Portal Serotonin Infusion and Glucose Disposal in Conscious Dogs

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Whether serotonin (5-hydroxytryptamine [5-HT]) enhances net hepatic glucose uptake (NHGU) during glucose infusion was examined in conscious 42-h–fasted dogs, using arteriovenous difference and tracer ([3-^{3}H]glucose) techniques. Experiments consisted of equilibration (−120 to −30 min), basal (−30 to 0 min), and experimental (0–390 min) periods. During the experimental period, somatostatin, fourfold basal intraportal insulin, basal intraportal glucagon, and peripheral glucose (to double the hepatic glucose load) were infused. In one group of dogs (SER; $n = 8$), saline was infused intraportally from 0 to 90 min (P1), and 5-HT was infused intraportally at 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ from 90 to 150 (P2), 150 to 210 (P3), and 210 to 270 (P4) min, respectively. In the other group (SAL; $n = 8$), saline was infused intraportally from 0 to 270 min. NHGU in SAL was 12.4 ± 2.3, 14.9 ± 2.7, 13.4 ± 2.1, and 15.1 ± 1.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in P1 to P4, respectively, whereas NHGU in SER averaged 13.2 ± 3.0, 16.4 ± 2.4, 19.0 ± 2.4 ($P < 0.05$ vs. SAL), and 22.0 ± 2.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.05$ vs. SAL). Nonhepatic glucose uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) in SAL was 31.7 ± 4.9, 43.9 ± 5.1, 55.1 ± 5.6, and 66.2 ± 8.6 during P1 to P4, respectively, whereas in SER, the corresponding values were 26.1 ± 5.7, 31.6 ± 9.4, 35.1 ± 7.6 ($P < 0.05$ vs. SAL), and 34.7 ± 7.7 ($P < 0.05$ vs. SAL). Intraportal 5-HT enhances NHGU but blunts nonhepatic glucose uptake, raising the possibility that hepatic-targeted 5-HT or 5-HT receptor agonists might reduce postprandial hyperglycemia. *Diabetes* 53:14–20, 2004

Poor control of postprandial glycemia in type 2 diabetes is associated with elevated rates of all-cause mortality (1), making postprandial hyperglycemia an attractive pharmaceutical target. Individuals who have type 2 diabetes and receive monoamine oxidase inhibitors demonstrate more frequent episodes of hypoglycemia (2), and those who take selective serotonin reuptake inhibitors have improved glucose tolerance (3) compared with similar individuals who do not take these drugs. Moreover, intraperitoneal administration of serotonin (5-hydroxytryptamine [5-HT]) or its precursor 5-hydroxytryptophan has a hypoglycemic effect in mice (4). 5-HT accumulated in both the liver and the brain of the mice, leaving a question of which tissue was responsible for the hypoglycemia. However, in subsequent studies, the mice were also treated with carbidopa, an inhibitor of peripheral but not central aromatic amino acid decarboxylase. Neither hypoglycemia nor hepatic accumulation of 5-HT was observed in carbidopa-treated mice, although brain levels of 5-HT were even higher than in the absence of carbidopa. Thus, it seemed that hypoglycemia was related to the elevation of hepatic 5-HT (5). We hypothesized that 5-HT can reduce postprandial glycemia by enhancing NHGU and examined this hypothesis in conscious dogs in which the pancreatic hormones and hepatic glucose load (HGL) could be fixed.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Studies were carried out on 16 conscious 42-h–fasted mongrel dogs of either sex with a mean weight of 24 ± 1 kg. Diet and housing were as previously described (6), and the protocol was approved by the Vanderbilt University Medical Center Animal Care Committee.

Approximately 16 days before study, each dog underwent a laparotomy for placement of ultrasonic flow probes around the portal vein and the hepatic artery, as well as for insertion of silicone rubber catheters for sampling in a hepatic vein, the portal vein, and a femoral artery and for infusion into a splenic and a jejunal vein as described in detail elsewhere (6,7). Criteria for study were as previously described (6,7).

On the morning of the study, catheters and flow probe leads were exteriorized from their subcutaneous pockets (6,7). The splenic and jejunal catheters were used for intraportal infusion of insulin (Eli Lilly & Co., Indianapolis, IN), glucagon (GlucaGen; Bedford Laboratories, Bedford, OH), and 5-HT (5-HT creatinine sulfate complex; Sigma, St. Louis, MO). Angiocaths (Deseret Medical, Sandy, UT) were inserted into three peripheral veins.

Experimental design. Each experiment consisted of a 90-min equilibration period (−120 to −30 min), a 30-min basal period (−30 to 0 min), and a 270-min experimental period (0 to 270 min) divided into four subperiods (P1, 0–90 min; P2, 90–150 min; P3, 150–210 min, and P4, 210–270 min). At −120 min, a primed, continuous infusion of [3-^{3}H]glucose and a continuous infusion of indocyanine green dye (5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were begun in all dogs, with the exception of two that did not receive [3-^{3}H]glucose. At 0 min, a constant peripheral infusion of somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was begun to suppress endogenous insulin and glucagon secretion. Insulin was infused intraportally at 1.2 $mU \cdot kg^{-1} \cdot min^{-1}$ (fourfold basal), and glucagon (0.55 ng $\cdot kg^{-1} \cdot min^{-1}$) was replaced intraportally in basal amounts. In addition, a primed continuous variable rate infusion of 50% dextrose was begun through a peripheral vein, to maintain the HGL twofold basal. During P1, all dogs received intraportal saline infusion. At the end of P1, the dogs were divided into two groups of eight each. In the SAL group, the intraportal saline infusion continued for the remainder of the study. In the SER group, 5-HT was infused into the portal vein at 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during P2, P3, and P4, respectively. 5-HT was infused intraportally because it appears in the portal vein after being absorbed from the intestinal lumen (where it is released by

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5-HT, 5-hydroxytryptamine; GIR, glucose infusion rate; HGL, hepatic glucose load; NEFA, nonesterified fatty acid; NHGU, net hepatic glucose uptake; nonHGU, nonhepatic glucose uptake.

TABLE 1
Serotonin concentrations and net hepatic balance and hormone concentrations in 42-h–fasted conscious dogs with insulin and glucagon clamped at fourfold basal and basal concentrations, respectively, hepatic glucose load at twofold basal, and saline or 5-HT infused intraportally

Parameter and group	Basal period	Experimental period			
		P1	P2	P3	P4
Arterial blood 5-HT (mg/l)					
SAL	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
SER	0.5 ± 0.1	0.5 ± 0.1	1.2 ± 0.3	1.9 ± 0.3*	2.5 ± 0.5*
Net hepatic 5-HT balance ($\mu g \cdot kg^{-1} \cdot min^{-1}$)					
SAL	0.6 ± 1.5	−0.5 ± 2.0	0.9 ± 1.2	2.1 ± 0.9	3.2 ± 1.3
SER	−1.5 ± 1.9	2.0 ± 1.8	−7.6 ± 5.3	−30.7 ± 13.7*	−47.7 ± 26.4*
Arterial plasma insulin (pmol/l)					
SAL	49 ± 8	107 ± 13	114 ± 12	134 ± 11	131 ± 10
SER	46 ± 6	103 ± 10	139 ± 10	139 ± 9	148 ± 10
Hepatic sinusoidal insulin (pmol/l)					
SAL	107 ± 19	384 ± 39	420 ± 46	360 ± 39	383 ± 30
SER	147 ± 21	370 ± 29	429 ± 35	426 ± 39	436 ± 33
Arterial plasma glucagon (ng/l)					
SAL	46 ± 8	41 ± 5	37 ± 5	37 ± 4	36 ± 5
SER	33 ± 3	32 ± 3	33 ± 3	30 ± 2	32 ± 3
Hepatic sinusoidal glucagon (ng/l)					
SAL	46 ± 7	48 ± 4	47 ± 5	45 ± 4	43 ± 4
SER	37 ± 5	39 ± 3	40 ± 3	39 ± 4	38 ± 4
Arterial plasma cortisol (nmol/l)					
SAL	69 ± 19	52 ± 16	44 ± 11	51 ± 6	47 ± 12
SER	84 ± 20	70 ± 6	91 ± 14	93 ± 14	194 ± 22*

Data are means ± SE, $n = 8$/group. SAL dogs received intraportal saline infusion during P1 to P4; SER dogs received saline during P1 and intraportal 5-HT at 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively, during P2 to P4. Negative values indicate net hepatic uptake. *$P < 0.05$ vs. SAL.

the enterochromaffin cells and certain neurons in the intestine) or secreted across the basolateral membrane of enterocytes (8,9). Moreover, the intraportal infusion allowed us to target the liver specifically with the 5-HT. The infusion rates were chosen to create a range of physiologic and pharmacologic 5-HT concentrations to allow us to determine whether 5-HT could alter carbohydrate metabolism. The 5-HT infusion rates during P2 and P3 gave rise to arterial blood 5-HT concentrations similar to those evident in four dogs during the first 2 h after an oral glucose tolerance test (1.5 mg glucose/kg; M.C.M. and A.D.C., unpublished observations). The infusion rate during P4 created 5-HT concentrations greater than those observed during the oral glucose tolerance test but similar to those reported in patients with irritable bowel syndrome (10).

Femoral artery, portal vein, and hepatic vein blood samples were taken every 15 to 30 min throughout the study as previously described (6,7). Arterial blood samples were also taken every 5 min throughout the experimental period to monitor the glucose level (6,7). After completion of each experiment, the animal was killed with an overdose of pentobarbital.

Processing and analysis of samples. Hematocrit; blood glucose, lactate, and glycerol; and plasma glucose, nonesterified fatty acids (NEFAs), insulin, glucagon, cortisol, catecholamines, and [^{3}H]glucose were measured as described previously (6,7,11). 5-HT concentrations were determined on whole blood by a high-performance liquid chromatography–amperometric assay (12) with a coefficient of variation of 4%. The assay results comprise the 5-HT present in plasma as well as that in the formed elements of the blood, including platelets. Methy-5-HT was added to each sample as an internal standard; in addition, a known amount of 5-HT was added to aliquots of blood to assess recovery. It is common to report platelet concentrations of 5-HT because platelets do not synthesize 5-HT, but they do exhibit transporter-mediated uptake of 5-HT from plasma, and once within the platelet, 5-HT is protected from monoamine oxidase activity. However, whole-blood and platelet concentrations have been shown to correlate well in a large population of humans, and measurement of blood concentrations avoids any errors introduced by alterations in platelet 5-HT transport (13–15).

Calculations and data analysis. Hepatic blood flow was measured using ultrasonic flow probes and by use of indocyanine green extraction. The two methods yielded similar results, but the data reported here were calculated with the ultrasonic-determined flows because their measurement did not require an assumption regarding the relative contribution of arterial and portal flow to total hepatic blood flow.

The rate of glucose delivery to the liver, or HGL; net hepatic substrate balance; net hepatic fractional substrate extraction; net hepatic carbon retention; hepatic sinusoidal insulin and glucagon concentrations; and nonhepatic glucose uptake (nonHGU) were calculated as described previously (16). During the first hour of glucose infusion, the nonHGU was corrected for the glucose required to fill the pool, using a pool fraction of 0.65 (17) and assuming that the volume of distribution for glucose equaled the volume of the extracellular fluid, or ~22% of the dog's weight (18). For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using correction factors (ratio of the blood to the plasma concentration) as previously established in our laboratory (6,19,20).

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

RESULTS

5-HT, insulin, glucagon, cortisol, and catecholamine concentrations. Arterial blood 5-HT concentrations remained basal throughout the experiments in SAL, but in SER, the concentrations increased progressively with increasing infusion rates ($P < 0.05$ for P2–P4 vs. basal and P1 in the same group; $P < 0.05$ for P3–P4 in SER vs. SAL; Table 1). Net hepatic 5-HT balance remained ~0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in SAL. In the SER group, net hepatic 5-HT balance was near 0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during the basal period and P1. The livers of this group then switched to progressively increasing net uptake ($P < 0.05$ for P3–P4 vs. basal and P1 in the same group and for SER vs. SAL).

The plasma insulin levels increased approximately three- to fourfold and remained stable during P1 to P4 in both groups (Table 1). Arterial and hepatic sinusoidal plasma glucagon concentrations were basal and indistinguishable in both groups throughout the experiments (Table 1). Arterial plasma cortisol concentrations remained basal in SAL throughout P1 to P4. In SER, the

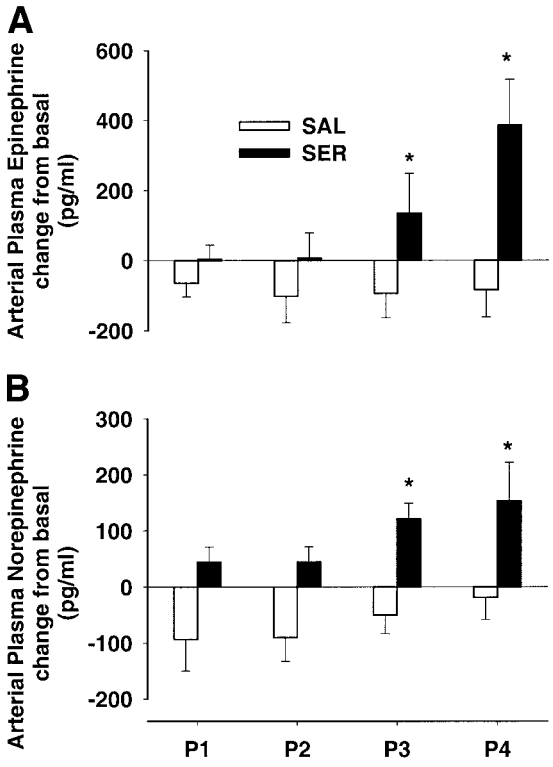

FIG. 1. Changes in arterial plasma epinephrine (*A*) and norepinephrine (*B*) concentrations in dogs that received somatostatin, intraportal infusions of insulin (fourfold basal) and glucagon (basal), and peripheral glucose infusion to double the HGL. During P1, both groups received intraportal saline, and this continued throughout P2 to P4 in the SAL group. The SER group received intraportal infusion of serotonin at 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively, during P2 to P4. Basal arterial plasma concentrations of epinephrine and norepinephrine were 219 ± 82 and 251 ± 101 pg/ml, respectively, in SAL and 315 ± 98 and 219 ± 38 pg/ml, respectively, in SER (NS between groups); *n* = 8/group; **P* < 0.05 between groups.

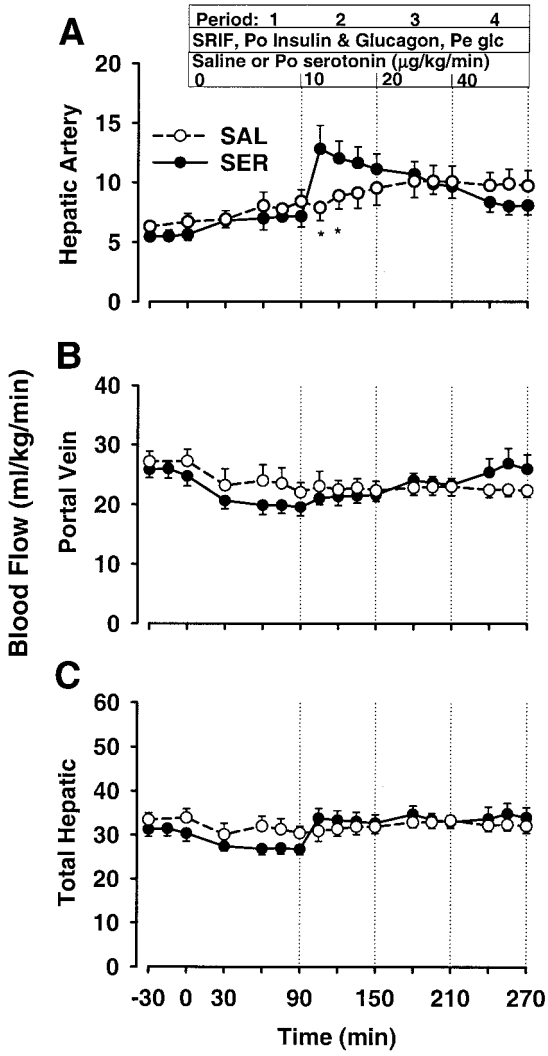

FIG. 2. Hepatic artery (*A*), portal vein (*B*), and total hepatic (*C*) blood flow in dogs that received somatostatin, intraportal (Po) infusions of insulin (fourfold basal) and glucagon (basal), peripheral (Pe) glucose infusion to double the HGL, and intraportal infusion of serotonin (SER) at the rates shown or saline (SAL); *n* = 8/group; **P* < 0.05 between groups.

cortisol concentrations remained at basal levels during P1 to P3 but increased significantly during P4 (194 ± 22 vs. basal values of 84 ± 20 nmol/l; $P < 0.05$).

Arterial plasma concentrations of epinephrine and norepinephrine decreased slightly from basal during P1 to P4 in SAL (Fig. 1). In SER, the concentrations remained near basal during P1 and P2 but rose significantly over basal concentrations during P3 and P4.

Hepatic blood flow, blood glucose concentrations, and HGL. Portal vein blood flow decreased significantly in SAL during P1 as a response to somatostatin infusion (from 27 ± 2 [basal] to 22 ± 2 $ml \cdot kg^{-1} \cdot min^{-1}$; $P < 0.05$) and did not change significantly thereafter (Fig. 2). There was a concomitant increase in hepatic artery flow (from 6 ± 1 [basal] to 8 ± 1 $ml \cdot kg^{-1} \cdot min^{-1}$ in P1, subsequently increasing to 9 ± 1 in P2 and to 10 ± 1 $ml \cdot kg^{-1} \cdot min^{-1}$ in both P3 and P4). As a consequence, total hepatic blood flow was relatively stable throughout the experiments. The SER group displayed a similar response to SAL during P1 (a fall in portal vein flow with a compensatory rise in hepatic artery flow). During P2, however, there was a sharp rise in hepatic artery flow (from 7 ± 1 to 12 ± 1 $ml \cdot kg^{-1} \cdot min^{-1}$; $P < 0.001$). Hepatic artery blood flow gradually declined during P3 and P4 (10 ± 1 and 8 ± 1 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). However, portal vein blood flow increased significantly during P3 and P4 (24 ± 1 and 27 ± 2 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively), so that total hepatic blood flow in the last three periods was significantly greater than that in P1.

Arterial blood glucose levels in SAL increased from a basal value of 4.5 ± 0.1 to 9.0 ± 0.1 mmol/l during all experimental periods (Fig. 3). In SER, the arterial glucose concentration increased from 4.5 ± 0.1 to 9.5 ± 0.1 mmol/l during P1 and then was reduced to 8.5 ± 0.2 mmol/l during P2 to P4 to maintain the HGL at a constant rate despite the changes in hepatic blood flow.

The HGLs were not significantly different between groups at any time (Fig. 3). In SAL, the HGL increased from 147 ± 8 to 266 ± 15 $mg \cdot kg^{-1} \cdot min^{-1}$ in P1, and it was stable at 286 ± 11 $mg \cdot kg^{-1} \cdot min^{-1}$ during P2 to P4. In SER, HGL increased from 137 ± 9 to 250 ± 14 $mg \cdot kg^{-1} \cdot min^{-1}$ in P1 and was maintained at 285 ± 16 $mg \cdot kg^{-1} \cdot min^{-1}$ during P2 to P4.

Net hepatic glucose balance and net hepatic fractional glucose extraction. The groups exhibited a similar rate of net hepatic glucose output during the basal period. Coincident with the start of the experimental

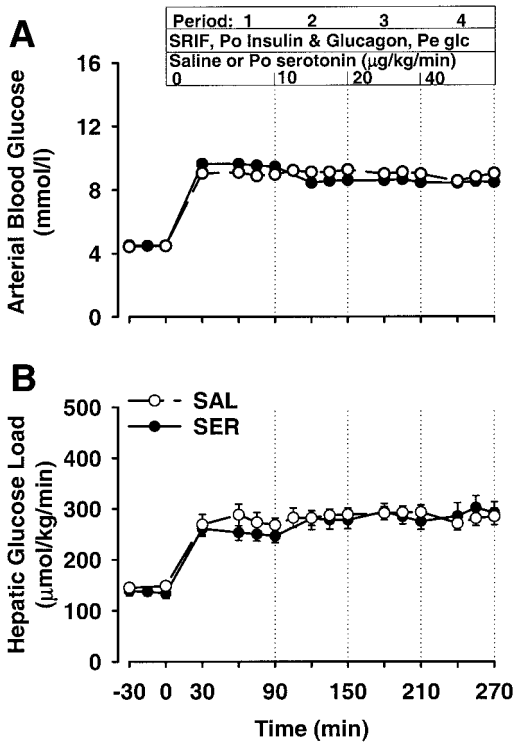

FIG. 3. Arterial blood glucose (*A*) and HGL (*B*). See legend to Fig. 2 for description of study conditions; *n* = 8/group. There were no significant differences between groups.

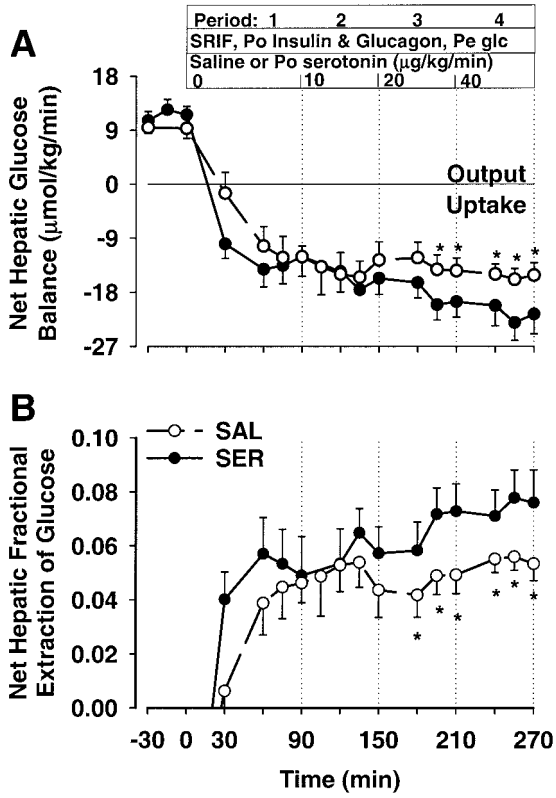

FIG. 4. NHGU (*A*) and fractional extraction (*B*) of glucose. See legend to Fig. 2 for description of study conditions; *n* = 8/group; **P* < 0.05 between groups.

period, they switched from net production to net uptake, with the rates being no different between groups during P1 and P2 (~13.5 μmol · kg^{-1} · min^{-1}; Fig. 4). Subsequently, the rate of NHGU remained relatively stable in SAL (14.4 ± 1.8 and 15.5 ± 1.8 μmol · kg^{-1} · min^{-1} in P3 and P4, respectively), whereas in SER, it increased to 19.0 ± 2.4 and 22.0 ± 2.9 μmol · kg^{-1} · min^{-1} during P3 and P4, respectively (both $P < 0.05$ vs. SAL). Similarly, the net hepatic fractional extraction of glucose did not differ significantly between groups during P1 and P2, but during P3 and P4, the fractional extraction in SER (0.069 ± 0.010 and 0.076 ± 0.010, respectively) was greater ($P < 0.05$) than that evident in SAL (0.049 ± 0.006 and 0.055 ± 0.005, respectively).

Glucose infusion rates, nonHGU, and glucose R_a and R_d. The glucose infusion rate (GIR) in SAL increased steadily in a time-dependent manner during P1 to P4 (44.1 ± 5.9, 59.4 ± 6.4, 68.7 ± 6.1, and 79.2 ± 9.3 μmol · kg^{-1} · min^{-1}, respectively; Fig. 5). In contrast, in SER, the time-dependent increase in GIR was blunted (43.9 ± 5.0, 48.0 ± 8.5, 54.1 ± 7.3 [$P < 0.05$ vs. SAL], and 56.7 ± 7.5 μmol · kg^{-1} · min^{-1} [$P < 0.05$ vs. SAL] during P1–P4, respectively). Similar to GIR, nonHGU increased over time in SAL (31.7 ± 4.9, 44.7 ± 4.7, 54.3 ± 5.5, and 63.7 ± 8.9 μmol · kg^{-1} · min^{-1} during P1–P4, respectively). In SER, the time-dependent increase in nonHGU was significantly reduced (26.1 ± 5.7, 31.6 ± 9.4, 35.1 ± 7.6 [$P < 0.05$ vs. SAL], and 34.7 ± 7.7 μmol · kg^{-1} · min^{-1} [$P < 0.05$ vs. SAL] in P1–P4, respectively).

Endogenous glucose R_a decreased similarly during the experimental period in both groups and did not differ significantly between groups at any time (Table 2). Glucose R_d did not differ between groups during the basal period, P1, or P2. However, during P3 and P4, the rates in SER were 28 and 38%, respectively, less than those in SAL ($P < 0.05$; Table 2).

Lactate metabolism and net hepatic carbon retention. The arterial blood lactate concentrations were similar in the two groups until P4, when the values in SER became significantly ($P < 0.05$) greater than those in SAL (Table 3). During the basal period, both groups exhibited a similar rate of net hepatic lactate uptake (Table 3). After the experimental period began, net hepatic lactate balance changed from uptake to output in both groups. After a peak in P1, net hepatic lactate output declined to ~4 μmol · kg^{-1} · min^{-1} during P2 to P4 in SAL. The SER group tended to reach a greater peak during P1 ($P = 0.055$ vs. SAL). This tendency toward greater net hepatic lactate output in SER versus SAL continued throughout P2 and P3, reaching statistical significance during P4.

Net hepatic carbon retention (data not shown) did not differ in SER and SAL during P1 (7.2 ± 2.3 vs. 9.4 ± 1.6 μmol · kg^{-1} · min^{-1}) and P2 (13.2 ± 3.1 vs. 12.6 ± 2.4 μmol · kg^{-1} · min^{-1}). However, net hepatic carbon retention tended to be enhanced in SER versus SAL during P3 (15.1 ± 2.3 vs. 11.4 ± 1.8 μmol · kg^{-1} · min^{-1}; $P = 0.10$) and P4 (17.4 ± 2.6 vs. 13.0 ± 1.5 μmol · kg^{-1} · min^{-1}; $P = 0.07$).

Glycerol and NEFA metabolism. Arterial glycerol concentrations and net hepatic glycerol uptake were reduced ~55% by hyperglycemia and hyperinsulinemia (Table 3). Neither parameter changed in SAL during P2 to P4, but both the arterial concentrations and net hepatic glycerol uptake increased significantly in SER during P3 and P4, returning to values no different from basal.

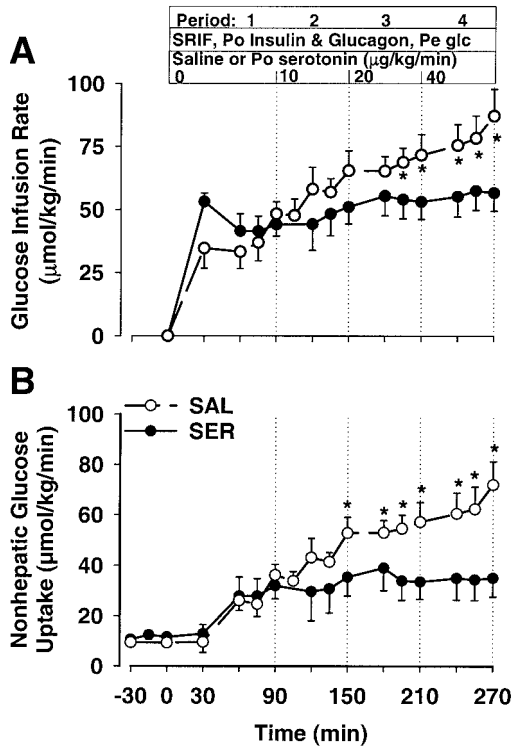

FIG. 5. GIR (*A*) and nonHGU (*B*). See legend to Fig. 2 for description of study conditions; n = 8/group; *P < 0.05 between groups.

Arterial NEFA concentrations and net hepatic NEFA uptake changed in a manner similar to glycerol, decreasing 89 to 90% during P1 in both groups and then remaining unchanged in SAL during P2 to P4. In SER, the concentrations and net hepatic uptake of NEFAs increased during P3 and P4, but they remained significantly below basal, and variability in the values precluded detection of a statistical difference from SAL.

DISCUSSION

A twofold increase in the HGL in the presence of fourfold basal insulin and basal glucagon concentrations resulted in NHGU of ~13 μmol · kg^{-1} · min^{-1} in both groups. Between P1 and P4, the rate of NHGU increased only 25% in SAL. During infusion of 5-HT at 10 μg · kg^{-1} · min^{-1}, there was no increase in NHGU compared with SAL. However, during 5-HT infusion at 20 and 40 μg · kg^{-1} · min^{-1}, NHGU increased 32 and 42%, respectively, over the corresponding rates in SAL ($P < 0.05$ for both increments). By the end of the experimental period, NHGU in SER had increased 67% over the rate during P1 ($P < 0.05$ vs. the increase in SAL). Similar to NHGU, net hepatic carbon retention was virtually identical between the groups during P1 and P2. During P3 and P4, net hepatic carbon retention was 32 and 34%, respectively, greater in SER than in SAL ($P = 0.10$ and 0.07, respectively). Because we administered 5-HT in three increasing steps during P2 to P4, it is not possible to separate the effects of time from those of dosage. Nevertheless, it is possible to conclude definitively that 5-HT had an impact on NHGU.

The effects of 5-HT on the liver could have resulted either from a direct effect on the hepatocytes or from a secondary signal initiated elsewhere. In regard to a potential direct effect of 5-HT on hepatocytes, the 5-HT$_{2B}$ receptor is known to be expressed in greatest abundance in the liver and kidney in humans (21). In regard to the second possibility, Niijima (22) determined that intraportal injection of 5-HT resulted in a decrease in the afferent firing rate in the hepatic branch of the vagus nerve and a stimulation of efferent firing in the pancreatic branch of the vagus, similar to the effect of intraportal glucose injection (23), suggesting a mechanism by which 5-HT could elicit a neural signal that could enhance NHGU.

A significant reduction of glucose uptake in the nonhepatic tissues occurred during 5-HT infusion at 20 and 40 μg · kg^{-1} · min^{-1} (35 and 46%, respectively, compared with the corresponding rates of nonHGU in SAL). Despite the concurrent increase in NHGU, total body glucose disposal was negatively affected. This was evident both in the reduction in GIR (21 and 28% with 5-HT 20 and 40 μg · kg^{-1} · min^{-1}, respectively) and in the blunting of glucose R_d (28 and 37%, respectively) in comparison with the rates in SAL. The cause of the blunting of nonHGU cannot be determined from the current data, but several possibilities exist. First, because the circulating 5-HT concentrations rose, it is possible that 5-HT had a direct effect to reduce glucose uptake by muscle, the predominant insulin-responsive peripheral tissue involved in glucose disposal in the dog. This explanation seems unlikely, however, because peripheral administration of the 5-HT precursor 5-hydroxytryptophan or various selective serotonin reuptake inhibitors to monoamine oxidase inhibitor–treated mice has a hypoglycemic rather than a hyperglycemic effect (24–27), an action apparently independent of changes in insulin concentrations (25–27). Moreover, 5-HT

TABLE 2
Endogenous glucose R_a and R_d in 42-h–fasted conscious dogs with insulin and glucagon clamped at fourfold basal and basal concentrations, respectively, hepatic glucose load at twofold basal, and saline or 5-HT infused intraportally

Group	Basal period	Experimental period			
		P1	P2	P3	P4
R_a (μmol · kg^{-1} · min^{-1})					
SAL	14.0 ± 1.4	5.4 ± 1.3	5.0 ± 1.9	2.5 ± 2.0	3.0 ± 2.7
SER	13.9 ± 0.5	4.8 ± 1.6	6.3 ± 1.2	4.1 ± 1.6	3.2 ± 2.0
R_d (μmol · kg^{-1} · min^{-1})					
SAL	13.6 ± 1.3	49.2 ± 7.2	58.0 ± 6.8	70.0 ± 6.4	84.1 ± 8.9
SER	14.2 ± 0.2	46.8 ± 2.3	51.0 ± 4.9	52.5 ± 4.5*	55.0 ± 3.1*

Values are means ± SE, n = 8 for SAL and n = 5 for SER (two dogs in SER did not receive tritiated glucose, and the data for one animal in that group were not usable). SAL dogs received intraportal saline infusion during P1 to P4; SER dogs received saline during P1 and intraportal 5-HT at 10, 20, and 40 μg · kg^{-1} · min^{-1}, respectively, during P2 to P4. *P < 0.05 vs. SAL.

TABLE 3
Arterial lactate, glycerol, and NEFA concentrations and net hepatic balances in 42-h–fasted conscious dogs with insulin and glucagon clamped at fourfold basal and basal concentrations, respectively, hepatic glucose load at twofold basal, and saline or 5-HT infused intraportally

Group	Basal period	Experimental period			
		P1	P2	P3	P4
Arterial blood lactate (μmol/l)					
SAL	465 ± 86	802 ± 31	848 ± 101	956 ± 70	1,093 ± 62
SER	680 ± 205	1,252 ± 190	1,116 ± 143	1,129 ± 95	1,555 ± 208*
Net hepatic lactate balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)					
SAL	−9.3 ± 1.9	6.0 ± 2.0	4.2 ± 1.4	3.9 ± 1.6	4.2 ± 1.9
SER	−9.4 ± 1.1	11.8 ± 2.2	7.6 ± 1.6	7.3 ± 0.9	9.5 ± 1.6*
Arterial blood glycerol (μmol/l)					
SAL	96.7 ± 14.9	41.4 ± 10.4	40.1 ± 10.8	39.7 ± 10.4	40.3 ± 11.4
SER	92.2 ± 7.1	46.3 ± 6.5	47.1 ± 10.8	74.5 ± 11.6*	82.7 ± 16.7*
NHGU ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)					
SAL	2.2 ± 0.4	0.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.3
SER	2.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	1.6 ± 0.4*	1.9 ± 0.5*
Arterial plasma NEFA (μmol/l)					
SAL	850 ± 86	102 ± 20	92 ± 14	85 ± 15	92 ± 22
SER	696 ± 79	142 ± 46	111 ± 24	154 ± 57	124 ± 40
Net hepatic NEFA uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)					
SAL	2.8 ± 0.6	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
SER	3.0 ± 0.5	0.4 ± 0.2	0.2 ± 0.1	0.7 ± 0.3	0.3 ± 0.2

Data are means ± SE, $n = 8$/group. SAL dogs received intraportal saline infusion during P1 to P4; SER dogs received saline during P1 and intraportal 5-HT at 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively, during P2 to P4. Negative values for balance indicate net hepatic lactate uptake; rates of net hepatic uptake are shown as positive values for substrates for which there was no net release by the liver. *$P < 0.05$ vs. SAL.

is reported to have either no effect (28) or a stimulatory effect (29) on glucose uptake in isolated skeletal muscle. The stimulatory effect of 5-HT can be reproduced with the 5-HT_{2A} agonist methylserotonin and apparently involves a mechanism other than the insulin signaling pathway (29).

The reduction of nonHGU with the higher rates of 5-HT infusion might also have resulted from a secondary effect of 5-HT, mediated via a change in muscle perfusion. 5-HT stimulates endothelial nitric oxide synthase, causing the release of nitric oxide and other labile compounds that relax smooth muscle. It has also been reported to stimulate the release of a vasoconstrictive eicosanoid from the endothelium (30). In addition, 5-HT has been shown to be a type B vasoconstrictor in skeletal muscle. Type B vasoconstrictors cause the channeling of blood away from the vessels that supply oxygen and nutrients to skeletal muscle ("nutritive flow") and into other vessels (those that supply connective tissue and associated adipocytes, creating "nonnutritive flow"), thus reducing muscle and hindlimb nutrient uptake (28,31). In this regard, the circulating concentrations of 5-HT achieved during P4 were higher than those that we have observed in four conscious dogs after intragastric glucose administration (peak value 1.8 ± 0.2 μg/ml; M.C.M. and A.D.C., unpublished data) and those reported in normal-fed rats (14). The high concentrations may have accentuated the peripheral effects of 5-HT, i.e., reduction in substrate uptake (28).

A final possible explanation for the suppression of nonHGU at the higher rates of 5-HT infusion is that the stress-induced increases in cortisol and catecholamines brought about a relative insulin resistance. Cortisol did not change during 5-HT infusion at 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$, but both epinephrine and norepinephrine increased. All three rose significantly during infusion of 5-HT at 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The association of elevated 5-HT with increased excretion of catecholamines and cortisol has been described in the clinical literature (32). That the increases in cortisol and the catecholamines were physiologically relevant is underscored by the fact that the glycerol and NEFA concentrations increased during infusion of 5-HT at 20 and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$, indicating that lipolysis was stimulated. The involvement of stress hormones in 5-HT effects is also demonstrated by that fact that activation of the peripheral 5-HT_{2A} receptor by 5-methoxytryptamine caused hyperglycemia in intact but not adrenalectomized rats (33). Thus, increases in these stress-response hormones could explain at least part of the blunting of nonHGU observed. It should be noted that they likely inhibited NHGU, causing the increase that we observed to be an underestimate of 5-HT action at the liver.

In conclusion, intraportal infusion of 5-HT enhanced NHGU under hyperglycemic, hyperinsulinemic conditions. We did not examine the effects of peripheral 5-HT infusion, and thus it is not possible to speculate on whether the effects observed were specific to the intraportal route of 5-HT delivery. The rise in circulating levels of catecholamines and cortisol and that higher rates of 5-HT infusion are likely to cause abdominal cramping limit the use of 5-HT in physiologic studies. Nevertheless, the effects of 5-HT on hepatic and whole-body glucose metabolism are intriguing and deserve further study, using pharmacologic agents that can elevate endogenous 5-HT levels, specific receptor agonists, or hepatic-targeted 5-HT (34). The presence of numerous subtypes of 5-HT receptors suggests that a targeted receptor agonist might serve to enhance NHGU while minimizing suppression of nonHGU.

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