Importance of the Route of Intravenous Glucose Delivery to Hepatic Glucose Balance in the Conscious Dog

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Abstract

To assess the importance of the route of glucose delivery in determining net hepatic glucose balance (NHGB) eight conscious overnight-fasted dogs were given glucose via the portal or a peripheral vein. NHGB was measured using the arteriovenous difference technique during a control and two 90-min glucose infusion periods. The sequence of infusions was randomized. Insulin and glucagon were held at constant basal levels using somatostatin and intraportal insulin and glucagon infusions during the control, portal, and peripheral glucose infusion periods (7±1, 7±1, 7±1 μU/ml; 100±3, 101±6, 101±3 pg/ml, respectively). In the three periods the hepatic blood flow, glucose infusion rate, arterial glucose level, hepatic glucose load, arterial-portal glucose difference and NHGB were 37±1, 34±1, 32±3 ml/kg per min; 0±0, 4.51±0.57, 4.23±0.34 mg/kg per min; 101±5, 200±15, 217±13 mg/dl; 28.5±3.5, 57.2±6.7, 54.0±6.4 mg/kg per min; +2±1, −22±3, +4±1 mg/dl; and 2.22±0.28, −1.41±0.31, and 0.08±0.23 mg/kg per min, respectively. Thus when glucose was delivered via a peripheral vein the liver did not take up glucose but when a similar glucose load was delivered intraportally the liver took up 32% ($P < 0.01$) of it. In conclusion portal glucose delivery provides a signal important for the normal hepatic-peripheral distribution of a glucose load.

Introduction

The effects of insulin and the blood glucose concentration on net hepatic or splanchnic glucose balance have been extensively explored in both man and the dog, but neither can completely explain the magnitude of hepatic glucose uptake observed after an oral glucose meal. DeFronzo et al. (1) found that in man with basal arterial glucose levels (94±2 mg/dl) and very high insulin concentrations (1,189±414 μU/ml), net splanchnic glucose uptake reached 0.68±0.13 mg/kg per min, but still accounted for only 6% of total glucose utilization. Studies from our lab have shown that in dogs in the presence of euglycemia, constant basal glucagon levels, and portal vein hyperinsulinemia as high as 500 μU/ml, net hepatic glucose uptake did not exceed 0.56±0.27 mg/kg per min (Frizzell, R. T., G. K. Hendrick, L. L. Brown, D. B. Lacy, P. E. Donahue, A. F. Parlow, R. W. Stevenson, P. E. Williams, and A. D. Cherrington. Manuscript in preparation.). Likewise, hyperglycemia brought about by peripheral intravenous glucose infusion in the presence of basal insulin levels cannot cause significant net hepatic glucose uptake. In man, marked hyperglycemia (224±2 mg/dl) in the presence of basal insulin and glucagon levels produced net splanchnic glucose uptake of only 0.58 mg/kg per min (2). In the dog under similar conditions net hepatic glucose production was 0.90±0.10 mg/kg per min and there was no net hepatic glucose uptake (3). It is clear, therefore, that within the physiologic range neither hyperinsulinemia nor hyperglycemia alone can cause significant net hepatic or splanchnic glucose uptake.

The data are more variable in studies in which hyperinsulinemia and hyperglycemia of the magnitude noted above were combined. In man Sacca et al. (4) and DeFronzo et al. (5) observed net splanchnic glucose uptake of 1.7 and 1.1 mg/kg per min, respectively, in response to combined hyperglycemia and hyperinsulinemia. In dogs made hyperglycemic and hyperinsulinemic net hepatic glucose uptake ranged from 0.9 to 3.8 mg/kg-min (6–8). Despite the range of response it is apparent that even combined hyperglycemia and hyperinsulinemia cannot account for the rate of net hepatic and splanchnic glucose uptake seen following oral glucose administration. DeFronzo et al. (5) reported a net splanchnic uptake of 5.9±0.6 mg/kg per min after oral glucose administration in man. Net hepatic glucose uptake ranged from 2.3±0.4 to 5.4±0.5 mg/kg per min (6, 8–10) in dogs given glucose orally. In those studies insulin levels 1 h after the glucose meal ranged from 41 to 153 μU/ml and glucose levels ranged from 173 to 224 mg/dl clearly not greater than those achieved in the aforementioned studies in which intravenous hyperglycemia and hyperinsulinemia were combined. Such findings led DeFronzo et al. (11) to propose the existence of a "gut factor," the presence of which could augment net hepatic glucose uptake.

Since the proposition of this gut factor, other studies have cast doubt on its importance by producing hyperglycemia via an intraportal glucose infusion (6, 8, 10) and triggering rates of hepatic glucose uptake similar to those seen after oral glucose feeding. When hyperinsulinemia and hyperglycemia of portal intravenous origin were combined net hepatic glucose uptakes were as large as those observed after oral glucose administration (2.5±0.8 vs. 2.3±0.4 (10), 6.0±1.4 vs. 5.4±0.5 (8), 5.7±1.2 vs. 5.4±0.5 (8), and 6.0±1.1 vs. 4.4±1.3 (6) mg/kg per min for portal intravenous and oral administration, respectively.) Thus, it appears as though the site of glucose entry into the circulation may play a key role in regulating the disposition of the glucose load within the body.

The present study compares the effect of peripheral venous glucose delivery with that of intraportal glucose delivery under conditions in which both insulin and glucagon were basal and fixed. The aim of the study was to determine whether hyperglycemia alone (i.e., without extra insulin release) can cause significant net hepatic glucose uptake if the glucose is delivered into the hepatic portal system.

Methods

Animals and surgical procedures. Experiments were carried out on eight overnight-fasted (18 h) conscious dogs (17–22 kg) of either sex that had

Presented at the European Association for the Study of Diabetes Meeting 13 September 1984, London, England.

Received for publication 27 May 1986 and in revised form 15 September 1986.

J. Clin. Invest.

0021-9738/87/02/0557/09 $1.00
Volume 79, February 1987, 557–565

been fed once daily a diet of meat and chow (Kal Kan meat [Kal Kan Foods, Vernon, CA] and Wayne Lab Blox [Allied Mills, Inc., Chicago, IL]): 31% protein, 52% carbohydrate, 11% fat, and 6% fiber based on dry weight).

16 d before each experiment, a laparotomy was performed under general anesthesia (sodium pentobarbitol, 25 mg/kg), and Silastic catheters were inserted into a splenic vein, the portal vein, the left common hepatic vein, and a jejunal vein. The tips of the splenic and jejunal catheters were such that they were 1 cm beyond the first site of coalescence of the catheterized vein with another vessel. The tip of the portain vein catheter was placed 2 cm from the point at which the vessel enters the liver, and the tip of the hepatic vein catheter was placed 1 cm inside the left common hepatic vein. In the dog the left common hepatic vein drains the blood from almost half of the liver, the largest portion of liver drained by any of the hepatic veins (12). Another catheter was placed in the left femoral artery following a cut-down in the left inguinal region. After the catheters were inserted, they were filled with saline containing heparin (200 U/ml, Abbott Laboratories, North Chicago, IL), their free ends were knotted, and they were placed in subcutaneous pockets so that complete closure of the incisions was possible. Two weeks after surgery, blood was drawn to determine the leukocyte count and the hematocrit of the animal. Only animals that had (*a*) a leukocyte count below 16,000/mm^3, (*b*) a hematocrit above 38%, (*c*) a good appetite (consuming all of the daily ration), and (*d*) normal stools were used.

On the day of an experiment, the subcutaneous ends of the catheters were freed through a small skin incision made under local anesthesia (2% Lidocaine, Astra Pharmaceutical Products, Worcester, MA). The contents of each catheter were aspirated and the catheters were flushed with saline. The catheters in the splenic and jejunal veins were used for intraportal infusion of insulin, glucagon, and glucose, while the portal vein, hepatic vein, and femoral artery catheters were used for blood sampling. Three Angiocaths (18-gauge, Abbott Laboratories) were then inserted: one in the right cephalic vein for indocyanine green and 3-[^{3}H]glucose infusion, one in the left cephalic vein for peripheral glucose infusion, and one in the left saphenous vein for somatostatin infusion. After preexperimental preparation, each dog was allowed to stand quietly in a Pavlov harness for 20–30 min before beginning the experiment.

Experimental design. Each experiment consisted of a 120 min (−160 to −40 min) period of tracer equilibration and hormone adjustment, a 40-min (−40 to 0 min) control period, and two 90-min test periods (0–90 min and 90–180 min). At $t = -160$ min, a primed-constant infusion of 3-[^{3}H]glucose (0.36 μCi/min) was begun and continued throughout the study. The priming dose of 3-[^{3}H]glucose equaled 42 μCi. At $t = -120$ min an infusion of somatostatin (0.8 μg/kg per min) was started to inhibit endogenous insulin and glucagon secretion. Concurrently, intraportal replacement infusions of insulin (200 μU/kg per min) and glucagon (0.65 ng/kg per min) were started. The plasma glucose level was monitored every 5 min and the rate of insulin infusion was adjusted until the plasma glucose level was stabilized at a euglycemic value. The average insulin infusion rate used in the eight experiments was 184 μU/kg per min. The last alteration in the insulin infusion rate was made at least 30 min before the start of the control period. In the first group of animals (protocol I) glucose was infused initially via the left cephalic vein so that the arterial glucose level approximately doubled during the first test period (0–90 min). During the second test period (90–180 min), glucose was infused via the splenic and jejunal veins so that the total load of glucose reaching the liver was the same as that seen during the first test period. The second group of animals (protocol II) received glucose via the intraportal route first and the peripheral route second. Blood samples were taken every 10 min during the control period and every 15 min thereafter. The collection and immediate processing of blood samples have been described previously (13). The arterial and portal blood samples were collected simultaneously, ~30 s before collection of the hepatic venous sample. This was done in an attempt to compensate for the time required for the blood to pass through the liver (14) and thus allow accurate estimates of hepatic balance to be made even under nonsteady state conditions.

Analytical procedures. Plasma glucose concentrations were determined using the glucose-oxidase method in a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Blood glucose concentrations were assumed to be 73% of the plasma concentrations given by the Beckman glucose analyzer based on comparison of values obtained for plasma by the glucose-oxidase method with values obtained for whole blood using an Auto-Analyzer (Technicon Instruments Corp., Tarrytown, NY) according to the method developed by Lloyd et al. (15). Blood glucose values were then used in the hepatic balance calculations. The radioactivity of plasma glucose was determined by established procedures (16). Whole blood lactate concentrations were determined using a Technicon Auto-Analyzer according to the method of Lloyd et al. (15). The immunoreactive glucagon concentration in plasma samples to which 500 U/ml Trasylol had been added was determined using the 30 K antiserum of Unger (17). Immunoreactive insulin was measured using the Sephadex bound antibody procedure (18). Indocyanine green (Hynson, Westcott, and Dunning, Inc., Baltimore, MD) was measured spectrophotometrically at 810 nm to estimate hepatic blood flow according to the method of Leevy (19).

Calculations and data analysis. Net hepatic glucose balance (NHGB)[1] was determined using three separate methods. In the first, referred to as the "direct" calculation, NHGB was determined by the formula $[0.28A + 0.72P - H] \times$ HBF, where A is the arterial glucose concentration, P the portal vein glucose concentration, H the hepatic vein glucose concentration, and HBF the hepatic blood flow. The proportion of hepatic blood supply provided by the hepatic artery was assumed to be 28% based on a compilation of data from many sources by Greenway and Stark (20). It is unlikely that somatostatin at the dose employed, would have significantly altered this ratio since in all of our published studies (21, 22) hepatic blood flow in the dog was unchanged when somatostatin and basal replacement amounts of insulin and glucagon were given. Furthermore in the present study the mean blood flow was not significantly different from those observed in overnight fasted dogs given saline only. It is unlikely that the peripheral or portal infusion of glucose would have altered this ratio since total blood flow did not change in our study and neither total flow nor the distribution of flow in the two vessels was altered by similar infusions in earlier dog studies by Ishida et al. (8) in which Doppler flow probes were used to measure flow in the portal vein and hepatic artery directly.

To calculate NHGB during the portal glucose infusion without relying on the portal vein glucose concentration or on the assumption that 72% of hepatic blood flow is derived from the portal vein, the following formula, referred to as the "indirect" method was used: NHGB = NSGB + NGGB − GI; NSGB being net splanchnic glucose balance (calculated by multiplying the splanchnic A − V difference for glucose by hepatic blood flow), GI being the amount of glucose infused into the portal system and NGGB being net gut glucose balance (calculated by multiplying the gut A − V difference for glucose by 0.72 HBF). During the portal glucose infusion period the portal vein glucose concentration cannot be used to calculate NGGB since the portal blood sample is taken downstream from the site of entry of the glucose infusion. Therefore, NGGB during portal glucose delivery was assumed to be the same as NGGB during peripheral glucose delivery, since the glucose concentration in arterial blood was similar during the two infusion periods.

NHGB was also estimated using tritiated glucose data in a method referred to as the "tracer-derived NHGB." This was done by dividing the net hepatic uptake of [^{3}H]glucose counts by the specific activity of glucose reaching the liver according to the following equation: [HBF × (0.28 Adpm + 0.72 Pdpm − Hdpm)]/[(0.28 Adpm + 0.72 dpm)/(0.28 A + 0.72 P)] where Adpm, Pdpm, and Hdpm are the [^{3}H]glucose counts in the artery, portal vein, and hepatic vein plasma, respectively.

The amount of glucose presented to the liver, called the hepatic glucose load (HGL), was calculated using the formula HGL = (0.72 P + 0.28

1. *Abbreviations used in this paper:* Adpm, artery disintegrations per minute; Hdpm, hepatic disintegrations per minute; HGL, hepatic glucose load; NGGB, net gut glucose balance; NHGB, net hepatic glucose balance; NSGB, net splanchnic glucose balance; Pdpm, portal disintegrations per minute; PGU, peripheral glucose uptake, R_a, rate of glucose production; THGU, total hepatic glucose uptake.

A) × HBF. Glucose uptake by the peripheral tissues (PGU), including all tissues other than the liver, was calculated using the formula PGU = HI − NHGB. Fractional extraction of glucose by the liver was calculated using the formula NHGB/HGL. NHGB used in the above calculations was that determined using the direct method.

The total rate of glucose production (R_a) was determined by means of a primed tracer infusion. Calculation of the rate was carried out according to the method of Wall et al. (23) as simplified by Debodo et al. (24). This method is based on a single-compartment analysis of glucose kinetics in which it is assumed that rapid changes in the specific activity and concentration of glucose do not occur uniformly within the entire glucose pool. To compensate for this nonuniform mixing, the nonsteady state term of the equation was multiplied by a correction factor (pool fraction) of 0.65 as suggested by Cowan and Hetenyi (25). Total hepatic glucose uptake (THGU) can be calculated by subtracting NHGB from R_a. The direct NHGB was used to calculate THGU.

Net splanchnic, hepatic, and gut lactate balances were calculated using hepatic blood flow and blood lactate values and the formulae given for determination of glucose balance.

It should be noted that steady state conditions existed for the control period and the latter part of each test period thus the mean±SEM data shown in Figs. 2–6 represent the data from the control period and the last 30 min of each test period for both protocols. Statistical significance was determined using the paired t test (26).

Results

Insulin and glucagon levels. The arterial plasma insulin and glucagon levels remained unchanged throughout each study regardless of the protocol employed (Fig. 1 and Fig. 2). The mean

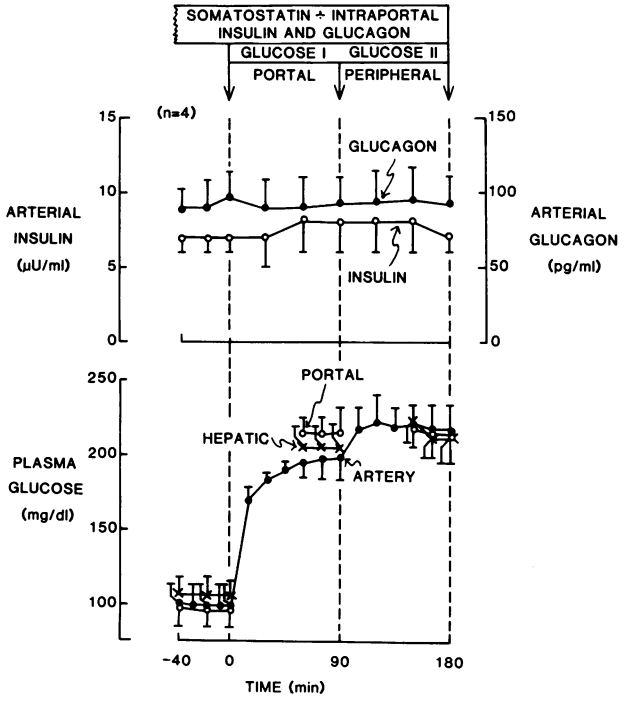

Figure 1. The effects of somatostatin, basal intraportal replacement amounts of insulin and glucagon, and infusion of glucose first into the portal vein then into a peripheral vein on arterial plasma insulin and glucagon, and on plasma glucose levels in an artery, the portal vein, and the hepatic vein. Data are expressed as the mean±SEM (n = 4). All glucose values were significantly ($P < 0.01$) elevated during glucose infusion.

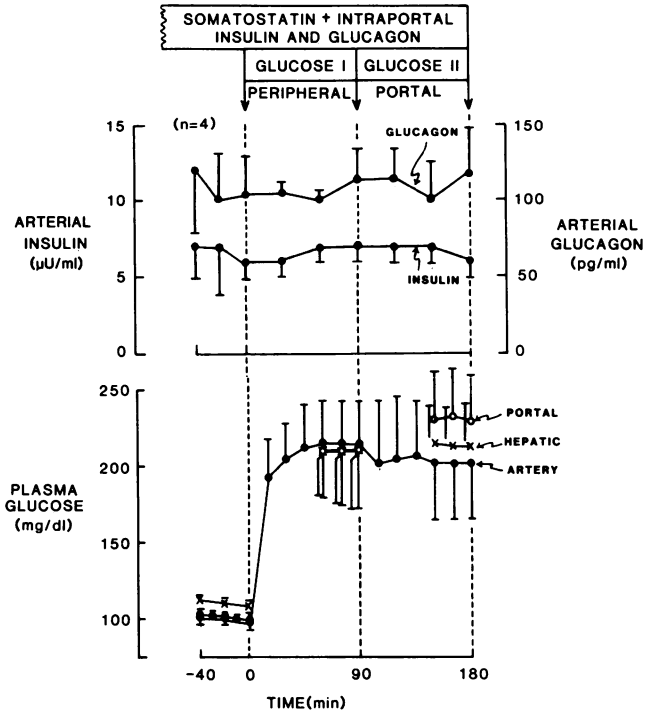

Figure 2. The effects of somatostatin, basal intraportal replacement amounts of insulin and glucagon, and infusion of glucose first into a peripheral vein then into the portal vein on arterial plasma insulin and glucagon and on plasma glucose levels in an artery, the portal vein, and the hepatic vein. Data are expressed as the mean±SEM (n = 4). All glucose values were significantly ($P < 0.01$) elevated during glucose infusion.

(n = 8) portal vein plasma insulin and glucagon levels (data not shown) were 22±3, 24±2, and 24±3 μU/ml; and 176±42, 181±30, and 178±31 pg/ml during the control period and the peripheral and portal glucose infusion periods, respectively. Both the load of insulin reaching the liver and the hepatic fractional extraction of insulin were similar during the two glucose infusion periods (Table I). Thus, any differences in hepatic glucose balance could not be attributed to a difference in the level, load, or fractional extraction of insulin.

Glucose levels and the hepatic glucose load. Plasma glucose levels in the artery, portal vein, and hepatic vein are shown in Figs. 1 and 2. The glucose levels were close to steady state during the control period and the last 30 min of each experimental period. For all eight dogs, glucose levels in the artery, portal vein, and hepatic vein were 101±5, 99±5, and 108±5 mg/dl, respectively, during the control period, 200±15, 222±15, and 210±15 mg/dl during the last 30 min of the intraportal infusion period, and 217±13, 213±14, and 214±15 mg/dl during the last 30 min of the peripheral infusion period (Fig. 3). Hepatic blood flow was similar during the two glucose infusion periods (Fig. 3), consequently, the hepatic glucose load was not significantly ($P > 0.05$) different in the two periods (Fig. 3, Table I).

NHGB and the fractional extraction of glucose by the liver. The net hepatic glucose balance (n = 8) was 2.22±0.28 and 0.08±0.23 mg/kg per min during the control period and the peripheral glucose infusion period, respectively (Fig. 4). During the portal glucose infusion period, the liver switched to net hepatic glucose uptake of −1.41±0.31 mg/kg per min as determined by the direct method. When net hepatic glucose balance was calculated using the indirect method, a balance of

Table I. Loads of Glucose and Insulin Reaching the Liver During Portal and Peripheral Glucose Infusion in the Conscious Overnight Fasted Dog and the Resulting Fractional Extraction of Both by the Liver

	Portal glucose infusion route				Peripheral glucose infusion route			
Dog	Load of glucose	Fractional glucose extraction	Load of insulin	Fractional insulin extraction	Load of glucose	Fractional glucose extraction	Load of insulin	Fractional insulin extraction
	mg/kg per min	%	*μU/kg per min*	%	*mg/kg per min*	%	*μU/kg per min*	%
1	86.9	2.1	454	48.1	80.0	0.0	304	49.3
2	54.9	3.9	308	43.2	39.9	1.8	258	40.2
3	51.9	4.7	418	24.1	63.4	0.3	687	38.9
4	38.9	4.0	340	35.4	30.9	0.4	278	61.2
5	42.3	0.0	495	34.3	40.7	0.7	410	36.9
6	43.9	2.7	395	10.2	51.0	0.0	362	15.7
7	57.5	2.1	450	43.9	71.6	0.5	579	45.8
8	81.3	1.4	303	26.4	54.4	0.0	254	36.7
Mean	57.2	2.6	395	33.2	54.0	0.5	392	40.6
±SEM	±6.7	±0.6	±27	±4.7	±6.4	±0.2	±61	±4.9
$n = 8$								

Values given for individual dogs represent the mean value for the final 30 min of each glucose infusion. When glucose output continued fractional glucose extraction was deemed to be 0.0.

−1.31±0.39 mg/kg per min was observed, while calculation of tracer-derived NHGB gave −2.02±0.47 mg/kg per min (Table II). Clearly, peripheral glucose infusion was associated with a cessation of glucose production but not with net hepatic glucose uptake. On the other hand, when the same glucose load was delivered via the portal route, significant ($P < 0.01$) net hepatic glucose uptake occurred. The fractional extraction of glucose was more than five times greater (2.6±0.5 vs. 0.5±0.2; percent

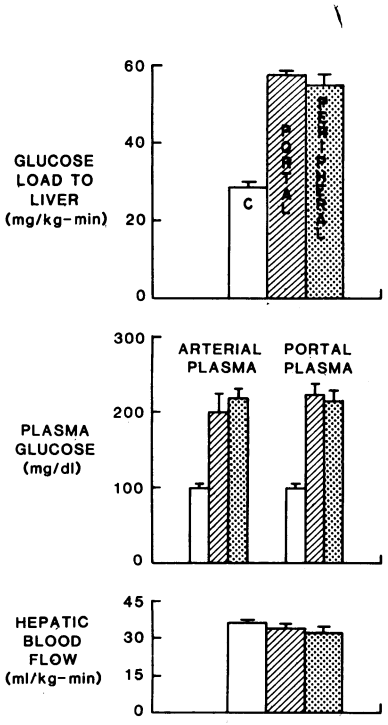

Figure 3. The effects of somatostatin, basal intraportal replacement amounts of insulin and glucagon, and intravenous infusion of glucose via the peripheral or portal route on arterial and portal glucose levels, hepatic blood flow, and glucose load to the liver. Data shown are for the 40-min control period and the last 30 min of each glucose infusion period. Data are expressed as the mean±SEM ($n = 8$). The glucose levels and load to the liver are significantly ($P < 0.01$) above control values regardless of the route of glucose infusion. The route of delivery, on the other hand, did not result in a significant difference in any parameter.

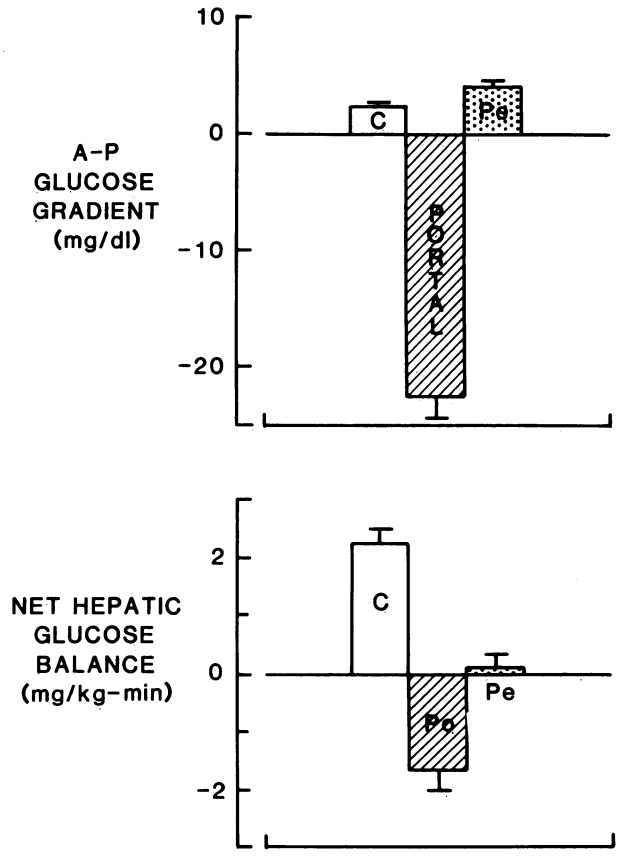

Figure 4. The effects of somatostatin, basal intraportal replacement amounts of insulin and glucagon, and intravenous infusion of glucose via the peripheral (*Pe*, $P < 0.01$) or portal (*Po*, $P < 0.01$) route on the arterial-portal plasma glucose gradient and the net hepatic glucose balance. Data are expressed as the mean±SEM ($n = 8$). NHGB was significantly suppressed below control value ($P < 0.01$) during Pe and Po infusion. Furthermore, NHGB during Po glucose infusion was significantly ($P < 0.01$) below NHGB Pe glucose infusion.

Table II. Net Hepatic Glucose Balance (mg/kg per min) During the Control Period and During Intraportal and Peripheral Intravenous Glucose Delivery in the Overnight Fasted Conscious Dog

		Intraportal glucose infusion			Peripheral glucose infusion
Dog	Control	Direct NHGB	Indirect NHGB	Tracer-determined NHGB	Direct NHGB
1	1.67	−1.82	−0.54	NM*	0.70
2	1.48	−2.14	−2.14	−1.67	−0.72
3	3.77	−2.46	−1.43	−1.70	−0.17
4	2.75	−1.54	−3.38	−1.68	−0.11
5	1.91	0.29	−0.47	−1.81	−0.27
6	1.85	−1.18	−1.18	−1.22	0.52
7	1.99	−1.23	−0.32	−3.12	−0.34
8	2.32	−1.16	−1.02	−3.97	1.05
Mean	2.22	−1.41	−1.31	−2.17	0.08
NHGB	±0.28	±0.31	±0.39	±0.40	±0.23
$n = 8$					

Values given for individual dogs represent the mean value over the entire control period and over the last 30 min of the glucose infusion periods.
^{3}H-balance by the liver during the control period was −0.74±0.26 mg/kg per min.
* Not measured.

$P < 0.05$) during portal glucose infusion than during peripheral glucose infusion (Table I). The arterial-portal glucose gradient was 2±1, 4±1, and −22±3 mg/dl during the control period, peripheral infusion period, and portal infusion period, respectively (Fig. 4). There was a significant correlation between the arterial-portal glucose gradient and net hepatic glucose balance ($r = 0.82$, $P < 0.05$) (Fig. 5).

Glucose turnover. The tracer-determined R_a was 3.50±0.74 mg/kg per min (Table III) in the four dogs in protocol I. R_a was suppressed by ~65% regardless of the route of glucose infusion. Total hepatic glucose uptake was 1.08±0.93 mg/kg per min in

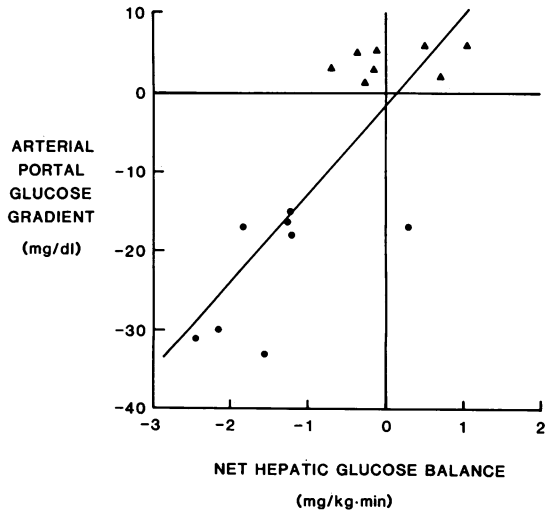

Figure 5. The correlation between the plasma arterial-portal glucose gradient and net hepatic glucose balance (direct) during peripheral (*closed triangle*) and portal (*closed circle*) glucose infusion ($n = 8$).

Table III. Tracer-determined Rate of Appearance of Glucose (R_a), Direct Net Hepatic Glucose Balance, Total Hepatic Glucose Uptake (THGU = R_a-"direct" NHGB), and the Tracer-determined Net Hepatic Glucose Balance During the Control Period and the Peripheral and Portal Glucose Infusion Periods of the Four Overnight Fasted Conscious Dogs in Which Glucose Was Infused Peripherally and Then Portally

	Control	Peripheral glucose	Portal glucose
R_a *(mg/kg per min)*	3.50±0.74	1.29±0.42*	1.16±0.35*
NHGB direct *(mg/kg per min)*	2.42±0.61	−0.08±0.34*	−1.99±0.23*‡
THGU *mg/kg per min*	1.08±0.93	1.37±0.75	3.15±0.55*‡
NHGB Tracer-determined *mg/kg per min*	−1.02±0.56	−0.58±0.42	−2.14±0.21‡

Values are mean±SEM, $n = 4$, for R_a, THGU; and $n = 3$ for tracer-determined NHGB; (not measured in one of the four dogs shown above).
Negative NHGB indicates hepatic uptake.
* Significantly different ($P < 0.05$) from control period.
‡ Significantly different ($P < 0.05$) from peripheral glucose infusion period.

the control period, and did not increase significantly during the peripheral glucose infusion period. However, during the portal glucose influsion period, total hepatic glucose uptake increased to 3.15±0.55 mg/kg per min ($P < 0.05$). "Tracer-determined" NHGB was −1.02±0.56 mg/kg per min during the control period and did not change significantly in the peripheral glucose period. During the portal infusion period, however, it increased significantly (−2.14±0.21 mg/kg per min). The fractional extraction of tritiated glucose was 2.3±1% during peripheral glucose infusion and 4.4±0.7% during portal glucose infusion (data not shown; $P < 0.05$). Meaningful tracer data could not be calculated for the other protocol because the peripheral infusion period followed the portal infusion period and [^{3}H]glucose was stored in glycogen during the latter thus preventing use of the tracer method during the second period.

Peripheral glucose uptake. When 4.23±0.34 mg/kg per min of glucose was infused into a peripheral vein, the liver exhibited net glucose output of 0.08±0.23 mg/kg per min (Fig. 6). Since steady state conditions existed, one can conclude that in a net sense 100% of the infused glucose was taken up by the peripheral tissues. However, when approximately the same amount of glucose (4.51±0.57 mg/kg per min) was infused intraportally, the liver took up 1.41±0.31 mg/kg per min, or 31% of the infused glucose. The peripheral tissues on the other hand reduced their uptake to 3.10±0.57 mg/kg per min, or 69% ($P < 0.05$) of the infused glucose (Fig. 6).

Lactate metabolism. The liver produced lactate throughout the control period of each protocol and significantly increased its lactate production in response to glucose loading (Table IV). There was no significant difference in lactate production during the two glucose infusion periods.

Discussion

This study demonstrates that hyperglycemia induced by intraportal glucose delivery can bring about significant net hepatic

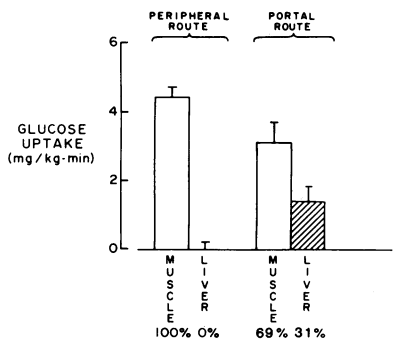

Figure 6. The effects of somatostatin, basal intraportal replacement amounts of insulin and glucagon, and intravenous infusion of glucose via the peripheral or portal route on glucose uptake by the peripheral tissues (primarily muscle) and by the liver. Data are expressed as the mean±SEM (n = 8).

glucose uptake even in the absence of increased insulin secretion. The same degree of hyperglycemia brought about by peripheral intravenous glucose delivery did not cause net hepatic glucose uptake, although net hepatic glucose production was effectively suppressed, a finding consistent with previous work (6). Remarkably, the extrahepatic tissues showed significantly less (30%, $P < 0.05$) glucose uptake during intraportal glucose delivery than during peripheral delivery (3.10±0.57 vs. 4.31±0.57 mg/kg per min) despite minimal differences (8%) in the arterial glucose level. The distribution of glucose to the liver and the peripheral tissues during intraportal glucose infusion was similar to the distribution reported by Abumrad et al. (9) and Bergman et al. (10) following an oral glucose load. These findings refine the hypothesis that a "gut factor" is responsible for the augmented net hepatic glucose balance seen after oral glucose feeding (11) by suggesting that a "portal" factor may be involved.

The magnitude of net hepatic glucose uptake seen during intraportal glucose delivery (1.4 mg/kg per min) is smaller than that seen in earlier studies by Bergman et al. (10), Ishida et al. (8), or Barrett et al. (6): 2.5, 5.7 to 6.0, and 6.0 mg/kg per min, respectively. In each of those studies, however, the insulin level was markedly elevated thus explaining the larger net hepatic glucose uptake. It is important to note that changes in the hepatic handling of insulin could not account for the difference in net hepatic glucose balance observed between the two routes of glucose delivery in the present study since both the level and load of the hormone reaching the liver, as well as its extraction by the liver, were the same regardless of the route of glucose infusion. Ishida et al. (8) noted a similar difference between the effects of portal and peripheral glucose delivery in the presence of elevated insulin values. Since the latter authors did not fix the insulin level in their study, conclusions regarding the relationship between insulin and a portal factor remain unclear.

Three technical issues require consideration relative to interpretation of the present data. The first is the possibility that the glucose infused intraportally streamed in the slow, laminar flow of portal venous blood. Such streaming could theoretically have affected the net hepatic glucose balance calculation by giving falsely high or low portal vein glucose levels or by allowing the glucose to stream to a part of the liver not drained by the catheterized hepatic vein. We have recently shown using paraminohippuric acid (PAH) added to the glucose infusate that, using the surgical approach described, mixing of the glucose is complete ~ 60% of the time (Myers et al., manuscript in preparation). Furthermore, the measured arterial-portal glucose gradient is a good predictor of mixing. In other words, when the measured arterial-portal glucose difference is equal to the difference predicted by dividing the glucose infusion rate by portal blood flow, the infused glucose can be considered to have mixed well. In the present study, three measurements of the arterial-portal glucose gradient were made during intraportal glucose infusion in each of eight dogs, giving a total of 24 such measurements. In 13 of these, the measured arterial-portal glucose difference was within ± 10% of the predicted difference. In five the measured difference was moderately higher than predicted, and in six, moderately lower than predicted. The error caused by streaming, when it occurred, thus was random and disappeared when means of the data were established, (the measured plasma arterial-portal glucose difference was 22±3 mg/dl, whereas the predicted was 26±5 mg/dl). Furthermore, if only the data from the 13 points in which the measured arterial-portal glucose difference equaled the predicted difference were used in calculating the data NHGB was − 1.51 mg/kg per min again indicating significant glucose uptake by the liver. Lastly, the NHGB calculated using the tritiated glucose data should not be subject to the streaming problem as the labeled glucose was infused peripherally and was well mixed before reaching the portal system and it too indicated significant hepatic glucose uptake during intraportal glucose infusion.

The individual variation in NHGB from dog to dog and the variation in a given dog when the rate was estimated using the three different approaches (Table II) is not unexpected. The first equation for NHGB ([0.28 A + 0.72 P] − H) × HBF) (Table II) involves four separate measurements, each of which carries with it some degree of error, and the second and third equations require five and six measured parameters, respectively. In ad-

Table IV. Arterial, Portal Venous, and Hepatic Venous Lactate Concentrations (mM) and Net Lactate Balance for Gut, Liver, and Splanchnic Bed (μmol/kg per min) During Glucose Infusion via the Peripheral or Portal Veins in the Overnight Fasted Conscious Dog

		Concentration			Net hepatic lactate balance		
		Artery	Portal vein	Hepatic vein	Gut	Liver	Splanchnic bed
Control period	Mean	0.603	0.622	0.747	0.771	4.446	5.217
	SEM	±0.218	±0.205	±0.272	±0.750	±2.476	±2.149
Peripheral glucose infusion period	Mean	0.763	0.802	1.017	1.166	7.677	8.843
	SEM	±0.163	±0.179	±0.204	±0.980	±2.332	±2.248
Portal glucose infusion period	Mean	0.873	0.904	1.107	1.282	6.636	7.918
	SEM	±0.244	±0.231	±0.299	±0.992	±4.097	±4.069

Values are mean±SEM, n = 8. Positive balance indicates net hepatic production.

dition, if the distribution of flow in the artery and portal vein which we assumed to be similar in each dog varied somewhat from dog to dog the error introduced would alter the direct NHGB somewhat while having little or no effect on the indirect or tracer-derived data. Given the random nature of these errors however the means obtained with the direct and indirect methods should correlate well and they do. The mean tracer derived value for NHGB (-2.17 ± 0.40 mg/kg per min) is somewhat higher than the other two values but that was to be expected since the tracer-derived NHGB was -0.74 ± 0.26 mg/kg per min during the control period.

The second technical issue of interest relates to the ratio of blood flow reaching the liver via the portal vein versus the hepatic artery. In the present study the hepatic artery was assumed to supply 28% of the blood flow to the liver based on data compiled by Greenway and Stark (20). This is slightly different from the 20% measured by Ishida et al. (8) using Doppler flow probes, or the 20% found by Barrett et al. (6) using a bromosulfophthalein dilution technique. Assuming a 20% contribution by the hepatic artery, however, would only increase the difference in glucose balance seen with the different routes of glucose delivery and thus strengthen our conclusion. In addition, when net hepatic glucose balance was calculated using the indirect method (i.e., without the portal glucose concentration or an assumption regarding flow distribution our conclusions were similar.

The final technical issue concerns the tracer data presented in Table III. The rate of glucose appearance (R_a) and the total hepatic glucose uptake (THGU = R_a − NHGB) are presented with the following caveats: first, data from protocol II are not presented because in that protocol glucose was given via the portal route first. During portal delivery, net hepatic glucose uptake was significant, suggesting that glycogen synthesis was occurring and that [^{3}H]glucose was being incorporated into glycogen. Thus the glucose being produced via glycogenolysis during the subsequent period (i.e., the peripheral glucose delivery period) contained labeled glucose, falsely lowering the tracer-determined rate of glucose appearance. The second caveat relates to problems encountered using the pool fraction model to analyze glucose kinetics in the presence of an infusion of a relatively large amount of cold glucose. There is an acknowledged but undefined error in the method (27) that is apparent from the frequent finding of negative rates of endogenous glucose appearance (R_a-glucose infusion rate) thus indicating an underestimate of R_a. In the data given here, negative values for the rate of glucose appearance were changed to a value of zero thereby minimizing but not eliminating this error. It should also be noted that the error would have been similar in the two glucose infusion periods because the rates of glucose infusion were virtually identical.

In spite of these problems, the tracer data do offer some insight into the effect of the route of glucose delivery on NHGB. Both peripheral and portal glucose delivery caused a suppression of the rate of hepatic glucose production to less than half of the control rate, but peripheral glucose delivery did not increase THGU. Thus, the suppression of NHGB by peripheral glucose delivery was due solely to a decrease in rate of glucose production. Portal glucose infusion, however, caused a greater than twofold increase in THGU in addition to the suppression of glucose production.

The liver produced lactate throughout the control period, a finding consistent with data from other postabsorptive dogs (28), and the production of lactate by the liver was greater during glucose delivery. However, if anything, it was slightly less during portal glucose infusion than during peripheral glucose infusion, suggesting that the glucose being taken up was not being lost as lactate, but was probably being stored as glycogen.

The finding of a smaller uptake of glucose by peripheral tissues during portal glucose delivery as compared with peripheral delivery suggests that the "portal factor" that enhances hepatic glucose uptake also induces some peripheral insulin resistance. The nature of this effect is not clear from the present study although it suggests that either a neural or hormonal factor in involved in that coordination of the response occurs.

The mechanism by which the route of glucose delivery triggers net hepatic glucose uptake is also not known. Neither the load of glucose nor the load of insulin reaching the liver could be factors, as these two parameters were the same during the two infusion periods. Likewise, the small (4%) increase in the portal plasma glucose level is insufficient to explain the switch from a slightly positive net hepatic glucose balance to significant uptake since increasing the glucose level in the portal vein further by peripheral infusion did not trigger net hepatic glucose uptake (29).

A gut factor is not involved as the gut was bypassed by the intraportal glucose infusion, however a portal factor cannot be discounted. The change in the arterial-portal glucose gradient correlates with the change in net hepatic glucose balance (Fig. 5), suggesting that a gradient may generate a signal that increases net hepatic glucose uptake. The glucose gradient has previously been related to the control of anorexia and food intake by Russek (30), and has recently been reported as a signal for insulin-dependent net hepatic glucose uptake in rat liver perfused simultaneously via the hepatic artery and the portal vein (31). In view of the simultaneous effect on glucose uptake by the liver and the extrahepatic tissues, and because of the speed with which the effect is manifest, a centrally mediated neural mechanism represents an attractive explanation for the phenomenon. Although the perfused liver data mentioned above would seem to contradict this hypothesis, some evidence supports the neural mechanism. Niijima (32) found afferent fibers in the hepatic branch of the vagus nerve of guinea pigs that showed a discharge rate inversely related to the concentration of glucose in the portal vein. Schmitt (33) reported finding lateral hypothalamic neurons that changed their rate of firing upon injection of glucose into the portal vein. This response was obliterated by cutting the splanchnic nerves, but not by severing the vagi. Shimazu (34, 35) found that electrical stimulation of the vagus nerve activated glycogen synthase in both intact and pancreatectomized rabbits, and Shimazu and Fujimoto (36) reported an increased rate of incorporation of [^{14}C]glucose into liver glycogen with electrical stimulation of the vagus in intact and pancreatectomized rabbits. Most interestingly, Lautt (37) proposed that the gut factor previously discussed is actually an autonomic reflex arc. Although current evidence disputes the existence of a gut factor, a portal factor may very well exist, at least in the dog. Furthermore, it may be an autonomic reflex that senses the presence of an arterial-portal glucose gradient and acts on the liver to cause net glucose uptake. Recent work by Chap et al. (38, 39) offers some support to this hypothesis, suggesting that atropine and adrenergic blockers may alter net hepatic glucose uptake after oral glucose in dogs. Other possible mechanisms include a humoral factor or some form of local autoregulation. For example, Drapanas (40) reported a marked increase in portal vein serotonin

levels after delivery of hypertonic glucose into the duodenum or jejunum. Gastric inhibitory peptide does not seem to affect the hepatic response to peripherally infused glucose (38).

In summary, this study shows that in overnight-fasted, conscious dogs the intraportal route of glucose delivery brings about net hepatic glucose uptake even in the presence of basal insulin levels. The distribution of the intraportally infused glucose to the liver and peripheral tissues is similar to that seen after an oral glucose load, and is significantly different from that seen with peripheral glucose delivery. The arterial-portal glucose gradient may be the signal that triggers net hepatic glucose uptake during intraportal glucose delivery. This study indicates that a gut factor is not involved in triggering net hepatic glucose uptake after intraportal or oral glucose delivery, but that a portal factor is necessary for normal glucose distribution. Such a factor would be advantageous to the animal in that it would provide a means of differentiating hyperglycemia of exogenous origin, i.e., a meal, from hyperglycemia of endogenous origin, for example, a response to stress.

Acknowledgments

The authors acknowledge the excellent technical assistance of Ray Green, Jon Hastings, Laurel Brown, and Keith Carr. We also thank Patsy Raymer for her excellent secretarial assistance.

This investigation was supported by National Institutes of Health grant AM18243-12.

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