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Role of the hepatic sympathetic nerves in the regulation of net hepatic glucose uptake and the mediation of the portal glucose signal

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DiCostanzo, Catherine A., Dominique P. Dardevet, Doss W. Neal, Margaret Lautz, Eric Allen, Wanda Snead, and Alan D. Cherrington. Role of the hepatic sympathetic nerves in the regulation of net hepatic glucose uptake and the mediation of the portal glucose signal. *Am J Physiol Endocrinol Metab* 290: E9–E16, 2006. First published August 16, 2005; doi:10.1152/ajpendo.00184.2005.—Portal glucose delivery enhances net hepatic glucose uptake (NHGU) relative to peripheral glucose delivery. We hypothesize that the sympathetic nervous system normally restrains NHGU, and portal glucose delivery relieves the inhibition. Two groups of 42-h-fasted conscious dogs were studied using arteriovenous difference techniques. Denervated dogs (DEN; $n = 10$) underwent selective sympathetic denervation by cutting the nerves at the celiac nerve bundle near the common hepatic artery; control dogs (CON; $n = 10$) underwent a sham procedure. After a 140-min basal period, somatostatin was given along with basal intraportal infusions of insulin and glucagon. Glucose was infused peripherally to double the hepatic glucose load (HGL) for 90 min (*P1*). In *P2*, glucose was infused intraportally (3–4 mg·kg^{-1}·min^{-1}), and the peripheral glucose infusion was reduced to maintain the HGL for 90 min. This was followed by 90 min (*P3*) in which portal glucose infusion was terminated and peripheral glucose infusion was increased to maintain the HGL. *P1* and *P3* were averaged as the peripheral glucose infusion period (PE). The average HGLs (mg·kg^{-1}·min^{-1}) in CON and DEN were 55 ± 3 and 54 ± 4 in the peripheral periods and 55 ± 3 and 55 ± 4 in *P2*, respectively. The arterial insulin and glucagon levels remained basal in both groups. NHGU (mg·kg^{-1}·min^{-1}) in CON averaged 1.7 ± 0.3 during PE and increased to 2.9 ± 0.3 during *P2*. NHGU (mg·kg^{-1}·min^{-1}) was greater in DEN than CON ($P < 0.05$) during PE (2.9 ± 0.4) and failed to increase significantly (3.2 ± 0.2) during *P2* (not significant vs. CON). Selective sympathetic denervation increased NHGU during hyperglycemia but significantly blunted the response to portal glucose delivery.

autonomic nervous system; liver; canine

POSTPRANDIAL HYPERGLYCEMIA is a concern for individuals with type 2 diabetes. The ability of the liver and peripheral tissues to increase their uptake of glucose after food injestion is therefore an area of interest. There are three major determinants of net hepatic glucose uptake (NHGU): the levels of insulin and glucagon in the blood, the glucose load reaching the liver, and the route of glucose delivery. NHGU increases with an increase in insulin concentration (31) and decreases with an elevation of glucagon (17). Likewise, hepatic glucose uptake is positively correlated with the glucose load. Finally, it has been shown that, when the glucose level is higher in the portal vein than in the hepatic artery, NHGU is augmented (2).

The stimulus for this response has been termed the portal glucose signal. Previous studies carried out in our laboratory have shown that activation of the portal signal not only increases NHGU but also reduces glucose uptake by muscle (2, 18, 33).

In the 1960s, Shimazu et al. (43, 44) showed that the autonomic nerves from the hypothalamus control glycogen metabolism. Fluro gold and transsynaptic tracer pseudorabies virus techniques have confirmed the presence of both parasympathetic and sympathetic nerve terminals within the liver, as well as direct connections between hypothalamic centers and the liver (6, 12, 23). These autonomic nerves innervate the liver along three routes: the portal vein, the hepatic artery, and the bile ducts. Efferent innervation by both parasympathetic and sympathetic nerve fibers has been shown to be responsible for hepatic hemodynamics, bile flow regulation, and control of carbohydrate and lipid metabolism (50). Stimulation of the sympathetic efferents results in an increase in glucose output by the liver through rapid activation of glycogen phosphorylase (39–41, 50) as well as an increase in phospho*enol*pyruvate carboxykinase (PEPCK) activity. Stimulation of the parasympathetic efferents results in an increase in glycogen synthesis because of activation of glycogen synthase and a decrease or complete inactivation of PEPCK activity (39–42). Afferent signals also emanate from the liver. Afferent fibers constitute 90% of the fibers in the hepatic vagus nerve (48). They have been shown to convey information regarding plasma glucose (37) and other nutrients as well as to be responsible for osmoregulation, ionoreception, and baroreception (51).

Although extensive research has been carried out relating to hepatic afferents and efferents in the euglycemic and hypoglycemic states (8, 16, 21, 22, 24, 26, 34), less is known about the hyperglycemic state, and it remains unclear how the portal signal may be mediated by these nerves. Total hepatic denervation eliminates the ability of the liver to discriminate between portal and peripheral glucose delivery (3), reinforcing the notion that the response to the portal glucose signal is neurally mediated (32, 36). In addition, glucose-sensitive neurons in the portal vein (32) have a discharge rate that is inversely correlated with portal vein glucose concentration. Because stimulation of either the parasympathetic or sympathetic nerves affects glucose metabolism, it is likely that these nerves play a role in mediating the effect of the portal glucose signal.

Several studies have examined the role of the parasympathetic nerves in bringing about the effect of the portal glucose

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signal. Stumpel and Jungermann (48) showed in the perfused rat liver that the effect of the portal signal could be abolished by the addition of atropine but mimicked by an infusion of ACh in the portal vein, leading to the conclusion that the effect was mediated by the parasympathetic nerves. We showed that an adrenergic blockade (portal vein phentolamine and propanolol infusion) and coincident cholinergic stimulation (portal vein ACh infusion), brought about in the presence of hyperinsulinemic, hyperglycemic conditions, increased NHGU by 1.8 mg·kg^{-1}·min^{-1} when compared with controls (46). These results were inconclusive, however, because portal vein administration of ACh caused an increase in hepatic artery blood flow, leading to a rise in the glucose and insulin loads to the liver. Thus the increase in NHGU was in part load dependent. The possibility also exists that the mediator which increased flow may have also increased NHGU. Because increased hepatic artery flow does not occur in response to portal glucose delivery, this raises a question as to the relevance of the above finding. In line with this, our laboratory has recently shown (7) that cooling the vagus nerves, which interrupts parasympathetic firing (22), had no effect on NHGU in the presence of the portal signal. This suggests that the parasympathetic efferents may not be involved in the transmission of the portal signal.

Our attention has thus turned to the sympathetic nerves and their possible role in mediating the effects of the portal glucose delivery. We hypothesize that the sympathetic nervous system exerts a restraining effect on hepatic glucose uptake that can be reversed by the entry of glucose in the portal vein. We suggest that eliminating the sympathetic nerves using selective hepatic sympathectomy would result in an increase in NHGU in response to peripheral glucose infusion and a reduction in the augmentation of NHGU in response to the portal glucose signal.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Studies were carried out on conscious 42-h-fasted mongrel dogs with a mean weight of 23.1 ± 0.4 kg. A fast of this duration was chosen because it produces a metabolic state resembling that in the overnight-fasted human and results in liver glycogen levels in the dog that are at a stable minimum. All animals were maintained on a diet of meat (Kal Kan, Vernon, CA) and chow (Purina Lab Canine Diet No. 5006; Purina Mills, St. Louis, MO) composed of 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight. The animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the protocol was approved by the Vanderbilt University Medical Center Animal Care Committee.

Approximately 16 days before study, each dog underwent a laparotomy under general anesthesia (0.02 mg/kg buprenorphine hydrochloride presurgery and ~1% isoflurane inhalation anesthetic during surgery), and silicone rubber catheters for blood sampling were inserted in the hepatic vein, the portal vein, and a femoral artery as described in detail elsewhere (30). Catheters for hormone infusion were also placed in a splenic and a jejunal vein. Selective sympathetic denervation (DEN, *n* = 10) or sham denervation (CON, *n* = 10) occurred at the celiac nerve bundle near the common hepatic artery. The nerve fibers that heavily encased the common hepatic artery were resected from 1–2 cm distal to the origin of the hepatic artery at the celiac trunk to several centimeters past the hepatic branches of the common hepatic artery. The hepatic arterial branches themselves were also skeletonized, and the gastroduodenal artery was stripped of nerves. All nerves surrounding the portal vein and its branches, as well as the vagus nerve, were left intact. Transonic flow probes

(Transonic Systems, Ithaca, NY) were placed around the portal vein and the hepatic artery. The catheters were filled with saline containing heparin (200,000 U/l; Abbott Laboratories, North Chicago, IL), their free ends were knotted, and they, along with the free ends of the Doppler leads, were placed in two separate subcutaneous pockets.

Approximately 2 days before each study, blood was drawn to determine the leukocyte count and the hematocrit for each animal. The dog was studied only if it had a leukocyte count <18,000/mm³, a hematocrit >35%, a good appetite (as evidenced by consumption of the entire daily ration), and normal stools.

On the morning of the study, the catheters and Doppler leads were exteriorized from their subcutaneous pockets using local anesthesia (2% lidocaine; Abbott). The contents of each catheter were aspirated, and the catheters were flushed with saline. The splenic and jejunal catheters were used for intraportal infusion of insulin (Eli Lilly, Indianapolis, IN) and glucagon (Glucagen; Novo Nordisk, Bagsvaerd, Denmark). Angiocaths (Deseret Medical; Becton-Dickinson, Sandy, UT) were inserted in the left cephalic vein for indocyanine green (ICG) infusion (Sigma Immunochemicals, St. Louis, MO), the right cephalic vein for peripheral glucose infusion, and the left saphenous vein for somatostatin (Bachem, Torrance, CA) and *p*-aminohippuric acid (PAH; Sigma) infusions. Each dog was allowed to stand quietly in a Pavlov harness throughout the experiment.

Experimental design. Each experiment consisted of a 100-min equilibration period (−140 to −40 min), a 40-min basal period (−40 to 0 min), and a 270-min experimental period (0 to 270 min) that was divided into three 90-min periods denoted *P1*, *P2*, and *P3*. In all experiments, a constant infusion of ICG dye (0.076 mg/min) was initiated at −140 min. At 0 min, a constant infusion of somatostatin (0.8 µg·kg^{-1}·min^{-1}) was begun to suppress endogenous insulin and glucagon secretion. Glucagon (0.57 ng·kg^{-1}·min^{-1}) and insulin (0.3 mU·kg^{-1}·min^{-1}) were then replaced intraportally at basal rates. In addition, a primed continuous peripheral infusion of 50% dextrose was begun at *time 0* so that the blood glucose could quickly be clamped at the desired hyperglycemic level (~235 mg/dl). During *P1* [time (*t*) = 0 to *t* = 90], glucose was infused peripherally to double the hepatic glucose load (HGL) in both groups. During *P2* (*t* = 90 to *t* = 180 min), a portal glucose infusion of 20% dextrose at (3–4 mg·kg^{-1}·min^{-1}) was initiated to activate the portal signal, and the peripheral glucose infusion was decreased so that the same glucose load to the liver seen in *P1* was maintained in *P2*. During *P3* (*t* = 180 to *t* = 270 min), the portal glucose infusion was terminated, and the peripheral glucose infusion was again adjusted to match the HGLs across the three periods. The peripheral glucose infusion rate was adjusted based on the plasma glucose levels and the hepatic blood flow in each individual dog. PAH was added to the portal glucose infusate to assess proper mixing with the blood in the portal and hepatic veins, as previously described (33). *P1* and *P3* will be referred to as peripheral glucose periods, whereas *P2* will be referred to as the portal glucose delivery period.

Femoral artery, portal vein, and hepatic vein blood samples were taken every 20 min during the basal period (−40 to 0 min) and every 15 min for the last 0.5 h of each experimental period (*P1*, *P2*, and *P3*). The arterial and portal samples were taken simultaneously, and hepatic vein samples were collected ~30 s later to compensate for transit time of glucose through the liver. Arterial blood samples were also taken every 5 min from 0 to 270 min of the experimental period to allow changes to be made in the glucose infusion rate as necessary. The total volume of blood withdrawn did not exceed 20% of the animal's blood volume, and two volumes of normal saline were infused for each volume of blood withdrawn.

After completion of each experiment, the animal was killed with an overdose of pentobarbital sodium, tissue samples from all seven liver lobes were frozen rapidly with clamps kept in liquid nitrogen, and samples were stored at −70°C. This tissue was then used to assess the completeness of the denervation. The norepinephrine content in each liver lobe was measured by HPLC, as previously described (28).

Processing and analysis of samples. The collection and immediate processing of blood samples have been described previously (15). Four to eight 10-μl aliquots of plasma from each sample were immediately analyzed for glucose using the glucose oxidase technique in a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin and glucagon concentrations were determined by RIA, as previously described (33). PAH was also measured in perchloric acid-deproteinized blood, as previously described (33). The methods by which the cortisol, lactate, and glycerol concentrations were measured have also been previously described (33).

Calculations and data analysis. An intraportal infusion of glucose has the possibility of mixing poorly with the blood in the laminar flow of the portal circulation. Thus it was critical to assess the mixing of the portal infusate in *P2*. This was accomplished by comparing the recovery of PAH (which was mixed with the portal glucose infusion of D20) in the portal and hepatic veins with the PAH infusion rate using an approach described elsewhere (33). In each group, 13 dogs were studied, and 3 dogs were not included because of poor mixing, as defined previously (29). In the control and denervated animals that were retained, the ratio of PAH recovery in the portal vein to the PAH infusion rate was 1.0 ± 0.1 to 1.0 ± 0.1, whereas the ratio of PAH recovery in the hepatic vein to the PAH infusion rate was 0.9 ± 0.03 to 1.0 ± 0.04, respectively (a ratio of 1.0 would represent perfect mixing). Hepatic blood flow (HBF) was measured using transonic flow probes and by use of ICG according to the method of Leevy et al. (25). The results obtained with transonic flow probes and ICG were not significantly different, but the data reported here were calculated using the transonic-determined flows because their measurement did not require an assumption regarding the distribution of the arterial and portal vein contribution to hepatic blood flow.

An indirect (I) method was used to assess NHGU to minimize the potential errors that arise due to any imperfect mixing of the infused glucose in the blood. Thus HGL was calculated as

$$load_{in}(I) = (G_A \times HBF) + GIR_{PO} - GUG$$

where Load$_{in}$ is the load of glucose entering the liver, G_A is the arterial blood glucose concentration, GIR_{PO} is the portal glucose infusion rate, and GUG is the uptake of glucose by the gastrointestinal tract, derived from the data in the peripheral infusion period.

The load of a substrate exiting (Load$_{out}$) the liver was calculated as

$$load_{out} = (G_H \times HBF)$$

where G_H represents the hepatic vein glucose concentration.

Net hepatic glucose balance (NHGB) was thus calculated as

$$NHGB = load_{out} - load_{in} \text{ (I).}$$

Hepatic glucose balance and fractional extraction were also calculated using a direct calculation that has been described previously (38). The results obtained did not differ significantly from those obtained using the indirect calculation. Net fractional substrate extraction by the liver was calculated directly as the ratio of NHGB to Load$_{in}$. Nonhepatic glucose uptake was calculated as previously described (13). The hepatic substrate load and net hepatic balance for lactate and glycerol, hepatic sinusoidal insulin and glucagon concentrations, and net gut glucose uptake were calculated as described previously (38).

For all glucose balance calculations, glucose concentrations were converted from plasma to blood values using previously determined (18, 19) conversion factors (the mean of the ratio of the blood value to the plasma concentration). The use of whole blood glucose values ensures accurate hepatic balance measurements regardless of the characteristics of glucose entry in the erythrocyte.

Statistical analysis. All data are presented as means $\pm$ SE. Time course data were analyzed with repeated-measures ANOVA. Independent *t*-tests were used for any comparisons of mean data. Statistical significance was accepted at $P < 0.05$.

RESULTS

Hepatic sympathetic denervation. Liver norepinephrine levels (Table 1) were assessed using HPLC. The average norepinephrine level in the CON group was 658 ± 68 ng/g liver, whereas, in the DEN group, it was only 10 ± 4 ng/g liver. These values indicate a >98% denervation.

Arterial and portal plasma glucose levels. The arterial plasma glucose levels in the control group were 238 ± 3, 236 ± 7, and 242 ± 3 mg/dl during *P1*, *P2*, and *P3*, respectively, whereas the portal vein plasma glucose levels were 235 ± 3, 255 ± 8, and 239 ± 3 mg/dl, respectively (Fig. 1*A*). The arterial plasma glucose levels (236 ± 3, 223 ± 3, 235 ± 2 mg/dl) in the DEN group were similar to those seen in the control group, as were the portal vein glucose levels (234 ± 3, 243 ± 4, and 233 ± 2 mg/dl) during *P1*, *P2*, and *P3*, respectively (Fig. 1*B*).

Arterial plasma and liver sinusoidal insulin and glucagon. Arterial and liver sinusoidal plasma insulin and glucagon levels were maintained at basal values throughout the experiment in both groups (Fig. 2).

Hepatic blood flow and glucose load. Hepatic blood flow (ml·kg^{-1}·min^{-1}) was similar in all three periods (*P1*, *P2*, and *P3*) in CON (32 ± 2, 30 ± 2, and 32 ± 2) and DEN (31 ± 2, 32 ± 2, and 33 ± 2), respectively (Table 2). Because the glucose levels and hepatic blood flows were similar in the two groups, there were no differences in the HGL across time or between treatments (Table 2). The average HGLs (mg·kg^{-1}·min^{-1}) in the CON and DEN groups were 53 ± 3 and 54 ± 4 in *P1*, 55 ± 3 and 55 ± 4 in *P2*, and 57 ± 3 and 55 ± 5 in *P3*, respectively. There were no statistical differences in the total glucose infusion rates between the two groups across the three periods. The total glucose infusion rates (mg·kg^{-1}·min^{-1}) in *P1*, *P2*, and *P3* were 3.5 ± 0.4, 4.1 ± 0.2, and 4.1 ± 0.2 in the CON group, respectively. The total glucose infusion rates in the DEN group were 4.3 ± 0.4, 4.8 ± 0.5, and 4.6 ± 0.3 in *P1*, *P2*, and *P3*, respectively.

Blood levels and net hepatic balance of lactate and glycerol. Both groups exhibited net lactate uptake in the basal period. Hyperglycemia resulted in a switch to net hepatic lactate output that was similar in both groups and constant over time. The arterial blood lactate levels rose steadily over the three periods in both groups because of net hepatic lactate output (Table 3).

Arterial glycerol levels and net hepatic glycerol uptake fell rapidly in response to hyperglycemia in both groups and

Table 1. *Hepatic norepinephrine content in each of the seven lobes of 42-h-fasted dogs with innervated or denervated livers*

Lobe	Norepinephrine Content, ng/g liver		NE Remaining, %
	CON	DEN	
Caudate	476 ± 83	2.5 ± 2.7	0.5
Left central	524 ± 126	4.3 ± 3.8	0.8
Left lateral	545 ± 90	3.8 ± 3.2	0.6
Left posterior	939 ± 202	3.8 ± 2.3	0.4
Quadrate	582 ± 82	29.1 ± 21.4	5.0
Right lateral	546 ± 94	27.6 ± 12.6	5.0
Right central	716 ± 149	4.4 ± 3.6	0.6

Data are means $\pm$ SE; $n = 10$ dogs in each group. Con, control; DEN, denervated; NE, norepinephrine.
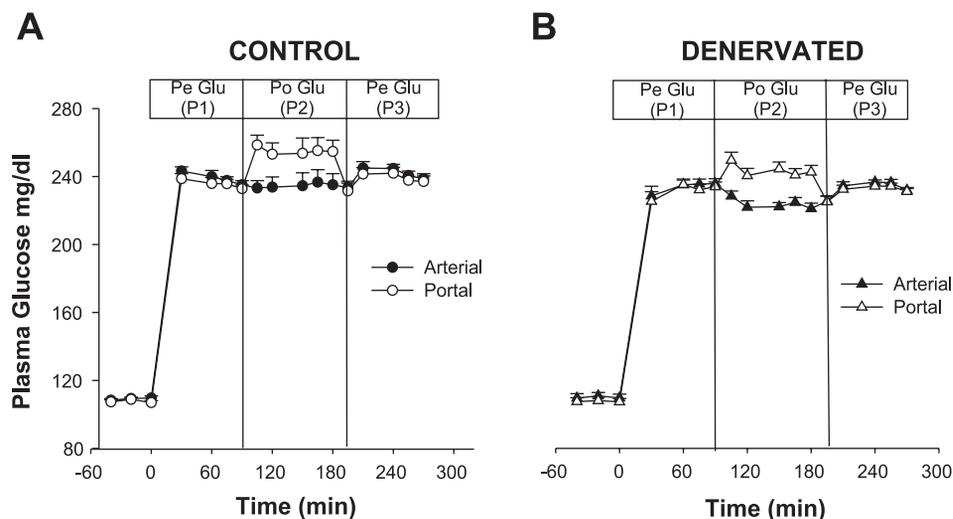
Fig. 1. Arterial and portal plasma glucose levels in control (*A*) and hepatic denervated (*B*) 42-h-fasted conscious dogs maintained on a pancreatic clamp during the basal and experimental periods (*n* = 10/group). Data are means ± SE. Pe, peripheral; Po, portal; *P1–P3*, *periods 1–3*.

eventually plateaued at an average of ~50 μmol/l and ~1 μmol·kg^{-1}·min^{-1} (Table 3), respectively (no differences between groups).

NHGB and nonhepatic glucose uptake. Basal net hepatic glucose ouput was not statistically different between the two groups (1.8 ± 0.3 and 1.9 ± 0.2 mg·kg^{-1}·min^{-1} in the CON and DEN groups, respectively). Because of the drift that is typically seen in NHGU over time (Table 4), *P1* and *P3* can be averaged to obtain the mean NHGU response to peripheral

hyperglycemia. Thus the average NHGU during peripheral glucose infusion in the CON group was 1.7 ± 0.3 mg·kg^{-1}·min^{-1}. NHGU in response to peripheral glucose infusion was significantly greater ($P < 0.05$) in the DEN group (2.9 ± 0.4 mg·kg^{-1}·min^{-1}; Fig. 3*A*). NHGU significantly increased during *P2* (portal glucose infusion) in the CON group, increasing to 2.9 ± 0.3 (Δ1.2) mg·kg^{-1}·min^{-1}, but did not change significantly in the DEN group [3.2 ± 0.2 (Δ0.3) mg·kg^{-1}·min^{-1}].
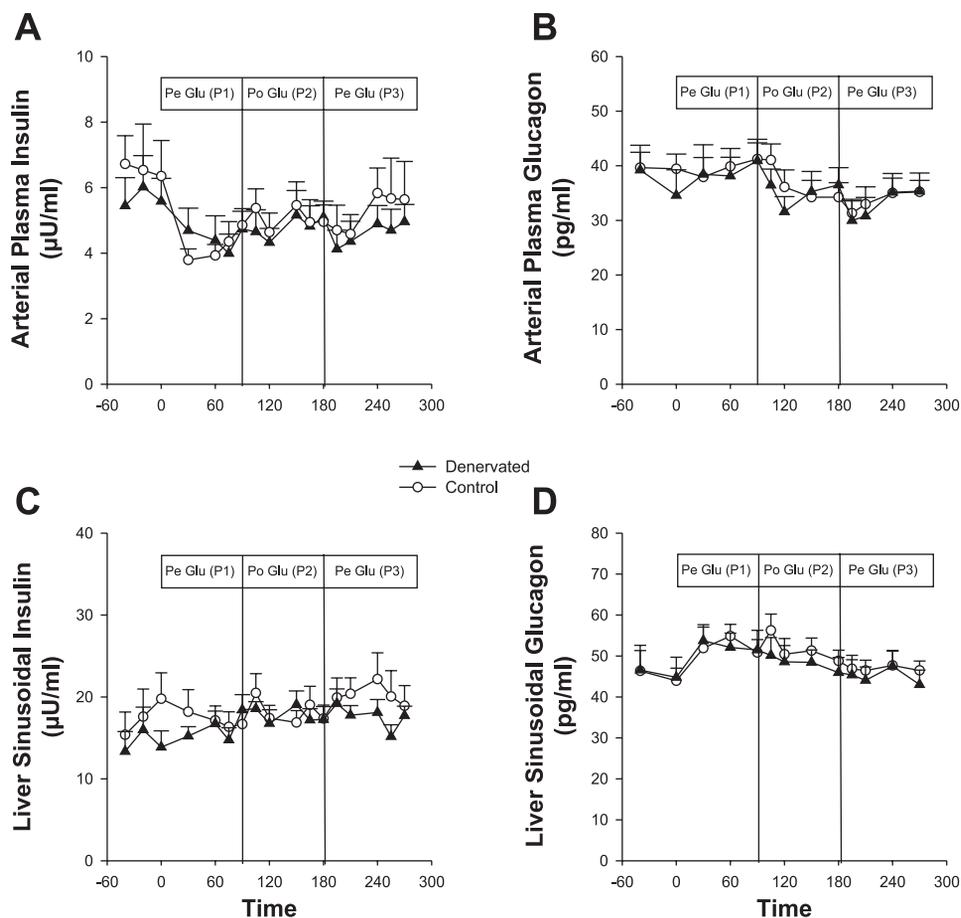

Fig. 2. *A*: arterial plasma insulin. *B*: arterial plasma glucagon. *C*: liver sinusoidal insulin. *D*: liver sinusoidal glucagon levels in 42-h-fasted conscious dogs maintained on a pancreatic clamp during the basal and experimental periods in both the control (CON) and denervated (DEN) groups (*n* = 10/group). Data are means ± SE.

Table 2. *Total hepatic blood flow and hepatic glucose load during the experimental periods in 42-h-fasted dogs with innervated or denervated livers*

Time, min	Period 1				Period 2					Period 3				
	30	60	75	90	105	120	150	165	180	195	210	240	255	270
Total hepatic blood flow, ml·kg^{-1}·min^{-1}														
CON	30±2	31±2	32±2	32±3	31±2	30±2	30±2	30±2	30±3	31±3	31±2	32±2	31±3	32±2
DEN	31±2	32±2	31±2	31±2	32±2	32±2	32±3	31±2	32±2	33±2	32±2	33±2	34±2	34±2
Hepatic glucose load (mg/kg/min)														
CON	52±3	54±4	55±4	55±5	56±4	56±5	54±4	56±4	55±5	52±5	56±5	56±4	55±5	56±5
DEN	52±4	54±4	53±4	53±3	56±3	55±3	56±4	55±3	56±3	53±3	55±3	56±3	58±4	58±3

Data are means ± SE; n = 10 dogs in each group.

Net hepatic fractional glucose extraction was 3.1 and 5.4% in response to peripheral glucose infusion in the CON and DEN groups, respectively. It increased in response to portal glucose infusion in the CON group (5.4%; $P < 0.05$) but failed to increase significantly (6.5%) in the DEN group (Table 4).

The average nonhepatic glucose uptake rate seen in response to peripheral glucose infusion was 2.2 ± 0.1 mg·kg^{-1}·min^{-1} in the CON group and 1.6 ± 0.4 mg·kg^{-1}·min^{-1} in the DEN group (Table 4). Nonhepatic glucose uptake decreased significantly ($P < 0.05$) during portal glucose infusion ($P2$) in the CON group to 1.2 ± 0.4 ($\Delta 1.0$) mg·kg^{-1}·min^{-1}, but it did not change during the same time period in the DEN group [1.6 ± 0.4 ($\Delta 0.0$) mg·kg^{-1}·min^{-1}; Fig. 3*B*].

DISCUSSION

In this study, we tested the hypothesis that a basal inhibitory sympathetic tone to the liver blocks NHGU and that this inhibition is relieved by entry of glucose in the portal vein. Our findings support this concept. There was almost a doubling of NHGU during peripheral glucose infusion in dogs that had undergone selective hepatic sympathectomy compared with those that had not. Furthermore, when the liver was sympathetically denervated, the response to portal glucose delivery was reduced by ≈75%. The data regarding nonhepatic glucose uptake are less clear. Although nonhepatic glucose uptake was lower during peripheral glucose infusion in DEN and it did not change in response to portal glucose infusion, the study lacked the statistical power to allow definitive conclusions to be drawn.

Sympathetic nerve fibers reach the liver through the celiac ganglia, celiac plexus, and the splanchnic nerves (18, 19, 39–41). The sympathetic fibers form an anterior plexus around the hepatic artery, and Alexander (4) showed that the hepatic artery receives only sympathetic fibers (4). The parasympathetic fibers form a posterior plexus around the portal vein. The postganglionic parasympathetic nerves are derived from ganglia located at the hepatic hilus and within the portal spaces (14). These parasympathetic fibers are separate from any aminergic nerves found in the area (5, 11, 27, 35, 47, 49). To selectively eliminate sympathetic input to the liver, we removed the nerves associated with the hepatic arterial circulation. The success of the denervation was confirmed by a reduction of norepinephrine in all seven lobes of the liver, which averaged 98%. Neural regeneration and hepatic reinnervation of the dog liver requires 3 mo (20), and thus the denervation was still virtually complete 2 wk postsurgery. It is possible that some vagal innervation may have been compromised during surgery, but given the association of parasympathetic nerves with the portal vein, we believe that most of the parasympathetic fibers remained intact. They were thus still able to exert any regulatory effects that they might have on NHGU and nonhepatic glucose uptake. It should be noted that there were no differences in body weight, food consumption, or basal NHGU between the two groups, suggesting that the chronic decrease in hepatic sympathetic tone did not have any demonstrable baseline effect on the animals. It is important to note that we did not directly measure hepatic sympathetic tone during these experiments but assumed that the level of nor-

Table 3. *Arterial blood lactate and glycerol and net hepatic balance of lactate and glycerol during the basal and experimental periods on 42-h-fasted dogs with innervated or denervated livers*

	Basal	*P1*	*P2*	*P3*
Arterial blood lactate, μmol/l				
CON	384±52	845±104	1,064±78	1,036±107
DEN	532±114	1,195±153*	1,318±131*	1,331±128*
Net hepatic lactate balance, μmol·kg^{-1}·min^{-1}				
CON	−5.9±0.8	5.8±2.4	6.9±1.5	5.4±1.6
DEN	−7.0±1.5	8.3±2.7	7.3±1.9	7.7±1.8
Arterial blood glycerol, μmol/l				
CON	84±10	53±10	47±8	48±6
DEN	84±10	56±7	50±7	49±7
Net hepatic glycerol uptake, μmol·kg^{-1}·min^{-1}				
CON	1.8±0.2	1.1±0.2	1.0±0.2	1.0±0.2
DEN	2.1±0.3	1.2±0.2	1.1±0.2	1.0±0.2

Data are means ± SE; *n* = 10 dogs in each group. *P1–P3, periods 1–3;* −, uptake. *Significant statistical difference ($P < 0.05$) from CON.

Table 4. *Net hepatic glucose uptake, net hepatic glucose fractional extraction, and nonhepatic glucose uptake during experimental periods in 42-h-fasted dogs with innervated or denervated livers*

	P1	P2	P3
Net hepatic glucose uptake, mg·kg^{-1}·min^{-1}			
CON	1.4±0.4	2.9±0.3†	2.0±0.3
DEN	2.5±0.4*	3.2±0.2	3.3±0.5*
Net hepatic glucose fractional extraction, %			
CON	2.6±0.6	5.4±0.4†	3.6±0.3
DEN	4.9±0.5*	6.5±0.7	6.1±0.5*
Nonhepatic glucose uptake, mg·kg^{-1}·min^{-1}			
CON	2.1±0.2	1.2±0.4†	2.3±0.2
DEN	1.9±0.2	1.6±0.4	1.4±0.5

Data are means ± SE; $n = 10$ dogs in each group. $P < 0.05$, significant statistical difference from CON (*) and from *P1* and *P3* (†).

epinephrine in the liver reflected an elimination of sympathetic input to the liver.

Several variables that affect NHGU were minimized to accurately assess the effect of the selective sympathetic denervation on NHGU. Arterial insulin was maintained at a basal level in both groups to avoid the variability seen in the response of NHGU to a rise in insulin. Likewise, the arterial plasma glucagon concentrations were kept constant and basal. The arterial plasma glucose concentration was doubled and clamped across the three periods in both groups. Because there was no change in hepatic blood flow, the HGL to the liver was the same throughout the three experimental periods and was equal in both groups. Thus the differences in NHGU between the two groups cannot be explained by differences in plasma insulin or glucagon levels or the glucose load to the liver.

Prior work in our laboratory has examined the effect of portal glucose delivery on NHGU. In hyperglycemic experiments performed by both Pagliassotti et al. (33) and Hsieh et al. (18) in which both insulin and glucagon were kept basal while plasma glucose was doubled, peripheral glucose delivery resulted in average NHGUs (mg·kg^{-1}·min^{-1}) of 0.4 ± 0.3 and 1.5 ± 0.3, respectively. Thus the NHGU of 1.7 ± 0.3 mg·kg^{-1}·min^{-1} that we observed in response to peripheral glucose administration in the control animals of the present study is consistent with previous data. In our earlier studies (18, 33), when glucose was given intraportally at ~3.5 mg·kg^{-1}·min^{-1} and the HGLs were matched to those seen during peripheral glucose administration,

NHGU (mg·kg^{-1}·min^{-1}) increased to 2.3 ± 0.7 and 3.4 ± 0.5, respectively. Thus we have shown previously that the portal signal, on the background of hyperglycemia, basal insulin, and basal glucagon, increases NHGU an average of ~1.9 mg·kg^{-1}·min^{-1}. In the present control experiments, the increase in NHGU during portal vs. peripheral glucose delivery was 1.2 ± 0.2 mg·kg^{-1}·min^{-1}, in line with the increase we have observed previously.

The dogs with sympathetically denervated livers responded to hyperglycemia quite differently from the normal animals. The average NHGU during the peripheral glucose infusion period was 2.9 ± 0.4 mg·kg^{-1}·min^{-1}, a value almost double ($P < 0.05$) that seen under the same conditions in the dogs with innervated livers. With portal glucose delivery, NHGU increased to 3.2 ± 0.2 mg·kg^{-1}·min^{-1} in the DEN group, which is a similar value to that seen in the control animals (2.9 ± 0.3 mg·kg^{-1}·min^{-1}), yet only ~0.3 mg·kg^{-1}·min^{-1} greater than that seen during average peripheral glucose administration. This limited increase is not the result of a saturation of the hepatic response, since these rates of NHGU are significantly lower than rates we have previously shown to occur during portal glucose infusion (45) and lower than the peak rates observed during oral glucose delivery (1). Thus we conclude that the sympathetic efferents play an important role in the regulation of NHGU by exerting a basal inhibitory tone that limits glucose uptake in response to hyperglycemia of peripheral origin. This is consistent with previous work from our laboratory (8), which showed that cooling the vagus nerves (decreasing afferent vagal firing) in the presence of euglycemia and euinsulinemia decreased net hepatic glucose output, presumably by reflexively decreasing the efferent sympathetic outflow to the liver. It is interesting to note that we saw no differences in basal net hepatic glucose output between the two groups in this current study despite hepatic denervation. There are several possible explanations for the discrepancy between our two studies. First, it is possible that vagal cooling results in changes in addition to reduced sympathetic input to the liver. Alternatively, there may be a difference between acute and chronic sympathetcomy.

The question arises as to whether cutting the sympathetic efferents changed NHGU in response to glucose per se (i.e., regardless of the site of delivery) or if, in addition, it blocked the effect of the portal glucose signal. If the response was to glucose per se, then the portal glucose signal should have caused a change of ~1.2 mg·kg^{-1}·min^{-1} when glucose was
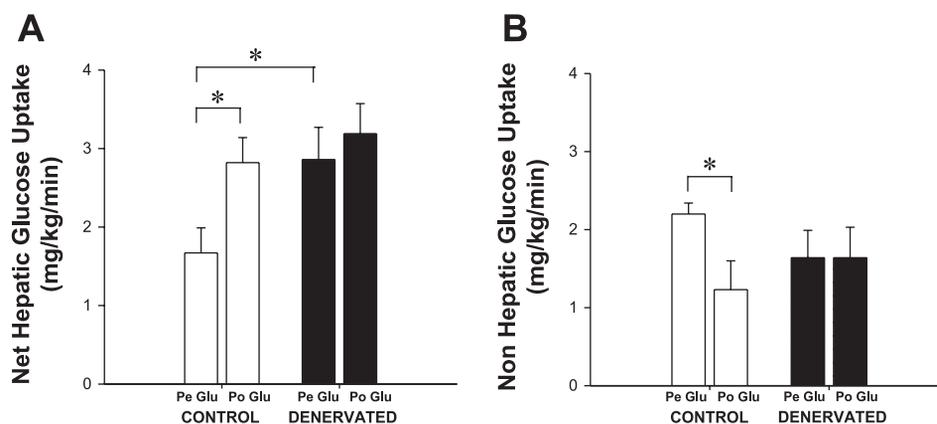
Fig. 3. Net hepatic glucose uptake (*A*) and nonhepatic glucose uptake (*B*) in 42-h-fasted conscious dogs maintained on a pancreatic clamp during the peripheral and portal glucose infusion periods in both the CON and DEN groups (*n* = 10/group). Data are means ± SE. *$P < 0.05$ compared with the CON group.

delivered intraportally in the hepatic denervated dogs. Because the change was only 0.3 mg·kg^{-1}·min^{-1}, these data suggest that reduced sympathetic tone to the liver is responsible for a significant portion of the increase in NHGU attributed to portal glucose delivery. It must be remembered, however, that we chronically removed sympathetic efferents to the liver. Chronic removal of sympathetic input may result in changes that increase the ability of glucose to increase NHGU, which might not occur in response to the acute removal of sympathetic input associated with portal glucose delivery. It is also possible that sympathetic denervation resulted in a saturation of the response to the portal glucose signal, thus masking any further input (i.e, positive stimuli) generated by portal glucose delivery. Thus, although our data suggest that the portal glucose signal increases NHGU by causing a decrease in sympathetic tone, further proof of this concept is still required.

Based on our data, we cannot draw a definitive conclusion about the additional involvement of a stimulatory signal in the response to portal glucose administration. In the control dogs, the response to portal glucose infusion was ~1.2 mg·kg^{-1}·min^{-1}, whereas in the denervated animals it was ~0.3 mg·kg^{-1}·min^{-1}. Taken at face value, this suggests that 75% of the response to portal glucose delivery is caused by removal of an inhibitory tone and 25% is because of the activation of a stimulatory signal. In the presence of the portal signal, there is an increase in glycogen synthase activation that could act as the stimulatory factor (33). It has also been suggested that the portal signal may cause the translocation of glucokinase, thus also contributing a stimulatory input (9). Unfortunately, the present study lacks the power needed to determine the quantitative contribution of any putative stimulatory signal to the overall response so that equal contribution by inhibitory and stimulatory factors remains possible.

Previous work in our laboratory by Shiota et al. (46) showed that an intraportal infusion of α- and β-adrenergic receptor antagonists (phentolamine and propanolol, respectively) did not enhance NHGU during hyperinsulinemia and hyperglycemia (achieved via peripheral glucose infusion). This is in apparent conflict with our current findings, but several issues should be noted. Intraportal infusion of the adrenergic blockers would have caused only a partial blockade of sympathetic tone to the liver if the dose of the blockers used was inadequate. Likewise, it is not clear if blockers delivered via the portal vein can reach the synapse and block endogenously released norepinephrine effectively. Additionally, phentolamine has been shown to stimulate postsynaptic (α$_1$) receptors while inhibiting presynaptic (α$_2$) receptors, resulting in an enhanced output of norepinephrine (52). It has also been shown that propanolol can have an intrinsic (partial agonist) effect on β-adrenergic receptors in the liver (10). The partial agonistic properties of these adrenergic antagonists may thus have counteracted any effects attributable to adrenergic blockade. Another way to reconcile our current observations and our previous findings would be to conclude that, in the present study, we removed afferent nerve fibers that travel with the efferent sympathetic nerves. For that to be the case, the afferent fibers would initiate an inhibitory signal to the liver through nonadrenergic mechanisms that would be blocked by sympathetic nerve section. This seems unlikely, since it has been shown that glucose-sensitive afferent fibers originating in the liver travel with the vagus nerves.

It has been shown that portal glucose delivery not only leads to an increase in hepatic glucose uptake but also to a decrease in nonhepatic glucose uptake (2, 18, 33). Under conditions similar to those seen in this experiment, Hsieh et al. (18, 19) showed an average nonhepatic glucose uptake of 4.3 ± 0.5 mg·kg^{-1}·min^{-1} during peripheral glucose administration, which decreased to 1.0 ± 0.3 mg·kg^{-1}·min^{-1} during portal glucose administration and then returned to 4.6 ± 0.7 mg·kg^{-1}·min^{-1} at the termination of the portal glucose signal. In the control animals of the current study, the average nonhepatic glucose uptake during the first peripheral glucose infusion period was 2.1 ± 0.2 mg·kg^{-1}·min^{-1}, decreasing significantly to 1.2 ± 0.4 mg·kg^{-1}·min^{-1} with portal glucose delivery. At the termination of the portal glucose administration, nonhepatic glucose uptake returned to 2.3 ± 0.2 mg·kg^{-1}·min^{-1}. Thus the response to portal glucose delivery in the control animals was consistent with previous data, although the nonhepatic glucose uptake was lower in *P1* and *P3* than in earlier studies for reasons that are not clear. In the animals with denervated livers, nonhepatic glucose uptake was only slightly less in response to peripheral glucose infusion (1.6 ± 0.4 mg·kg^{-1}·min^{-1}) than in the control dogs. There was no change in nonhepatic glucose balance in response to portal glucose infusion in the DEN group. Given the subtle difference noted above, however, one cannot draw a definitive conclusion regarding the role of sympathetic nerves in the mediation of the change in nonhepatic glucose uptake.

In summary, the sympathetic nerves exert an inhibitory tone on liver glucose uptake. Removal of this inhibition by selective hepatic sympathetic denervation leads to an increase in NHGU in response to hyperglycemia induced by peripheral glucose infusion. It also results in a blunting of the increase in NHGU seen in response to intraportal glucose delivery. The portal signal, therefore, leads to the removal of an inhibitory tone to the liver, which in turn allows NHGU to increase. Whether hepatic sympathetic nerves act in concert with an additional stimulatory signal still needs to be determined.

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