significance was accepted at P < 0.05.

RESULTS

Dearterialization of the liver, hepatic oxygenation, and hepatic norepinephrine. Microspheres with a 15.5-μm diameter were used because the diameter of canine mesenteric capillaries has been measured at 7.4 ± 1.4 (SD) μm (24). Thus the microspheres were sufficiently large to ensure that virtually all were trapped in the capillary beds, and any microspheres reaching the liver tissue or vasculature must have been carried there by arterial flow. In two control dogs examined, the liver tissue contained 2,519 and 2,516 microspheres/g tissue. Of the five HAL animals undergoing microsphere injection, the liver of one animal contained 125 microspheres/g tissue (≈5% of the normal controls) and the other four livers contained no microspheres [mean for the group: 25 ± 28 (SE) microspheres/g liver]. In the two HAL dogs in which postmicrosphere blood samples were drawn, there were no microspheres in the portal or hepatic vein blood, indicating that the hepatic sinusoids were not receiving arterial blood. Thus the dearterialization procedure appeared virtually complete, with no evidence of revascularization during the postoperative period.

Hepatic oxygen delivery was not diminished significantly in HAL dogs (4.19 ± 1.3 vs. 4.58 ± 0.9 ml·kg⁻¹·min⁻¹ in HAL vs. control dogs, respectively). Hepatic oxygen extraction was ≈25% in both HAL and control dogs (data not shown).
Hepatic concentrations of norepinephrine were not different in the three HAL and three control dogs in which these were examined (572 ± 86 and 650 ± 74 ng/g liver, respectively), indicating that hepatic sympathetic innervation was intact in the HAL animals.

**Insulin and glucagon data.** The arterial plasma insulin concentrations in HALpe and HALpo, respectively, averaged 6 ± 1 and 6 ± 2 μU/ml in the basal period, 21 ± 2 and 18 ± 2 μU/ml in period 1, and 26 ± 3 and 20 ± 1 μU/ml during period 2 (Fig. 1). The portal vein plasma insulin concentrations in HALpe and HALpo were 21 ± 5 and 21 ± 8, 92 ± 14 and 97 ± 8, and 109 ± 10 and 106 ± 17 μU/ml during the basal period and periods 1 and 2, respectively. There were no differences between the two groups, and the insulin levels in HAL dogs did not differ from those in the control animals.

The arterial and portal vein plasma glucagon concentrations remained basal throughout the experiment in all groups (Table 2).

**Glucose concentrations, HBF, and hepatic glucose load.** The arterial blood glucose concentrations in HALpe and HALpo were 76 ± 1 and 82 ± 2 mg/dl during the basal period, increasing to 164 ± 3 and 170 ± 3 mg/dl during period 1 (Fig. 2). During period 2, the arterial blood glucose concentration in HALpe was 166 ± 3 mg/dl, but it was reduced to 153 ± 4 mg/dl in HALpo to keep the hepatic glucose load constant during infusion of glucose in the portal vein. In the control group, the arterial blood glucose concentration increased from 78 ± 2 to 152 ± 3 mg/dl (P < 0.05 vs. HALpe during period 2). Portal vein blood glucose concentrations within each group were the same throughout periods 1 and 2 (167 ± 8, 164 ± 3, and 149 ± 3 mg/dl in HALpe, HALpo, and control, respectively). The portal vein blood glucose level in the control group was significantly (P < 0.05) lower than those in the HAL groups because both the hepatic artery and the portal veins were contributing to the hepatic glucose load, and it was the glucose load that was controlled.

HBF (ICG-determined portal vein flow) in HALpe averaged 27 ± 3 ml·kg⁻¹·min⁻¹ in the basal period, tended to decrease after that (25 ± 1 and 24 ± 1 ml·kg⁻¹·min⁻¹ in periods 1 and 2, respectively; data not shown). Blood flow in HALpo averaged 32 ± 2 ml·kg⁻¹·min⁻¹ in the basal period and 27 ± 2 ml·kg⁻¹·min⁻¹ during both periods 1 and 2 (P = 0.4 vs. HALpe; P < 0.05 vs. basal period in HALpo). ICG-determined total HBF in the control group was not different from that observed in the HAL groups (27 ± 2 ml·kg⁻¹·min⁻¹ in the basal period and period 1 and 28 ± 2 ml·kg⁻¹·min⁻¹ in period 2). Portal vein blood flow in the control group was 22 ± 2, 20 ± 1, and 21 ± 2 ml·kg⁻¹·min⁻¹ in the basal period and periods 1 and 2, respectively. Thus it appears that portal vein flow increased in the HAL dogs to compensate for the loss of hepatic artery flow.

The hepatic glucose loads during the basal period and periods 1 and 2 were 20.2 ± 1.7, 39.9 ± 1.8, and 39.1 ± 2.5 mg·kg⁻¹·min⁻¹, respectively, in HALpe; 25.7 ± 1.8, 43.8 ± 3.0, and 43.8 ± 2.9 μmol·kg⁻¹·min⁻¹ in HALpo, and 19.9 ± 3.0, 37.3 ± 3.7, and 37.7 ± 3.7 mg·kg⁻¹·min⁻¹ in control [not significant (NS) among groups].

**Net hepatic glucose balance and fractional extraction.** All groups exhibited net hepatic glucose output during the basal period (1.8 ± 0.2, 1.9 ± 0.2, and 1.5 ± 0.2 mg·kg⁻¹·min⁻¹ in HALpe, HALpo, and control, respectively; Fig. 3). They all shifted to NHGU within 30 min of initiating the hyperglycemic-hyperinsulinemic clamp. During period 1, NHGU averaged 1.3 ± 0.4, 1.7 ± 0.3, and 1.5 ± 0.3 mg·kg⁻¹·min⁻¹ in HALpe, HALpo, and control, respectively.
HALpo, and control, respectively (NS among groups). During period 2, the mean NHGU was 2.0 ± 0.4, 3.2 ± 0.4, and 2.0 ± 0.1 mg·kg⁻¹·min⁻¹ in HALpe, HALpo, and control, respectively, using the indirect calculation (P < 0.05 between HALpo and the other groups). With the use of the direct calculation, NHGU in HALpo during period 2 was 3.6 ± 0.5 mg·kg⁻¹·min⁻¹. The net fractional extraction of glucose by the liver during period 1 was not significantly different among the groups. During period 2, fractional extraction tended to be less (P < 0.1) in HALpe and control than in HALpo (0.05 ± 0.01 and 0.05 ± 0.00 vs. 0.07 ± 0.01, respectively).

The total glucose infusion rate (peripheral plus portal if applicable) did not differ significantly among the groups (11.9 ± 1.5, 10.8 ± 1.6, and 9.0 ± 1.3 mg·kg⁻¹·min⁻¹ in HALpe, HALpo, and control, respectively, during period 2; Fig. 4). Nonhepatic glucose uptake during period 2 tended to be greater in HALpe than in HALpo and control (9.9 ± 1.5 vs. 7.8 ± 1.6 and 6.9 ± 1.2 mg·kg⁻¹·min⁻¹, respectively, P = 0.2 for HALpe vs. the other groups).

Lactate concentrations and net hepatic lactate balance. Arterial blood lactate concentrations increased from 425 ± 40 to a maximum of 1,163 ± 119 µmol/l in HALpe and from 554 ± 101 to 1,190 ± 119 µmol/l in HALpo (Fig. 5). Both groups exhibited net hepatic lactate uptake during the basal period, and both shifted to net hepatic lactate release during period 1, with a peak (6.9 ± 2.3 and 10.5 ± 1.2 µmol·kg⁻¹·min⁻¹ in HALpe and HALpo, respectively; NS between groups) occurring at 45–60 min. Net hepatic lactate output diminished until, by the end of period 2, both groups exhibited a low rate of net hepatic lactate uptake (−0.1 ± 1.2 and −0.8 ± 1.6 µmol·kg⁻¹·min⁻¹ in HALpe and HALpo, respectively). Neither arterial blood lactate nor net hepatic lactate balance in the two HAL groups differed significantly from those observed in control dogs.

Net hepatic carbon retention. Net hepatic carbon retention (Fig. 5) during period 2 was significantly greater in HALpo than in the HALpe and control groups (3.1 ± 0.4 vs. 2.1 ± 0.4 and 1.9 ± 0.2 mg glucose equivalents·kg⁻¹·min⁻¹; P < 0.05).

DISCUSSION

When insulin and glucagon concentrations and the hepatic glucose load are kept constant during portal glucose infusion, NHGU is linearly related to the mag-
magnitude of the gradient between the arterial and portal vein glucose concentrations over the physiological range (35). This indicates that the portal vein glucose concentration must be compared with the glucose concentration in an arterial reference site. Recently, Hsieh et al. (18) demonstrated that the hepatic artery glucose level could serve as a reference value for comparison with the portal vein glucose concentration. In those studies, glucose was infused in the portal vein with concomitant hepatic artery infusions of saline or glucose. The enhancement of NHGU during portal vein glucose infusion was blocked when glucose as opposed to saline was infused in the hepatic artery (2.5 ± 0.8 vs. 4.3 ± 0.6 mg·kg⁻¹·min⁻¹, hepatic artery glucose infusion vs. saline infusion, respectively, P < 0.05). Moreover, during simultaneous hepatic artery and portal vein glucose infusion, the rate of NHGU was not different from that observed during peripheral glucose infusion alone (2.3 ± 0.4 mg·kg⁻¹·min⁻¹). Consistent with these findings, no glucose uptake was evident in bivascularly perfused rat livers when the hepatic arterial and portal vein glucose concentrations were maintained at 10 and 5 mM, respectively, or when the glucose concentrations in the two blood vessels were equal (10 mM); however, when the arterial and portal vein glucose concentrations were 5 and 10 mM, respectively, substantial rates of hepatic glucose uptake were achieved (44).

It should be noted that Horikawa et al. (16) reported that NHGU did not differ among three infusion periods when conscious dogs received glucose infusions via the portal vein at 10 mg·kg⁻¹·min⁻¹, simultaneously via the portal vein and hepatic artery at 5 mg·kg⁻¹·min⁻¹ each, and via the hepatic artery at 10 mg·kg⁻¹·min⁻¹. Their study suggests that a glucose gradient between the hepatic artery and portal vein is not necessary to stimulate NHGU, but their report has numerous limitations. These include failure to assess mixing of the glucose infused with the blood in the portal vein or hepatic artery; high hepatic artery and portal vein glucose infusion rates, which increase the noise-to-signal ratio and impede accurate assessment of NHGU because the exogenous glucose makes a major contribution to hepatic glucose load; and a significant rise in hepatic plasma flow during hepatic artery infusion, a phenomenon observed under conditions in which nitric oxide concentrations can be anticipated to be elevated. The latter is the case during intraportal ACh infusion (28, 42) and sepsis (8, 37, 45), but it did not occur in our hands during portal vein or hepatic artery glucose infusion (18, 29, 34). Elevation of HBF in itself may enhance NHGU (42). Thus conclusions regarding the role of the hepatic artery glucose level in generating the portal signal are difficult to draw from the data of Horikawa et al. (16).

Even though Hsieh et al. (18) demonstrated that the hepatic artery can serve as a reference site for comparison with the portal vein glucose level, our current data are consistent with the existence of at least one other sensing site. NHGU in response to glucose delivered via a peripheral vein was identical in the dogs with

![Fig. 4. Glucose infusion rate and nonhepatic glucose uptake in dogs that had undergone hepatic artery ligation. Study conditions were as described in the legend to Fig. 1. There were no significant differences among the groups.](image)

![Fig. 5. Arterial blood lactate, net hepatic lactate balance, and net hepatic carbon retention in dogs that had undergone hepatic artery ligation. Study conditions were as described in the legend to Fig. 1. *P < 0.05 for HALpo vs. the other 2 groups.](image)
ablation of the hepatic arterial blood supply and in dogs with intact hepatic arteries. Importantly, in comparison with peripheral glucose delivery, portal delivery of glucose to dogs lacking a hepatic artery still resulted in significant enhancement of NHGU (change from period 1 = 0.7 ± 0.4 vs. 1.5 ± 0.2 mg·kg⁻¹·min⁻¹ in HALₚ and HALₚₚ, respectively, P < 0.05), as well as a tendency toward suppression of nonhepatic glucose uptake. Suppression of nonhepatic (primarily skeletal muscle) glucose uptake is normally a consequence of the portal signal in dogs with intact hepatic arteries (13, 19). Taken together, these findings indicate that the portal signal exhibited effects on both the liver and the nonhepatic tissues in dogs with HAL. It should be noted that the enhancement of NHGU during portal glucose infusion in HAL dogs was modest in comparison with our previous findings in normal dogs. Dogs with intact hepatic arteries usually exhibit a doubling of NHGU or an increase of ∼2–2.5 mg·kg⁻¹·min⁻¹ during portal glucose delivery compared with dogs receiving peripheral glucose infusion (17, 18, 29, 34). The apparent modest enhancement of NHGU by portal glucose delivery in hepatic artery-ligated dogs might be related to the nature of glucose sensing in the hepatic region or to alterations in intrahepatic circulation.

First, in regard to glucose sensing, it is known that the afferent firing rate in the hepatic branch of the vagus is inversely related to the portal vein glucose concentration (31). However, it has not been established whether the suppression of afferent signaling is purely dependent on the portal vein glucose concentration or whether the signal actually transmits a “referred” glucose value (i.e., the rate of firing could be determined not by the portal vein glucose concentration per se but instead by an intrahepatic comparison of the portal vein and hepatic artery concentrations). If the afferent firing rate in the vagus is determined solely by the portal vein glucose concentration, then our previous work (18) suggests that there must also be a neural signal from the hepatic artery under normal conditions. The hepatic artery is well innervated, just as the portal vein is (9, 21), and hepatic artery nerves apparently bring about an increase in pancreatic islet blood flow during portal glucose infusion (10). The role of hepatic artery innervation in the response to portal glucose delivery has not been explored in depth, however. If hepatic artery sensors normally signal the brain regarding glycemic concentrations, it follows that HAL necessitated reliance on glucosensors in other parts of the body [e.g., the brain (41)] that are either not as efficient as those in the hepatic artery or not coupled as closely to signals from the hepatoporal sensor. The alternative scenario (i.e., afferent signaling in the vagus represents a referenced intrahepatic glucose concentration, and there is no specific hepatic artery signal) would imply that the unusually large intrahepatic A-P glucose gradient (i.e., 0 vs. 163 mg/dl) produced by HAL partially attenuated NHGU. A-P gradients larger than those normally encountered physiologically do not suppress NHGU in dogs with intact hepatic arteries (32, 35). No studies have been done with gradients as large as −163 mg/dl, however, so a bell-shaped response remains possible. A second likely explanation for the blunting of the response to the portal signal in the HALₚ dogs may lie in the importance of the hepatic artery in regulation of hepatic circulation (4, 5). It has been proposed that the architecture of the liver allows shunting of portal vein blood away from the areas of hepatic glucose uptake (zone III), and the presence of hepatic artery flow decreases intrahepatic shunting and thus stimulates glucose uptake (5). Nevertheless, the fact that the HAL dogs responded qualitatively, even if not completely quantitatively, to both peripheral and portal glucose infusion implies that redundant sensing sites are normally operational or that chronic adaptation to the loss of the hepatic artery allowed other sensing sites to become evident, even though the redundant site or sites may not be as effective as the intrahepatic site.

This raises the question of the location(s) of an additional reference site or sites. The brain, once again, emerges as a likely candidate. In particular, glucose-sensitive neurons are known to exist in the lateral hypothalamus (33). Hsieh et al. (17) determined, during acute studies using bilateral infusion of glucose in the vertebral and carotid arteries to prevent any negative A-P glucose gradient between the brain and the portal vein, that the brain is not the primary reference site. Nevertheless, studies of glucose perfusion of isolated rat brain, liver, and combined brain and liver preparations strongly suggest a cooperative relationship between the brain and the liver in regulation of glucose uptake (2, 3). Perfusate glucose concentrations did not change during perfusion of the brain alone or during univascular (portal vein) perfusion of the liver with a recirculating system. However, the glucose in the perfusate fell from 200 to 45–60 mg/dl during the combined organ perfusion, although whether the brain and/or the liver extracted the glucose was not determined (3).

Glucose-sensing neurons responding both to hyperglycemia and hypoglycemia have been identified in the myenteric plexus (23, 46), and evidence exists for a neurogenic gastroinsular axis with possible glucose-sensing capabilities in the short gastric veins (30). Although it is not likely that these findings could be related to neural signaling during portal vein glucose infusions, since neither the gastric veins nor the intestines would have been contacted by the infusate, they do indicate that, under usual postprandial conditions, neural signals are likely to arise from splanchnic sites outside the liver. Thus the potential exists for an arterial reference site to be present within the splanchnic bed but outside the liver. Nevertheless, the site of HAL in the current investigation was the same as the implantation site of the hepatic artery infusion catheter in the studies of Hsieh et al. (18). Those investigators were able to block the effects of the portal signal by infusing glucose in the hepatic artery (18), indicating that the primary arterial reference site for the portal vein glucose concentration is at the location of the
hepatic artery infusion catheter and the HAL or distal to that point, i.e., within the liver itself.

The HALs were carefully carried out to avoid as much as possible the removal of the numerous nerves that run along the course of the hepatic artery. We have previously found that surgical hepatic denervation (resulting in hepatic norepinephrine levels <3% of those in normally innervated dogs) results in a blunting of NHGU in response to the portal signal, and, conversely, a greater than normal rate of NHGU during peripheral glucose infusion (1). Functionally, it is clear that HAL did not enhance NHGU during peripheral glucose infusion (in comparison with NHGU exhibited by the control dogs), and it did not prevent a significant enhancement of NHGU during portal glucose infusion. Moreover, norepinephrine levels in the liver lobes of three HAL animals were 88% (P > 0.05) of those evident in control dogs that did not undergo HAL.

The liver is metabolically zoned so that the periporal region contains a higher concentration of enzymes active in glycogenolysis and gluconeogenesis, and the perivenous region contains more enzymes related to glycolysis and glycogen storage, a phenomenon related to the relative oxygenation of the tissues (20). In the HAL dogs, the oxygen delivery to the perivenous region was reduced slightly (not significantly), because this region no longer received mixed blood from the sinusoids. Thus one might expect the dogs with HAL to demonstrate alterations in glycolytic activity and glycogen storage. However, net hepatic lactate output, an indicator of glycolysis, was no different in the HAL dogs than in controls. Moreover, net hepatic carbon retention, reflecting the carbon available for glycogen synthesis, did not differ between HAL and control dogs and was significantly (~2-fold) greater in HAL than in control dogs. Thus the decrease in oxygen did not appear to produce changes in functional outcomes. This is likely because oxygenation was not reduced significantly in the HAL dogs, probably because of the enhancement of portal vein blood flow. In addition, the threshold oxygen concentrations for normal glycolytic and glycogen synthetic activity are well below those observed in the dogs with HAL (20).

Nonhepatic glucose uptake in both HAL and HAL was greater than might be expected. This might be interpreted as indicating that HAL in some way stimulates nonhepatic glucose uptake, although we have no explanation of how such stimulation might occur. It is more likely that the failure of nonhepatic uptake in HAL to be significantly suppressed, in comparison with HAL, was related to the slight blunting of NHGU in that group. We have previously emphasized the reciprocal nature of NHGU and nonhepatic glucose uptake, i.e., when NHGU is increased, nonhepatic glucose uptake is reciprocally decreased, and vice versa (26). How this precise reciprocal interaction is achieved is unclear, but neural (25) or humoral (22) mechanisms are both possible. Nonhepatic glucose uptake during period tended to be higher in HAL than in control dogs, a finding likely related to the difference in arterial blood glucose between the two groups during that period.

In summary, HAL did not appear to alter the response to peripheral glucose infusion, although it may have slightly truncated the response to portal glucose delivery. In addition, HAL did not alter metabolism of glucose via the glycogen synthetic or glycolytic pathways in a measurable way. These findings suggest the presence of one or more reference sites for comparison of portal vein glucose concentrations, in addition to the hepatic artery.

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DISCLOSURES

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REFERENCES


