Net Hepatic Gluconeogenic Amino Acid Uptake in Response to Peripheral versus Portal Amino Acid Infusion in Conscious Dogs

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ABSTRACT These studies were conducted to determine the effect of route of gluconeogenic amino acid delivery on the hepatic uptake of the amino acids. After a sampling period with no experimental intervention (basal period), conscious dogs deprived of food for 42 h received somatostatin, intraportal infusions of insulin (3-fold basal) and glucagon (basal), and a peripheral infusion of glucose to increase the hepatic glucose load 1.5-fold basal for 240 min. A mixture of alanine, glutamate, glutamine, glycine, serine, and threonine was infused intraportally at 7.6 μmol·kg⁻¹·min⁻¹ (PorAA group, n = 6) or peripherally at 8.1 μmol·kg⁻¹·min⁻¹ (PerAA, n = 6), to match the hepatic load of gluconeogenic amino acids in PorAA. During the infusion period, there were no differences in PerAA and PorAA, respectively, with regard to arterial plasma insulin (144 ± 18 and 162 ± 18 nmol/L), glucagon (51 ± 8 and 47 ± 11 ng/L), hepatic glucose load (199.8 ± 22.2 and 210.9 ± 16.6 μmol·kg⁻¹·min⁻¹), net hepatic glucose uptake (2.8 ± 2.2 and 2.2 ± 1.7 μmol·kg⁻¹·min⁻¹), hepatic load of amino acids (68 ± 14 and 62 ± 7 μmol·kg⁻¹·min⁻¹), or net hepatic glycogen synthesis (11.1 ± 2.2 and 8.9 ± 2.2 μmol·kg⁻¹·min⁻¹). The net hepatic uptake of glutamine (2.1 ± 0.4 vs. 0.8 ± 0.3 μmol·kg⁻¹·min⁻¹), or the net hepatic fractional extractions of glutamine (0.11 ± 0.02 vs. 0.05 ± 0.02) and serine (0.41 ± 0.03 vs. 0.34 ± 0.02) were greater in PorAA than in PerAA (P < 0.05). We speculate that one or more of the amino acids in the mixture causes enhancement of the net hepatic uptake and fractional extraction of glutamine, and perhaps other gluconeogenic amino acids, during intraportal amino acid delivery. J. Nutr. 129: 2218–2224, 1999.

KEY WORDS: • amino acids • glutamine • liver nerves • dogs

We recently reported the results of studies in conscious dogs receiving intraportal glucose infusion concomitant with intraportal or peripheral infusion of a mixture of gluconeogenic amino acids (alanine, glycine, glutamate, glutamine, serine and threonine) (Moore et al. 1999). Net hepatic glucose uptake (NHGU) was significantly greater with peripheral infusion of amino acids than with intraportal infusion. Moreover, the net hepatic uptakes (NHU) and/or fractional extractions (NHFE) of alanine, glutamate and glutamine were significantly greater during portal versus peripheral amino acid infusion. However, from those data we could not determine whether the NHU and/or NHFE of the amino acids were enhanced by portal amino acid administration per se or by an interaction between portal glucose and portal amino acid delivery.

Many amino acids are known to increase or decrease the afferent firing rate in the hepatic branch of the vagus nerve (Niijima and Meguid 1995). A decrease in the afferent firing rate was shown to be associated with changes in efferent vagal signaling (Niijima 1983, Niijima and Meguid 1989). We hypothesized that the gluconeogenic amino acids generate signals transmitted via the vagus that result in enhancement of their own net hepatic uptakes. As a first step in testing this hypothesis, we undertook the current studies to determine whether the NHU or NHFE of any of the gluconeogenic amino acids would be enhanced by their intraportal delivery in the absence of portal glucose infusion and thus in the absence of the portal glucose signal that results in enhanced NHGU.

**MATERIALS AND METHODS**

**Animals, diets and experimental preparation.** Studies were carried out on conscious adult dogs of either sex with a mean weight of 21 ± 2 kg with breeds as previously described (Moore et al. 1993). Dogs were housed in a facility approved by the American Association for the Accreditation of Laboratory Animal Care International, and the protocols were approved by the Vanderbilt University Animal Care Committee. The dogs were fed once daily equal parts by weight...
of canned meat [Pedigree Meat Food (Kal Kan, Vernon, CA)] and nonpurified diet [Purina Lab Canine diet no. 5026 (Purina, St. Louis, MO)] and provided, in g/100 g diet: protein, 17.5; fat, 7.9; carbohydrate, 23.5; crude fiber, 1.5; moisture, 44; and ash, 4.8. Micronutrient composition was as previously described (Moore et al. 1993). The animals were closely monitored, and the amount of food offered was adjusted to maintain a stable weight. However, these dogs were very homogeneous in terms of weight and activity level, and most received 400 g each of meat and nonpurified diet daily.

All dogs underwent a laparotomy under general anesthesia 16 d before study, for insertion of sampling catheters in the portal and left common hepatic veins and femoral artery and infusion catheters in a splenic and a jejunal vein to allow intraportal nutrient and hormone delivery (Pagliassotti et al. 1996). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were positioned around the portal vein and hepatic artery. Pre-study assessment and preparation were performed as previously described (Pagliassotti et al. 1996). Food was withheld for 48 h before each experiment, to allow glycogen to reach a stable minimum and all dogs to achieve a state of net hepatic lactate uptake.

**Experimental design.** At −120 min, a primed (1.48 MBq), continuous (14.8 kBq/min) infusion of i-[3-3H]glucose and a continuous infusion of indocyanine green dye (Becton Dickinson, Cockeysville, MD; 4 μg·kg⁻¹·min⁻¹) were begun via the left cephalic vein. After 80 min (−120 to 0) of tracer and dye equilibration, there was a 40 min (−40 to 0) control or basal period, followed by a 240 min (0 to 240) experimental period. At time 0, constant infusions of several solutions were begun, and they were continued throughout the experimental period. Somatostatin (0.8 μg·kg⁻¹·min⁻¹ [Bachem, Torrance, CA]) was infused via a portal saphenous vein to suppress endogenous insulin and glucagon secretion. Insulin (7.2 mmol·kg⁻¹·min⁻¹; 3-fold basal) and glucagon (basal; both hormones obtained from Eli Lilly & Co, Indianapolis, IN) were delivered intraportally. The dogs were divided into two groups (see below). Two dogs in each group received glucagon at 0.65 ng·kg⁻¹·min⁻¹, but since this rate was estimated based on the proportion of hepatic load that approximated the load in the PorAA group, the amino acid mixture was mixed with a tissue sample from each liver lobe was immediately freeze-clamped and stored at −70°C.

**Processing and analysis of samples.** Blood glucose, glutamine, glutamate, lactate, alanine, serine, threonine, glycine, glycerol and hematocrit; plasma glucose, insulin and glucagon concentrations; and liver glycogen concentrations were determined as described previously (Moore et al. 1991, 1998).

**Calculations.** In the PorAA group, mixing of the infused amino acids in the portal vein was assessed by comparing recovery of PAH in the portal and hepatic veins with the PAH infusion rate as previously described (Moore et al. 1999, Pagliassotti et al. 1996). Mixing was good, as evidenced by the ratios of recovered to infused PAH in the portal and hepatic veins (0.9 ± 0.1 in both veins, with a ratio of 1.0 representing ideal mixing).

The ultrasonic flow probe and the dye extraction technique (Moore et al. 1991), yielded hepatic blood flow (HBF) rates that were not significantly different. Since 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 when available. One or the other of the flow probes did not function in one dog in each group. In these animals indocyanine green-derived flows were used, and the portal vein was assumed to provide 80% of hepatic blood flow during the basal period and 74% during the experimental period (Moore et al. 1998, Pagliassotti et al. 1996).

The rate of substrate delivery to the liver, or hepatic substrate load, was calculated by a direct (d) method as:

\[
\text{load}_{\text{d}(i)} = (\text{C}_{\text{A}} \times \text{ABF}) + (\text{C}_{\text{P}} \times \text{PB} \times \text{HBF})
\]

where [S] is the substrate concentration, A and P refer to artery and portal vein respectively, and ABF and PB refer to blood flow through the hepatic artery and portal vein, respectively. To avoid any potential errors arising from either incomplete mixing of amino acids during intraportal infusion or lack of precise measurements of the distribution of hepatic blood flow, the hepatic amino acid load was also calculated by an indirect (i) method:

\[
\text{load}_{\text{i}(i)} = (G \times \text{HBF}) + \text{IR}_{\text{Por}} - \text{GU}
\]

where G is the blood amino acid concentration, IRPor is the intraportal amino acid infusion rate, and GU is the uptake of amino acids by the gastrointestinal tract during peripheral infusion.

The load of a substrate exiting the liver was calculated as:

\[
\text{load}_{\text{out}} = [\text{S}]_{\text{P}} \times \text{HBF}
\]

where H represents the hepatic vein.

Direct and indirect methods were used in calculation of net hepatic balance (NHB). The direct calculation was: NHB = load(i) - load(d). The indirect calculation was: NHB = load(i) - load(i). A negative value indicates net hepatic uptake (NHB); where only NHB is evident, the negative sign is omitted and NHB is described as NHU. Both equations were used in calculation of net hepatic amino acid balance, but only the direct calculation was employed for other substrates. The results for net hepatic amino acid balance did not differ regardless of the method used in calculation. The results given in this report utilize the indirect calculation, because this method is less likely to be affected by any inadequate mixing in the portal vein. Net hepatic fractional extraction of substrates was calculated as the ratio of NHB to load(i).

Plasma glucose concentrations were converted to blood glucose as previously described (Pagliassotti et al. 1996). Use of blood concentrations ensures accurate measurements of net hepatic glucose and amino acid balance regardless of the characteristics of glucose or amino acid entry into the erythrocyte.

Net hepatic glycogen synthesis was calculated as the difference between post-study glycogen concentrations in the PerAA and PorAA dogs and basal concentrations in 11 dogs which were killed after 42 h of food deprivation (corresponding to time 0 in the experimental animals) (Moore et al. 1991). The glycogen concentrations for each dog represent the mean of the values for the seven liver lobes, weighted for the percentage of liver mass accounted for by each lobe (Moore et al. 1991).
Glucagon concentrations did not differ from basal in either group during the experimental period. However, the mean NHU (2.1 ± 0.4 vs. 0.8 ± 0.3 μmol · kg⁻¹ · min⁻¹, P < 0.05 for both repeated-measures ANOVA and t-test of AUC) and NHFE (0.11 ± 0.02 vs. 0.05 ± 0.02, P < 0.05 for both repeated-measures ANOVA and t-test of AUC) of glutamine were greater in PorAA than in PerAA (Fig. 2).

For alanine, glycine, glutamate and threonine, there were no significant differences between PorAA and PerAA in hepatic loads, NHI, or NHFE (Table 2), although the net hepatic fractional extraction of alanine tended to be greater in PorAA than in PerAA throughout the experimental period (P = 0.08). There were no significant differences in serine concentrations, hepatic loads or NHI between PorAA and PerAA. However, the NHFE of serine was greater in PorAA than in PerAA during the experimental period (P < 0.05 for both repeated-measures ANOVA and t-test of AUC).

Lactate levels and balance data. There were no differences between the groups in concentration or net hepatic balance of lactate (data not shown). The arterial blood lactate concentrations rose progressively and similarly in both groups, from ~352 ± 50 μmol/L in the basal period to ~865 ± 90 μmol/L at the last sampling time. Both groups shifted from net hepatic lactate uptake (9.2 ± 1.7 μmol · kg⁻¹ · min⁻¹) to net lactate output within 30 min of beginning the hormone and substrate infusions (peak rate 3.6 ± 2.5 μmol · kg⁻¹ · min⁻¹). Both groups had returned to a low rate of net hepatic lactate uptake by 150 min, with the mean rate at the final sampling point being ~1.7 ± 1.3 μmol · kg⁻¹ · min⁻¹.

Glycogen data. The net hepatic glycogen synthetic rates were 8.9 ± 2.2 and 11.1 ± 2.7 μmol · kg⁻¹ · min⁻¹ in PorAA and PerAA, respectively (P = 0.3). The net rates of glycogen synthesis via the direct pathway were not different in the two groups (5.5 ± 1.7 and 3.9 ± 1.7 μmol · kg⁻¹ · min⁻¹ in PorAA and PerAA, respectively).

**DISCUSSION**

We showed that intraportal glucose delivery enhances net hepatic glucose uptake and suggested that this occurs as a result of the generation of a signal by glucose sensors in the
hepatoportal region (Adkins et al. 1987). Presumably this signal reaches the central nervous system via the hepatic branch of the vagus nerve (Adkins-Marshall et al. 1992). The afferent firing rate in the vagus nerve is inversely proportional to the portal vein glucose concentration (Niijima 1982 and 1996). However, the vagal firing rate is not modulated only by the portal glucose concentration. Niijima and Meguid (1995) found that intraportal injection or infusion of 15 common dietary amino acids also alters the afferent firing rate in the hepatic branch of the vagus nerve. Eight of the 15 amino acids studied by Niijima and Meguid (1995), including alanine and serine, had an excitatory effect on the afferent firing rate in the hepatic branch of the vagus nerve in the rat. On the other hand, glycine, threonine and five other amino acids had an inhibitory effect (Niijima and Meguid 1995). Niijima and
Meguid did not study glutamine and glutamate. We speculated that changes in afferent vagal firing might initiate a mechanism for directing selected substrates into the liver. Thus it might be possible for intraportal amino acids not only to modulate NHGU when administered concurrently with intraportal glucose (Moore et al. 1998 and 1999), but also to affect their own hepatic uptake.

To examine the effect of intraportal amino acid infusion on NHU and NHFE of the amino acids themselves, it was necessary to conduct the current studies in the absence of a portal glucose infusion (current data). Similarly, the NHFE of serine tended to be greater during portal amino acid infusion in the presence of portal glucose delivery (Moore et al. 1999), but it was significantly enhanced during portal amino acid infusions in the absence of portal glucose infusion (current data).

There are at least two possible explanations for our findings.

The first possibility is that the enhancement of hepatic amino acid uptake by portal amino acid delivery is actually a generalized phenomenon. In other words, the hepatic uptakes of all or most of the amino acids in our mixture may have been enhanced by portal delivery, but their hepatic loads and changes in net hepatic uptake were so small that only the findings for glutamine were consistently significant. The hepatic load of glutamine was 2- to 10-fold greater than for the other five amino acids infused, and glutamine was unique among these amino acids in that the liver shifted from net output of the amino acid in the basal period to net uptake in the infusion period. Thus glutamine had a large "signal-to-noise" ratio in comparison to the other amino acids. The second possibility is that glutamine was really the only amino acid whose NHU and NHFE were significantly enhanced by portal delivery (Moore et al. 1999), while there was only a tendency (P = 0.08) for NHFE of alanine to be enhanced in the PorAA group in the current report. Similarly, the NHFE of serine tended to be greater during portal amino acid infusion in the presence of portal glucose delivery (Moore et al. 1999), but it was significantly enhanced during portal amino acid infusions in the absence of portal glucose infusion (current data).

TABLE 2

Arterial and portal vein blood concentrations, hepatic loads, net hepatic uptakes and net hepatic fractional extractions of alanine, glutamate, glycine, serine and threonine in dogs in the basal state and during the experimental period, when they received infusions of somatostatin, insulin, glucagon, glucose and gluconeogenic amino acids via a peripheral vein (PerAA group) or the portal vein (PorAA group)1

<table>
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<tr>
<th>Amino acid parameter</th>
<th>Group</th>
<th>Basal period</th>
<th>Experimental period</th>
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<td>PerAA</td>
<td>263 ± 19</td>
<td>349 ± 21</td>
<td>55 ± 6</td>
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<td>307 ± 33</td>
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<td></td>
<td>PorAA</td>
<td>247 ± 38</td>
<td>293 ± 44</td>
<td>66 ± 9</td>
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<td>208 ± 23</td>
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<td>PorAA</td>
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<td>9.6 ± 1.8</td>
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<td>2.6 ± 0.4</td>
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<td></td>
<td>PerAA</td>
<td>2.6 ± 0.3</td>
<td>5.2 ± 0.8</td>
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<td>PerAA</td>
<td>0.25 ± 0.01</td>
<td>0.40 ± 0.03</td>
<td>0.15 ± 0.18</td>
<td>0.10 ± 0.01</td>
<td>0.18 ± 0.02</td>
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<td>0.15 ± 0.02</td>
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<td>PorAA</td>
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<td>0.47 ± 0.04</td>
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<td>0.07 ± 0.07</td>
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<td>0.30 ± 0.06</td>
<td>0.14 ± 0.04</td>
<td>0.41 ± 0.04*</td>
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1 Basal and experimental period values are the mean ± SEM of two and seven sampling points, respectively; n = 6/group. NHU, net hepatic uptake; NHFE, net hepatic fractional extraction (no units). * P < 0.05 vs. the PerAA group.
there are clear cut differences in hepatic balance data between the portal and peripheral routes of amino acid delivery. The question arises as to what metabolic event(s) would cause glutamine to be extracted by the liver more avidly during portal than peripheral amino acid infusion. The enhancement of glutamine uptake may result simply from the fact that the liver contains the enzymes for both synthesis and degradation of glutamine. The perportal region of the liver is high in glutaminase (Jungermann and Kietzmann 1996), and therefore the enhancement of hepatic glutamine uptake during portal amino acid delivery might have resulted from the ready availability of the enzyme for its degradation. However, glutamine apparently plays important roles in the regulation of key hepatic functions, including glycogen storage, protein synthesis, gluconeogenesis and urea cycle activity. Enhancement of glucagon synthase activity and glycogen synthetase by glutamine was documented in rat liver both in vitro (Lavoine et al. 1987) and in vivo (Niewoehner and Nuttal 1996). In addition, glutamine is known to stimulate the transcription of certain genes, including β-actin, phosphoenolpyruvate carboxykinase, and argininosuccinate synthetase, in hepatocytes (Husson et al. 1996, Lavoine et al. 1996, Quillard et al. 1997, Quillard et al. 1996). Glutamine’s enhanced uptake during portal amino acid delivery may have been related to one or more of these roles. Hepatic protein synthesis, in particular, was shown to be stimulated by portal, as opposed to peripheral, vein, delivery of amino acids (Bennet and Haymond, 1991, Bozetti et al. 1993).

In the current studies, the total rate of net hepatic substrate uptake was very similar and quite low in both groups, and it closely approximated the rate of net hepatic glyceroneogenesis. Thus it is not unexpected that glyceroneogenesis should be similar in the two groups. We did not measure proteolysis, ureagenesis or protein synthetic rates, and therefore it is impossible to know what effect portal amino acid delivery had upon these processes. The amino acid mixture was imbalanced, and thus it was likely to promote proteolysis. Nevertheless, because both groups received the same amino acid mixture, our findings are unlikely to be explained simply by the composition of the infusate.

The negative arterial-portal (A-P) glucose gradient (i.e., concentration higher in the portal vein than in the artery) evident during portal glucose delivery is a crucial factor in the generation of the portal glucose signal (Adkins et al. 1987, Gardemann et al. 1986, Pagliassotti et al. 1991, Stumpel and Jungermann 1997). It is possible that such a gradient between portal and peripheral amino acid delivery was much greater than for serine and alanine. Amino acid sensors were identified in the hepatopetal region (Niijima and Meguid 1995) and in the portal vein (Lavoinne et al. 1996). Glutamine inhibits the lowering effect of glucose on the level of phosphoenolpyruvate carboxykinase mRNA in isolated hepatocytes. Biochem. J. 248: 537–543.


In conclusion, intraportal infusion of gluconeogenic amino acids during peripheral infusion of glucose, with the insulin and glucagon concentrations clamped at 3-fold basal and basal, respectively, enhanced the net hepatic uptake and fractional extraction of glucose >2-fold (P < 0.05), compared with peripheral infusion of the same amino acid mixture. The net hepatic fractional extraction of serine was significantly increased and that of alanine tended to be increased with portal vs. peripheral amino acid delivery. However, the difference in the net hepatic fractional extraction of glutamine between portal and peripheral amino acid delivery was much greater than for serine and alanine. Amino acid sensors were identified in the hepatopetal region (Niijima and Meguid 1995) and in the portal vein (Lavoinne et al. 1996). Glutamine inhibits the lowering effect of glucose on the level of phosphoenolpyruvate carboxykinase mRNA in isolated hepatocytes. Biochem. J. 248: 537–543.


