Importance of the hepatic arterial glucose level in generation of the portal signal in conscious dogs

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Hsieh, Po-Shiuwan, Mary Courtney Moore, Doss W. Neal, and Alan D. Cherrington. Importance of the hepatic arterial glucose level in generation of the portal signal in conscious dogs. Am J Physiol Endocrinol Metab 279: E284–E292, 2000.—The aim of this study was to determine whether the elimination of the hepatic arterial-portal (A-P) glucose gradient would alter the effects of portal glucose delivery on hepatic or peripheral glucose uptake. Three groups of 42-h-fasted conscious dogs (n = 7/group) were studied. After a 40-min basal period, somatostatin was infused peripherally along with intraportal insulin (7.2 pmol·kg⁻¹·min⁻¹) and glucagon (0.65 ng·kg⁻¹·min⁻¹). In test period 1 (90 min), glucose was infused into a portal vein to double the hepatic glucose load (HGL) in all groups. In test period 2 (90 min) of the control group (CONT), saline was infused intraportally; in the other two groups, glucose was infused intraportally (22.2 μmol·kg⁻¹·min⁻¹). In the second group (PD), saline was simultaneously infused into the hepatic artery; in the third group (PD+HAD), glucose was infused into the hepatic artery to eliminate the negative hepatic A-P glucose gradient. HGL was twofold basal in each test period. Net hepatic glucose uptake (NHGU) was 10.1 ± 2.2 and 12.8 ± 2.1 vs. 11.5 ± 1.6 and 23.8 ± 3.3* vs. 9.0 ± 2.4 and 13.8 ± 4.2 μmol·kg⁻¹·min⁻¹ in the two periods of CONT, PD, and PD+HAD, respectively (*P < 0.05 vs. same test period in PD and PD+HAD). NHGU was 28.9 ± 1.2 and 39.5 ± 4.3 vs. 26.5 ± 3.7 and 24.5 ± 3.7* vs. 36.1 ± 3.8 and 53.3 ± 8.5 μmol·kg⁻¹·min⁻¹ in the first and second periods of CONT, PD, and PD+HAD, respectively (*P < 0.05 vs. same test period in PD and PD+HAD). Thus the increment in NHGU and decrement in extrahepatic glucose uptake caused by the portal signal were significantly reduced by hepatic arterial glucose infusion. These results suggest that the hepatic arterial glucose level plays an important role in generation of the effect of portal glucose delivery on glucose uptake by liver and muscle.

THE ROUTE OF GLUCOSE DELIVERY is one of the key determinants of net hepatic glucose uptake (NHGU), with NHGU being enhanced two- to threefold during infusion of glucose into the portal vein vs. a peripheral vein (11). The ability of intraportal glucose delivery to increase NHGU is not dictated by the absolute glucose concentration in the portal vein but is instead a function of the magnitude of the negative arterial-portal (A-P) glucose gradient. It seems likely, therefore, that after portal glucose delivery, the portal glucose level is sensed and compared with the arterial glucose level, which is simultaneously monitored at some as yet undetermined site. Three possible arterial reference sites have been suggested: the hepatic artery (6, 24), the arterial blood supply of the hypothalamus (4), and the arterial blood reaching the carotid bodies (2, 3).

Using isolated perfused rat liver, Gardemann et al. (6) and Stumpel and Jungermann (24) showed that a negative glucose gradient between the hepatic artery and the portal vein could be transformed into a metabolic signal locally within the liver and that the action of acetylcholine on the hepatocyte seemed to be involved in this signal transduction. Recently, Horikawa et al. (7) reported that both portal vein and hepatic arterial glucose infusion stimulated NHGU in the conscious dog. These authors went on to suggest that glucose sensors within the liver, rather than the portal vein, are involved in the augmentation of NHGU.

Data also exist to support the contention that the portal glucose level is compared with an arterial glucose level sensed outside the liver. Results from Matsuhisa et al. (9) suggested that minimizing the glucose gradient between the portal vein and the central nervous system diminished hepatic glucose uptake. Our previous study (8) overcame many of the problems associated with their study design, however, and failed to confirm their results. In our study, there was no diminution of NHGU during portal glucose delivery when glucose was infused simultaneously into the carotid and vertebral arteries to maintain the brain isoglycemic with the portal vein (8). Other studies (2, 3) have shown that changes in the blood glucose concentration within the carotid body can affect glucose homeostasis. This implies that the carotid bodies provide another potential

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reference site for arterial glucose sensing. However, infusion of glucose into the carotid and vertebral arteries, as in our earlier study (8), would also alter the glucose level in the carotid bodies. Our earlier data, therefore, do not support carotid body involvement in the operation of the portal signal.

The aim of the present study was to clarify this issue by determining whether elimination of the hepatic A-P venous glucose gradient within the liver would alter the changes in net hepatic and peripheral glucose uptake induced by portal glucose delivery in conscious dogs.

METHODS

Animals and surgical procedures. Studies were carried out on twenty-one 42-h-fasted conscious mongrel dogs of either sex weighing 21–28 kg. All animals were maintained on a diet of meat (Kal-Kan, Vernon, CA) and chow (Purina Lab Canine Diet no. 5006; Purina Mills, St. Louis, MO) composed of 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight. The protocol was approved by the Vanderbilt University Medical Center Animal Care Committee, and the animals were housed according to the guidelines of the American Association for the Accreditation of Laboratory Animal Care International. Approximately 16 days before the study, each dog underwent surgery under general anesthesia for placement of sampling catheters into a hepatic vein, the portal vein, and a femoral artery and infusion of glucose into the carotid and vertebral arteries. The distal end of the gastroduodenal artery was then fused to suppress endogenous insulin and glucagon secretion. In the first experimental period, glucose was infused only through a peripheral vein to double the glucose load to the liver in all groups. In the control study (CONT, n = 7) the conditions established in the first test period were continued throughout the second experimental period. During the second experimental period of the other two groups, glucose was infused intraportally (22.2 μmol·kg⁻¹·min⁻¹). In one group, saline was concurrently infused into the hepatic artery (PD, n = 7), but in the other group, glucose was concurrently infused into the hepatic artery (8.0 ± 5 μmol·kg⁻¹·min⁻¹) to eliminate the glucose gradient between the portal vein and the hepatic artery (PD+HAD, n = 7).

The peripheral glucose infusion rate was modified as required to maintain the hepatic glucose load equal to twofold basal in each protocol. Dextrose, 20 and 5%, was used for the portal and hepatic arterial glucose infusions, respectively, and p-aminohippuric acid (PAH; Sigma Chemicals, St. Louis, MO) was added to the infusates to assess mixing of the infused glucose with blood in the portal and hepatic veins, as described previously (11, 18). Blood sampling was performed as previously described (8).

Processing and analysis of samples. Plasma glucose was assayed by the glucose oxidase method with a Beckman glucose analyzer (Fullerton, CA). Plasma insulin and glucagon concentrations were determined by RIA, as previously described (8). Blood glucose and blood lactate levels were determined by fluorometric enzymatic assays on perchloric acid-treated samples, as previously described (8). PAH was also measured in perchloric acid-deproteinized blood (11, 18).

Calculations. When substrates are infused into the portal vein, the possibility of poor mixing with the blood in the laminar flow of the portal circulation is of concern. In addition, multiple branching patterns of the hepatic artery in the dog (21) raise concern about the mixing of arterially delivered PAH by the time the PD group, we used the equation

\[
\left([PAH]_P - [PAH]_A\right) \times PBF = \text{portal PAH infusion rate}
\]

where A and P represent the femoral arterial and portal venous blood concentrations, and PBF represents portal blood flow.

The mixing of the infused with blood by the time it reached the hepatic vein was assessed in a similar fashion. In the PD group, we used the equation

\[
\left([PAH]_P - [PAH]_A\right) \times HBF = \text{portal PAH infusion rate}
\]

whereas in the PD+HAD group, we used the equation

\[
\left([PAH]_P - [PAH]_A\right) \times HBF = \text{total PAH infusion rate}
\]

in portal vein and hepatic artery

where HBF represents hepatic blood flow.

In the PD+HAD group, we used an indirect (1) approach to assessing the mixing in the hepatic artery, because we could not sample hepatic arterial blood. To increase our ability to assess the mixing of arterially delivered PAH by the time the arterial blood reached the hepatic vein, we infused PAH at a higher rate into the hepatic artery than into the portal vein (0.37 ± 0.03 vs. 0.23 ± 0.01 mg·kg⁻¹·min⁻¹). Mixing of the
hepatic arterial infusate within the liver in the PD+HAD group was assessed using the equation
\[
([\text{PAH}]_{\text{H}} - [\text{PAH}]_{\text{A}}) \times \text{HBF} = ([\text{PAH}]_{\text{P}} - [\text{PAH}]_{\text{H}}) \times \text{PBF}
\]
+ hepatic arterial PAH infusion rate

This assumes that mixing of portally delivered PAH is similar in the portal and hepatic veins, which is usually the case. Samples were considered statistically unmixed (95% confidence that mixing did not occur) if hepatic or portal vein recovery of PAH was 40% greater or less than the actual amount of PAH infused (11, 18). An experiment was excluded from the database if poor mixing was observed at more than one of four time points in the portal glucose infusion period or if the average mixing in a dog was <80% or >120% of the expected value. In PD, 5 of 12 dogs studied were excluded because of poor mixing; in PD+HAD, 6 of 13 dogs studied were excluded because of poor mixing in the portal vein and/or the hepatic artery. In the dogs retained in the database, the average ratio of PAH recovery in the portal vein to the intraportal PAH infusion rate was 0.9 ± 0.1 in both PD and PD+HAD, with a ratio of 1.0 representing perfect mixing. The ratio of PAH recovery in the hepatic vein to the PAH infusion rate was 0.8 ± 0.1 and 0.9 ± 0.1 in PD and PD+HAD, respectively. The recovery of PAH infused in the hepatic artery was 1.0 ± 0.1 during the hepatic arterial glucose infusion period in the PD+HAD group. When a dog was retained in the database, all of the data points were used, whether they were mixed or not, because mixing errors occur randomly.

The mean results for HBF obtained with ultrasonic flow probes and ICG, respectively, in the two study periods for each group were not significantly different. The data shown in the figures are those obtained with the flow probes, because their use did not require an assumption regarding the distribution of arterial and portal contribution to hepatic blood flow. Plasma glucose values were converted to whole blood glucose values by use of a correction factor, as previously described (18).

The rate of substrate delivery to the liver, or hepatic substrate load, was calculated by a direct (D) method as
\[
\text{load}_{\text{in}} (D) = ([\text{S}]_{\text{H}} \times \text{ABF}) + ([\text{S}]_{\text{P}} \times \text{PBF})
\]
where [S] is the substrate concentration, and ABF refers to blood flow through the hepatic artery. A similar method was used to calculate the hepatic sinusoidal insulin and glucagon concentrations
\[
[\text{HS}]_{\text{H}} = ([\text{H}]_{\text{H}} \times \text{ABF}) + ([\text{H}]_{\text{P}} \times \text{PBF}) \div \text{(ABF + PBF)}
\]
where [H] is the hormone concentration, and HS refers to hepatic sinusoid. To minimize any potential errors arising from either incomplete mixing of glucose during intraportal glucose infusion or a lack of precise measurement of the distribution of hepatic blood flow, the hepatic glucose load was also calculated by an indirect method
\[
\text{load}_{\text{in}} (I) = (\text{G} \times \text{HBF}) + \text{GIR}_{\text{P}} + \text{GIR}_{\text{HA}} - \text{GUG}
\]
where G is the blood glucose concentration, GIR_{P} is the intraportal glucose infusion rate, and GUG is the uptake of glucose from the blood by the gastrointestinal tract, calculated on the basis of the previously described relationship between the arterial blood glucose concentration and GUG (11, 18).

The load of a substrate exiting the liver was calculated as
\[
\text{load}_{\text{out}} = [\text{S}]_{\text{H}} \times \text{HBF}
\]

Direct and indirect methods were used in calculation of net hepatic balance (NHB). The direct calculation was: NHB = load_{out} - load_{in} (D). The indirect calculation was: NHB = load_{out} - load_{in} (I). The data in the figures represent those calculated by the indirect method of net hepatic glucose balance (NHGB) during the portal glucose infusion periods so that we would be consistent with our previous publications; nevertheless, the mean values were not significantly different, regardless of the method used in calculation. Lactate balance was calculated by the direct method. Net fractional substrate extraction by the liver was calculated as the ratio of NHB (I) to load_{in} (I). Nonhepatic glucose uptake (non-HGU) was calculated by subtracting the rate of NHGU (I) from the total glucose infusión rate. The net hepatic balance of glucose equivalents was calculated as the sum of the balances of NHGB (I) and lactate when the latter had been converted to glucose equivalents. This calculation serves as an indicator of the carbon used for glycogen deposition; however, it ignores carbon derived from gluconeogenic precursor uptake (≥3 μmol·kg^{-1}·min^{-1}) and glucose used for oxidation (≥1.5 μmol·kg^{-1}·min^{-1}), which tend to offset each other.

The calculation of hepatic arterial glucose infusion rate was based on our desire to maintain the hepatic arterial glucose level at a value slightly above the portal glucose level

<table>
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<tr>
<th>portal glucose infusion rate,</th>
<th>mg·kg^{-1}·min^{-1}</th>
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<tr>
<th>portal plasma flow, ml·kg^{-1}·min^{-1}</th>
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\[
\text{hepatic arterial glucose infusion rate, mg·kg^{-1}·min^{-1}} \leq \frac{\text{hepatic arterial plasma flow, ml·kg^{-1}·min^{-1}}}{\text{hepatic arterial glucose infusion rate, mg·kg^{-1}·min^{-1}}}
\]

In the calculation of the hepatic arterial glucose infusion rate, the hepatic arterial and portal blood flows were measured by Transonic flow probes. Our goal was to maintain the positive A-P glucose gradient that exists in the presence of peripheral glucose delivery.

Data are presented as means ± SE. SYSTAT (SYSTAT, Evanston, IL) was used for statistical analysis. The time course data were analyzed by repeated-measures ANOVA with post hoc analysis and univariate F-tests. Results were considered statistically significant at P < 0.05.

RESULTS

Plasma insulin and glucagon concentrations. Arterial (data not shown) and liver sinusoidal insulin concentrations rose nearly three- to fourfold in all groups. Glucagon levels, on the other hand, remained basal. Neither hormone differed between groups (Fig. 1).

Blood glucose levels, the A-P glucose gradient and hepatic blood flow. Peripheral glucose infusion in the first test period doubled the blood glucose levels (Fig. 2) such that the arterial, portal venous, and hepatic venous glucose levels were not significantly different (NS) among the three groups. In the second test period, the arterial blood glucose level was slightly higher in CONT than in the other two groups (9.3 ± 0.2* vs. 8.6 ± 0.2 and 8.3 ± 0.2 mM, *P < 0.05 vs. PD and PD+HAD, respectively). The portal and hepatic glucose levels, on the other hand, were indistinguishable in the three groups. In PD, intraportal glucose infusion switched the A-P blood glucose gradient from 0.10 ± 0.04 (period 1) to −0.75 ± 0.08 mM and thereby pre-
Groups except in the latter test period when HBF was maintained in similar rates of NHGU in all three groups (11.5 ± 1.6, 9.0 ± 2.3, and 10.1 ± 2.2 μmol·kg⁻¹·min⁻¹ in PD, PD+HAD, and CONT, respectively). Peripheral glucose infusion in the presence of hyperinsulinemia resulted in similar rates of NHGU in all three groups (268 ± 29 and 264 ± 24 μmol·kg⁻¹·min⁻¹) between the two test periods in CONT. The HGLs in the other two groups were similar, although the HGL rose slightly in period 2 in these groups (252 ± 19 and 281 ± 18 vs. 233 ± 19 and 278 ± 21 μmol·kg⁻¹·min⁻¹ in PD and PD+HAD; *P < 0.05 vs. period 1; Fig. 4, top). Net hepatic glucose balance (NHGB, i.e., net hepatic glucose output and NHGU) is shown in Fig. 4 (bottom). In the basal period, net hepatic glucose output did not differ between PD, PD+HAD, and CONT (9.7 ± 1.1, 9.7 ± 1.6, and 9.3 ± 1.7 μmol·kg⁻¹·min⁻¹, respectively). Peripheral glucose infusion in the presence of hyperinsulinemia resulted in similar rates of NHGU in all three groups (11.5 ± 1.6, 9.0 ± 2.3, and 10.1 ± 2.2 μmol·kg⁻¹·min⁻¹ in PD, PD+HAD, and CONT, respectively). During the latter experimental period, NHGU increased to 23.8 ± 3.3 μmol·kg⁻¹·min⁻¹ in PD (Δ12.4 ± 3.2 μmol·kg⁻¹·min⁻¹, P < 0.05), to 13.8 ± 4.2 μmol·kg⁻¹·min⁻¹ in PD+HAD (Δ4.9 ± 2.4 μmol·kg⁻¹·min⁻¹), and to 12.8 ± 2.1 μmol·kg⁻¹·min⁻¹ in CONT (Δ2.7 ± 1.5 μmol·kg⁻¹·min⁻¹, NS). NHGU did not differ between PD+HAD and CONT at any time, indicating that elimination of the glucose difference between the hepatic artery and the portal vein markedly reduced the effect of portal glucose delivery on NHGU (Fig. 4, bottom).

When the data were analyzed with D (rather than I), NHGU increased to 20.5 ± 2.9 μmol·kg⁻¹·min⁻¹ in PD (Δ9.1 ± 2.4 μmol·kg⁻¹·min⁻¹, P < 0.05), to 8.4 ± 2.7 μmol·kg⁻¹·min⁻¹ in PD+HAD (Δ0.3 ± 0.7 μmol·kg⁻¹·min⁻¹, NS), and to 12.8 ± 2.1 μmol·kg⁻¹·min⁻¹ in CONT (Δ2.7 ± 1.5 μmol·kg⁻¹·min⁻¹, NS).

Net hepatic fractional extraction of glucose (NHFEG; Fig. 5) showed a similar trend. NHFEG values were

**Fig. 1.** Calculated plasma insulin (top) and glucagon (bottom) levels in the hepatic sinusoid in 42-h-fasted conscious dogs during the basal and two experimental periods in PD (portal vein glucose delivery), PD+HAD (portal vein and hepatic artery glucose delivery), and CONT (control) groups (n = 7/group). SRIF, somatostatin; PO+HA Glucose, intraportal and hepatic arterial glucose infusion. There are no significant differences between groups.
0.047 ± 0.008 and 0.092 ± 0.016 vs. 0.045 ± 0.012 and 0.051 ± 0.016 vs. 0.039 ± 0.007 and 0.051 ± 0.009 in the two test periods of PD, PD+HAD, and CONT, respectively (*P < 0.05 vs. period 1). In the latter experimental period, the increment of NHFEG induced by the portal signal was completely suppressed by hepatic arterial glucose infusion. NHFEG did not differ between PD₁HAD and CONT.

Nonhepatic glucose uptake. Peripheral glucose infusion during period 1 resulted in nonhepatic glucose uptake (non-HGU; Fig. 6) of 26.3 ± 3.7, 36.1 ± 3.8, and 28.9 ± 1.2 μmol·kg⁻¹·min⁻¹ in PD, PD+HAD, and CONT, respectively. In period 2, non-HGU was 24.5 ± 3.7, 53.3 ± 8.5, and 39.5 ± 4.3 μmol·kg⁻¹·min⁻¹ in PD, PD+HAD, and CONT, respectively. Non-HGU rose by 10.5 ± 4.2 μmol·kg⁻¹·min⁻¹ in period 2 of CONT. In PD, non-HGU fell by 1.7 ± 4.7 μmol·kg⁻¹·min⁻¹ in PD₁HAD vs. 0.045 ± 0.012 and 0.051 ± 0.009 in the two test periods of PD, PD+HAD, and CONT, respectively (*P, 0.05 vs. period 1). In the latter experimental period, the increment of NHFEG induced by the portal signal was completely suppressed by hepatic arterial glucose infusion. NHFEG did not differ between PD₁HAD and CONT.

The total glucose infusion rate increased moderately (20–50%) between periods 1 and 2 in each protocol (37.7 ± 3.5 and 48.4 ± 3.1 vs. 44.7 ± 4.3 and 67.4 ± 6.8 vs. 39.0 ± 2.2 and 52.3 ± 3.7 μmol·kg⁻¹·min⁻¹ in PD, PD+HAD, and CONT, respectively; data not shown).

Net hepatic lactate balance. In response to peripheral glucose infusion, net hepatic lactate balance switched from uptake (10.8 ± 1.7, 8.7 ± 1.3 and 5.9 ± 2.2 μmol·kg⁻¹·min⁻¹) to output (4.4 ± 1.3, 3.6 ± 2.4 and 5.8 ± 1.8 μmol·kg⁻¹·min⁻¹) in the first test period of PD, PD+HAD, and CONT, respectively. Net hepatic lactate output fell slightly in the latter experimental period.
period but was not significantly different among the three groups (Table 1). Blood lactate levels in the femoral artery, portal vein, and hepatic vein were also not different among the three groups (data not shown).

Net hepatic balance of glucose equivalents. The net balance of glucose equivalents across the liver represents the combination of glucose and lactate balance (after the latter is converted to glucose equivalents) and serves as an index of glycogen deposition. The net balance of glucose equivalents across the liver switched from output (4.0 ± 1.3, 5.4 ± 1.7, and 9.0 ± 1.7 μmol·kg⁻¹·min⁻¹) to uptake (9.2 ± 1.6, 6.9 ± 1.8, and 7.2 ± 1.8 μmol·kg⁻¹·min⁻¹) in PD, PD+HAD, and CONT, respectively, in response to peripheral glucose infusion (Fig. 7). In the latter experimental period, the uptakes of glucose equivalents in the three groups were 22.1 ± 3.2, *13.8 ± 4.0, and 10.9 ± 1.6 μmol·kg⁻¹·min⁻¹, respectively. Hepatic arterial glucose infusion markedly reduced the stimulatory effect of the portal signal on glycogen deposition (*P < 0.05 vs. the other two groups).

DISCUSSION

Previous studies have demonstrated that the portal signal is an important component of the metabolic response to feeding (4, 11, 12, 18). We have established that a negative A-P gradient is necessary for the initiation of the portal signal (19). However, the reference glucose concentration against which the portal glucose

Table 1. Net hepatic lactate balance during basal and two experimental periods in PD+HAD, PD and CONT groups of 42-h-fasted conscious dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Basal</th>
<th>Test 1</th>
<th>Test 2</th>
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<tr>
<td>PD+HAD</td>
<td></td>
<td>-8.7 ± 1.3</td>
<td>3.6 ± 2.4</td>
<td>0.0 ± 1.9</td>
</tr>
<tr>
<td>PD</td>
<td></td>
<td>-10.8 ± 1.7</td>
<td>4.4 ± 1.3</td>
<td>3.4 ± 1.3</td>
</tr>
<tr>
<td>CONT</td>
<td></td>
<td>-5.9 ± 2.2</td>
<td>5.8 ± 1.8</td>
<td>3.9 ± 1.5</td>
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</table>

Values are means ± SE. The data during the three periods are as follows: Basal, mean of two samples taken at -40 and 0 min; Test 1, mean of 3 samples taken between 60 and 90 min; Test 2, mean of 3 samples taken between 120 and 180 min. Negative values indicate net uptake. PD+HAD, portal vein + hepatic artery glucose delivery; PD, portal vein glucose delivery; CONT, control. No significant differences were observed among groups.

Fig. 5. Net hepatic fractional extraction in 42-h-fasted conscious dogs during the basal and two experimental periods in PD, PD+HAD, and CONT (n = 7/group). †P < 0.05, CONT vs. PD at this time point; ‡P < 0.05, PD+HAD vs. PD at this time point.

Fig. 6. Nonhepatic glucose uptake in 42-h-fasted conscious dogs during the basal and two experimental periods in PD, PD+HAD, and CONT (n = 7/group). †P < 0.05, CONT vs. PD at this time point; *P < 0.05, PD+HAD vs. PD at this time point.

Fig. 7. Net hepatic balance of glucose equivalents in 42-h-fasted conscious dogs during the basal and two experimental periods in PD, PD+HAD, and CONT (n = 7/group). The net balance of glucose equivalents is calculated as the sum of the net balance of glucose and lactate, the latter converted to glucose equivalents. †P < 0.05, CONT vs. PD at this time point; *P < 0.05, PD+HAD vs. PD at this time point.
concentration is compared is still not clear. This study sought to determine whether elimination of a negative glucose gradient between the hepatic artery and the portal vein would alter the effects of the portal signal on glucose uptake by the liver and/or peripheral tissues. The present data demonstrate that the effects of the portal signal on hepatic and peripheral glucose uptake and on the stimulation of hepatic glycogen storage were markedly reduced by eliminating the hepatic A-P glucose gradient within the liver in conscious dogs.

The results from the present study suggest that the portal signal is generated within the liver itself. They are consistent with the work of Gardemann et al. (6) and Stumpel and Jungermann (24) in the perfused liver. However, the intrahepatic mechanism by which the portal signal is generated and the way in which it is transduced into an effect on hepatic glucose uptake are still unknown. Stumpel et al. suggested that the parasympathetic nervous system may be involved. In their studies with isolated perfused rat livers, the increment in insulin-stimulated glucose uptake induced by a negative A-P glucose gradient was markedly reduced by either portal or arterial delivery of atropine. In addition, they showed that the effect of a negative A-P glucose gradient on NHGU could be mimicked by addition of acetylcholine to either the portal or arterial perfusate. The combination of acetylcholine and adrenergic blockers infused intraportally in the conscious dog rapidly stimulated NHGU in the presence of hyperinsulinemia and hyperglycemia, but adrenergic blockade alone did not alter NHGU (23). The above change in NHGU was consistent with those induced by the portal signal in terms of both time and magnitude. These observations suggest that the muscarinic nervous system within the liver is somehow involved in the generation of the hepatic effect of the portal signal and/or in the transduction of its effect into a biological action.

Neurophysiological data indicate that glucose-responsive neurons in specific hypothalamic regions and in the dorsomedial medulla oblongata modulate glucose metabolism in some organs (liver and pancreas) through autonomic efferent nerves (16, 17). However, only one study (9) has suggested that the head glucose level is involved in the generation of the portal signal. Furthermore, the quantitative accuracy of that study has been questioned on several grounds, including the mixing of their infusate in portal blood (8). In a previous study designed to examine the same question, we infused glucose (22.2 \( \mu \)mol\cdot kg\(^{-1}\)\cdot min\(^{-1}\)) intraportally at a rate known to increase NHGU maximally in the presence of a fourfold rise in insulin. Unlike Matsuhisa et al. (9), we used a rate that minimized potential problems of portal glucose mixing (a favorable noise-to-signal ratio). In addition, we used an independent method (PAH assay) to assess the mixing of the infused glucose in the portal vein and the hepatic vein, and this in turn allowed evaluation of the potential effects of imperfect mixing in the two individual protocols. Also, we infused glucose into the carotid and vertebral arteries bilaterally instead of unilaterally, as had Matsuhisa et al., to ensure a uniform elimination of the difference between brain and portal vein glucose. Our results clearly demonstrated that, under hyperglycemic hyperinsulinemic conditions, raising the head arterial glucose level did not modify the increase in NHGU seen in response to portal glucose delivery. Thus the brain arterial glucose level does not appear to provide the reference information used in generation of the portal signal in conscious dogs. To date, our data suggest that the liver, not the brain, is the organ critical for orchestration of the distribution of dietary glucose after a carbohydrate meal.

A recent report by Horikawa et al. (7) suggested that augmentation of hepatic glucose uptake was not dependent on the sign of the A-P venous glucose gradient and that a difference in either direction (\( A > P \) or \( P > A \)) was effective. They used conscious dogs in an experiment consisting of a 30-min control and three 90-min test periods. After the control period, glucose (55.6 \( \mu \)mol\cdot kg\(^{-1}\)\cdot min\(^{-1}\)) was first infused via the superior mesenteric vein; it was then infused into both the superior mesenteric vein and the gastroduodenal artery (27.8 \( \mu \)mol\cdot kg\(^{-1}\)\cdot min\(^{-1}\) in each vessel); and finally, it was infused solely into the gastroduodenal artery (55.6 \( \mu \)mol\cdot kg\(^{-1}\)\cdot min\(^{-1}\)). Unfortunately, the data from that study must be questioned on several grounds. First, the authors did not assess the mixing of their infusate with blood in any vessel, and poor portal glucose mixing was likely, given the extremely high glucose infusion rates used. This is further evident from the inconsistency between NHGU calculated by the direct and indirect methods in the second test period. When the indirect method of glucose balance calculation was used, it appeared that NHGU decreased 50% during concurrent infusion of glucose into the portal and arterial systems, suggesting an inhibition of NHGU by hepatic arterial glucose infusion. On the other hand, no decrease in NHGU was evident when the direct method of calculation was used, making it difficult to draw a conclusion. Second, the pancreatic hormones were not clamped, and as a result they varied somewhat over the course of the study. Third, the authors failed to include a control group that would account for changes over time during the study. Finally, the plasma glucose values were not in steady state, thus limiting the accuracy of the arteriovenous difference calculation.

In the present study, we made several improvements over the study design of Horikawa et al. (7) to minimize the above errors. First, the hepatic arterial glucose infusion (8.0 \( \pm \) 0.5 \( \mu \)mol\cdot kg\(^{-1}\)\cdot min\(^{-1}\)) was kept small to minimize the impact of any mixing problems (i.e., to improve the signal-to-noise ratio). Even under our carefully controlled conditions, mixing of the infusate in the hepatic artery was probably not perfect. This is evidenced by the fact that the positive gradient between the hepatic artery and the portal vein in PD+HAD was slightly greater during the latter experimental period than during the first. Second, we used PAH to assess mixing of both the portal and hepatic...
arterial infusates and only “mixed” dogs were utilized for data analysis. Third, we used two control groups so that we could isolate the effects of hyperglycemia, hyperinsulinemia, and the portal signal over time. Fourth, we clamped insulin, glucagon, and the HGL so that they did not differ among the protocols. Finally, we made our measurements in a steady-state period. The present results thus clearly demonstrate that a gradient between the hepatic arterial and portal vein glucose levels is critical for the generation of the hepatic and extrahepatic effects of the portal signal.

It is also possible, however, that the portal signal is sensed within the liver and that the transduction of its effect is mediated by the autonomic nervous system outside the liver. This is made more likely by the need to explain the coordinate but discrete responses of the liver and nonhepatic tissues (primarily muscle). It is clear that the glucose portal signal not only enhances hepatic glucose uptake but also suppresses nonhepatic glucose uptake (Fig. 6). If this were not true, the glucose infusion rate would have been the same in PD and PD+HAD. Earlier reports have described the existence of neural pathways that link the liver to the brain and the brain to the liver and various endocrine organs (5, 20). Afferent fibers in the hepatic branch of the vagus nerve (14) and neurons in the lateral hypothalamus (22) can respond to the presence of glucose in the portal vein. Functional studies also have demonstrated that an intact nerve supply to the liver appears to be vital for a normal hepatic response to intraduodenal or intraportal glucose delivery (1, 10). Nijima and colleagues (13, 15) reported that the stimulation of hepatic afferents can alter the efferent activity of the adrenal and vagal pancreatic nerves in the rabbit and rat. There is no doubt that an autonomic link between the liver and peripheral organs exists, but the extent to which this is involved in the initiation of the effects of the portal signal remains to be determined.

Recently, several reports from Xie and Lautt (26, 27) focused on the relationship between peripheral insulin resistance and the activity of hepatic parasympathetic nervous system. They reported that surgical denervation of the hepatic anterior plexus or intraportal atropine infusion reduced the magnitude of insulin’s effectiveness in skeletal muscle but had no effect on the liver or gut (25, 26). Complete hepatic denervation plus vagotomy did not cause further impairment, thus indicating that all of the relevant nerves reached the liver via the anterior plexus. Furthermore, intraportal, but not peripheral, acetylcholine infusion reversed insulin resistance produced by liver denervation (27). These studies suggest that the hepatic parasympathetic nerves regulate release of a liver-generated factor that selectively controls insulin effectiveness in skeletal muscle. Because the intrahepatic parasympathetic system has been suggested to be a key determinant in generation and/or transduction of the effect of the portal signal (24), the results from Xie and Lautt (25–27) further imply that the anterior plexus around the hepatic artery might be a potential reference site for generation of the suppressive effect of the portal signal on nonhepatic glucose uptake. This possibility needs to be investigated further.

In summary, the elimination of the negative glucose gradient between hepatic artery and portal vein markedly reduced the effects of the portal signal on hepatic and peripheral glucose uptake. This suggests that the liver plays a primary role in the regulation of postprandial glucose distribution, not only by augmenting its own uptake of glucose but by preventing glucose uptake by skeletal muscle.

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REFERENCES