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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 814–824
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

The effect of vagal cooling on canine hepatic glucose metabolism in the presence of hyperglycemia of peripheral origin

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Received 19 August 2006; accepted 19 January 2007

Abstract

We examined the role of vagus nerves in the transmission of the portal glucose signal in conscious dogs. At time 0, somatostatin infusion was started along with intraportal insulin and glucagon at 4-fold basal and basal rates, respectively. Glucose was infused via a peripheral vein to create hyperglycemia ($\simeq$2 fold basal). At t = 90, hollow coils around the vagus nerves were perfused with −10°C or 37°C solution in the vagally cooled (COOL) and sham-cooled (SHAM) groups, respectively (n = 6 per group). Effectiveness of vagal blockade was demonstrated by increase in heart rate during perfusion in the COOL vs SHAM groups (183 ± 3 vs 102 ± 5 beats per minute, respectively) and by prolapse of the third eyelid in the COOL group. Arterial plasma insulin (22 ± 2 and 24 ± 3 μU/mL) and glucagon (37 ± 5 and 40 ± 4 pg/mL) concentrations did not change significantly between the first experimental period and the coil perfusion period in either the SHAM or COOL group, respectively. The hepatic glucose load throughout the entire experiment was 46 ± 1 and 50 ± 2 mg · kg^{-1} · min^{-1} in the SHAM and COOL groups, respectively. Net hepatic glucose uptake (NHGU) did not differ in the SHAM and COOL groups before (2.2 ± 0.5 and 2.9 ± 0.8 mg · kg^{-1} · min^{-1}, respectively) or during the cooling period (3.0 ± 0.5 and 3.4 ± 0.6 mg · kg^{-1} · min^{-1}, respectively). Likewise, net hepatic glucose fractional extraction and nonhepatic glucose uptake and clearance were not different between groups during coil perfusion. Interruption of vagal signaling in the presence of hyperinsulinemia and hyperglycemia resulting from peripheral glucose infusion did not affect NHGU, further supporting our previous suggestion that vagal input to the liver is not a primary determinant of NHGU.

1. Introduction

Postprandial hyperglycemia is a concern for individuals with type 2 diabetes mellitus. The ability of the liver and peripheral tissues to increase their uptake of glucose after food ingestion is therefore an area of interest. There are 3 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. Net hepatic glucose uptake increases with an increase in insulin concentration [1] and decreases with an elevation of glucagon [2]. 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, net hepatic glucose uptake is augmented [3]. 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 net hepatic glucose uptake but also reduces glucose uptake by muscle [3-5].

In the 1960s, Shimazu et al [6,7] showed that the autonomic nerves from the hypothalamus control glycogen metabolism. These autonomic nerves innervate the liver along 3 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 [8]. Stimulation of the

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doi:10.1016/j.metabol.2007.01.012

sympathetic efferents results in an increase in glucose output by the liver through rapid activation of glycogen phosphorylase [8-11] as well as an increase in phosphoenolpyruvate carboxykinase (PEPCK) activity. Stimulation of the parasympathetic efferents results in an increase in glycogen synthesis due to an activation of glycogen synthase and a decrease or complete inactivation of PEPCK activity [9-12]. Afferent nerves also emanate from the liver. Afferent fibers constitute 90% of the total fibers in the hepatic vagus nerves [13], and they have been shown to convey information regarding plasma glucose [14] and other nutrients to the brain as well as to be responsible for osmoregulation, ionoreception, and baroreception [15].

It has been shown that glucose-sensitive neurons in the portal vein [16] have a discharge rate that is inversely correlated with portal vein glucose concentration. A change in afferent firing is accompanied by a change in efferent firing in the pancreatic branch of the vagus nerve, the splanchnic nerve, and the adrenal nerve [17].

Although extensive research has been carried out relating to hepatic afferents and efferents in the euglycemic and hypoglycemic states [18-24], less is known about them in the hyperglycemic state. Furthermore, it remains unclear how they are involved in the augmentation of NHGU by portal glucose delivery. Total hepatic denervation eliminates the ability of the liver to discriminate between portal and peripheral glucose delivery [25], reinforcing the notion that the response to the portal glucose signal is neurally mediated [16,26]. Glucose delivery into the portal vein, but not a peripheral vein, resulted in a significant fall in hepatic norepinephrine spillover [27], an index of sympathetic signaling [28]. In a recent article by DiCostanzo et al [29], it was shown that the sympathetic efferent nerve fibers exert a tonic inhibition on NHGU. When these fibers were chronically transected, under hyperinsulinemic and hyperglycemic (resulting from peripheral glucose infusion) conditions, NHGU doubled. Thus, the sympathetic nervous system appears to play an important role in bringing about the portal signal. Nevertheless, a substantial body of evidence implicates the parasympathetic nervous system in the regulation of hepatic glucose uptake and disposition. Stimulation of parasympathetic nerves reduced hepatic glucose output in the cat [30] and activated glycogen synthase in the rabbit liver [31]. In addition, acute vagotomy in rats reduced hepatic glycogen synthesis after a glucose load [32]. We have previously observed enhanced activation of glycogen synthase in the presence of portal vs peripheral glucose delivery [4]. Moreover, in studies completed by Shiota et al [33], an intraportal acetylcholine infusion (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$), in the presence of hyperglycemia (brought about by peripheral glucose infusion) and hyperinsulinemia, increased NHGU in comparison to control dogs receiving saline (4.6 $\pm$ 0.6 vs 3.0 $\pm$ 0.6 mg $\cdot kg^{-1} \cdot min^{-1}$, respectively). Taken together, these data led to the conclusion that the parasympathetic nerves are likely involved in the increase of net hepatic glucose uptake in the postprandial state.

We hypothesized that when the portal vein glucose concentration is greater than the arterial concentration, vagal afferent firing falls and this triggers a reduction in sympathetic neural input to the liver concurrently with an increase in parasympathetic input. In a recent study [34], we showed that acute vagal blockage (cooling coils) brought about under hyperglycemic, hyperinsulinemic conditions in the presence of portal glucose delivery failed to alter NHGU. One might have predicted that the augmented afferent signal (ie, a greater reduction in firing) would have resulted in an increase in the withdrawal of sympathetic tone and an augmentation of parasympathetic input and thus an increase in NHGU. However, it is also possible that in the presence of the portal signal, NHGU was maximally stimulated, and therefore, it was impossible to augment it further. To shed further light on this topic we repeated the experiment in the absence of the portal glucose signal. If our hypothesis is correct, then one would predict an increase in NHGU to result from vagal cooling under this condition.

2. Research design and methods

2.1. Animals and surgical procedures

Studies were carried out on conscious 42-hour fasted mongrel dogs with a mean weight of 23.1 $\pm$ 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 and Use Committee.

Approximately 16 days before study, each dog underwent a laparotomy under general anesthesia (15 mg/kg Pentothal sodium (Abbott Laboratories, North Chicago, IL) presurgery and ~1% isoflurane inhalation anesthetic during surgery), and silicone rubber catheters were inserted for sampling in the hepatic vein, the portal vein, and a femoral artery as described in detail elsewhere [35]. Catheters for hormone infusion were also placed in a splenic and jejunal vein. Stainless steel cooling coils, with Silastic (Dow Corning, Midland, MI) extension tubing attached, were placed around the vagus nerves in the neck of all dogs as described previously [19]. 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 Transonic leads, were placed in 2 separate subcutaneous pockets.

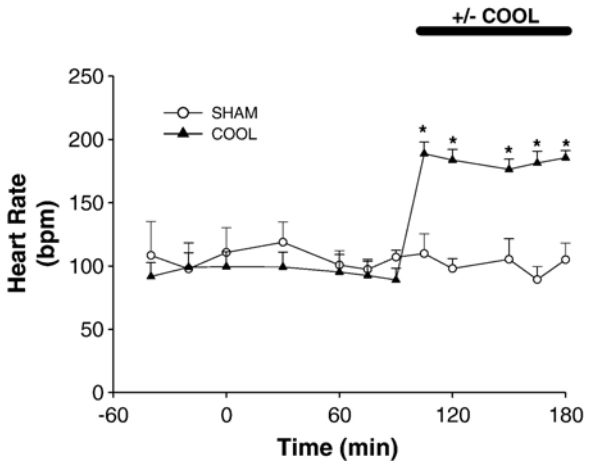

Fig. 1. Heart rate in SHAM and COOL 42-hour fasted conscious dogs maintained on a pancreatic clamp during the basal and experimental periods (n = 6 per group). Data are mean ± SEM. *$P < .05$.

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 of less than $<18\,000/mm^3$, a hematocrit of more than 35%, a good appetite (as evidenced by consumption of the entire daily ration), and normal stools.

On the morning of the study, catheters and Transonic 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 & Co, Indianapolis, IN) and glucagon (Glucagen, Novo Nordisk, Bagsvaerd, Denmark). Angiocaths (Deseret Medical, Becton Dickinson, Sandy, UT) were inserted into the left cephalic vein for indocyanine green infusion (Sigma Immunochemicals, St Louis, MO), the right cephalic vein for peripheral glucose infusion, and the left saphenous vein for somatostatin infusion (Bachem, Torrance, CA). Each dog was allowed to stand quietly in a Pavlov harness.

2.2. Experimental design

Each experiment consisted of a 100-minute equilibration period (−140 to −40 minutes), a 40-minute basal period (−40 to 0 minute), and a 180-minute experimental period (0-180 minutes), which was subsequently divided into two 90-minute periods. In all experiments, a constant infusion of indocyanine green dye (0.076 mg/min) was initiated at −140 minutes. At 0 minute, a constant infusion of somatostatin ($0.8\ \mu g \cdot kg^{-1} \cdot min^{-1}$) was begun to suppress endogenous insulin and glucagon secretion, and glucagon ($0.57\ ng \cdot kg^{-1} \cdot min^{-1}$) and insulin ($1.2\ mU \cdot kg^{-1} \cdot min^{-1}$) were replaced intraportally. 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). Glucose was infused peripherally to increase the hepatic glucose load 2-fold basal during the entire experimental period. At $t = 90$, the vagal coils were infused with a 50:50 methanol/saline solution at body temperature (37°C) in the sham-cooled (SHAM) dogs (n = 6) or at ≃10°C in the vagally cooled (COOL) dogs (n = 6), with the use of a digital temperature controller/circulator (Polyscience, Niles, IL). Effective cooling was confirmed by observation of a doubling in heart rate and bilateral Horner syndrome [36-38]. Earlier studies showed that the addition of atropine failed to induce any further change, suggesting a complete blockade was achieved with cooling alone [19]. A reservoir temperature of −10°C was associated with an exiting neck temperature of ~0°C and a bath return temperature of ~3°C.

Femoral artery, portal vein, and hepatic vein blood samples were taken every 20 minutes during the basal period (−40 to 0 minute) and every 15 minutes for the last half hour of each experimental period. The arterial and portal samples were taken simultaneously, and hepatic vein samples were collected ~30 seconds later to compensate for transit time of glucose through the liver. Arterial blood samples were also taken every 5 minutes from 0 to

Table 1
Arterial, portal, and total HBF in the basal and experimental periods in both SHAM and COOL 42-hour fasted conscious dogs

	Basal			exp + saline				exp ± COOL				
	−40	−20	0	30	60	75	90	105	120	150	165	180
Arterial blood flow ($mL \cdot kg^{-1} \cdot min^{-1}$)												
SHAM	5 ± 1	5 ± 1	5 ± 1	7 ± 1	7 ± 1	8 ± 1	7 ± 1	7 ± 2	7 ± 2	7 ± 2	8 ± 2	8 ± 1
COOL	6 ± 1	6 ± 1	7 ± 1	8 ± 1	9 ± 1	9 ± 1	9 ± 1	12 ± 2*	14 ± 2*,†	14 ± 2*,†	13 ± 2*,†	13 ± 2*,†
Portal blood flow ($mL \cdot kg^{-1} \cdot min^{-1}$)												
SHAM	27 ± 3	27 ± 2	27 ± 2	21 ± 1	21 ± 1	20 ± 2	21 ± 2	20 ± 1	21 ± 2	20 ± 1	20 ± 1	21 ± 1
COOL	27 ± 4	28 ± 4	26 ± 3	21 ± 3	20 ± 3	21 ± 3	21 ± 3	22 ± 3	22 ± 3	22 ± 3	23 ± 3	23 ± 3
Total HBF ($mL \cdot kg^{-1} \cdot min^{-1}$)												
SHAM	31 ± 3	31 ± 2	32 ± 2	27 ± 2	28 ± 2	27 ± 2	28 ± 3	27 ± 2	28 ± 2	27 ± 2	27 ± 1	28 ± 2
COOL	33 ± 4	34 ± 4	33 ± 3	29 ± 3	29 ± 3	30 ± 3	29 ± 3	34 ± 4	36 ± 4*	36 ± 4*	36 ± 4*	36 ± 4*

n = 6 in each group. exp indicates experimental period.
* $P < .05$ vs SHAM dogs.
† $P < .05$ vs exp + saline period in the respective group.

180 minutes of the experimental period to monitor the glucose concentration. The total volume of blood withdrawn did not exceed 20% of the animal's blood volume, and 2 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 and the catheter positions were verified.

2.3. Processing and analysis of samples

The collection and immediate processing of blood samples have been described previously [39].

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 radioimmunoassay, as previously described [4]. The methods by which the cortisol, lactate, and glycerol concentrations were measured have also been previously described [4].

2.4. Calculations and data analysis

Hepatic blood flow (HBF) was measured using transonic flow probes and by use of indocyanine green according to the method of Leevy et al [40]. The results obtained with transonic flow probes and dye 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 HBF.

The hepatic substrate load in ($Load_{in}$) was calculated as:

$$Load_{in} = (S_A \times AF) + (S_P \times PF)$$

where S_A and S_P are the arterial and portal vein substrate concentrations, respectively, and AF and PF are the hepatic arterial and portal vein blood or plasma flows, as appropriate for the substrate.

The load of substrate exiting the liver was calculated as

$$Load_{out} = (S_H \times HF)$$

where S_H represents the hepatic vein substrate concentration and HF is total hepatic blood or plasma flow (ie, AF + PF).

Net hepatic balance was thus calculated as

$$NHB = Load_{out} - Load_{in}.$$

Net fractional glucose extraction by the liver was calculated as the ratio of net hepatic glucose balance to glucose $Load_{in}$. Nonhepatic glucose uptake was calculated as the difference between the glucose infusion rate and the net hepatic glucose uptake, with the data adjusted for changes in the mass of the glucose pool. Nonhepatic glucose uptake was divided by the arterial glucose concentration to yield nonhepatic glucose clearance. Sinusoidal hormone concentrations were calculated as $Load_{in}$ ÷ HF, using plasma flows. The net glycogen synthetic rate (or net hepatic carbon retention) was calculated as NHGU − net hepatic lactate output, as previously explained in detail [41]. For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using previously determined [5,42] correction factors (the mean of the ratio of the blood value to the plasma concentration), which have been published previously [43]. In this report, blood glucose concentrations were thus used for the calculation of NHGB because the use of whole blood glucose ensures accurate hepatic balance measurements regardless of the characteristics of glucose entry into the erythrocyte.

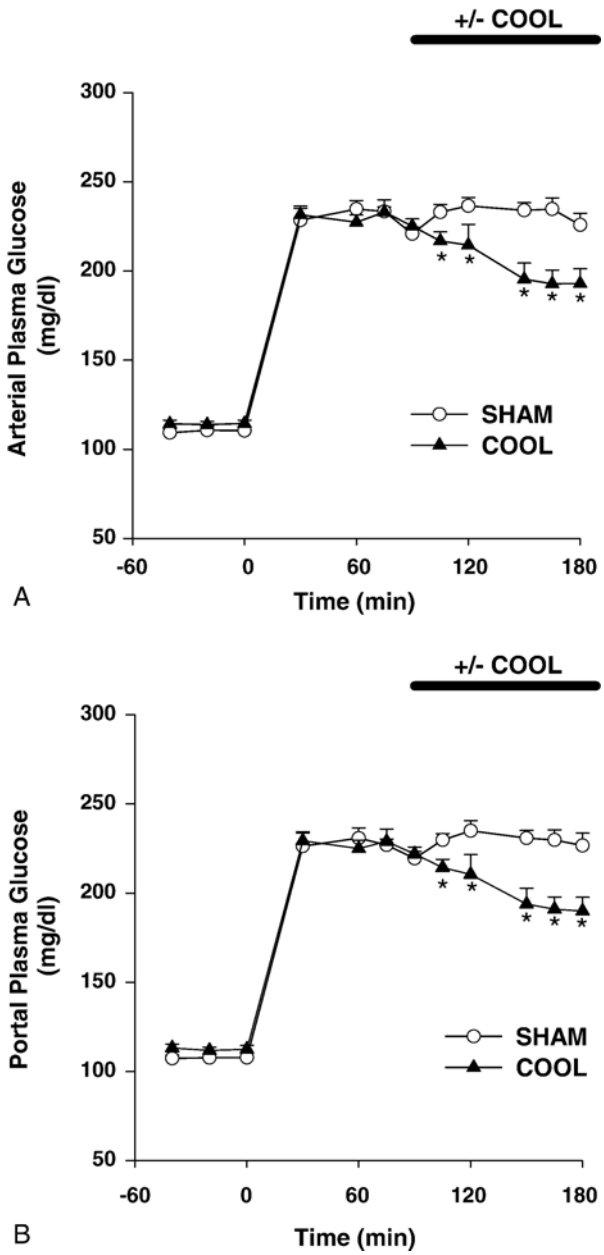

Fig. 2. Arterial (A) and portal (B) plasma glucose levels in SHAM and COOL dogs maintained on a pancreatic clamp during the basal and experimental periods (n = 6 per group). Data are mean ± SEM. *$P < .05$.

2.5. Statistical analysis

All data are presented as means ± SEM. Time course data were analyzed with repeated-measures analysis of variance. Independent *t* tests were used for any comparisons of mean data. Statistical significance was accepted at $P < .05$.

3. Results

3.1. Cardiovascular parameters and HBF

Blockade of vagal transmission during the cooling period was confirmed by the presence of a significantly greater heart rate in the COOL (183 ± 3 beats per minute) group when compared with the SHAM (102 ± 5 beats per minute) group (Fig. 1). In addition, all COOL dogs exhibited prolapse of the third eyelid (Horner sign) and a deepening and slowing of respirations during the cooling period. There were no significant changes in blood pressure during the basal or experimental periods in either group.

As expected, with the onset of somatostatin infusion, hepatic arterial blood flow increased slightly, whereas portal blood flow decreased modestly. Hepatic blood flow (Table 1) was initially similar in both groups and, as a result of the previously mentioned changes, fell slightly but not significantly during the first experimental period in both groups. It increased significantly (20%) during the cooling period because of a 50% rise in arterial blood flow (9 ± 1 to ~13 ± 2 mL · kg^{-1} · min^{-1}). Sham cooling was without effect on arterial blood flow.

3.2. Arterial and portal plasma glucose levels

The average arterial plasma glucose level (mg/dL) in the COOL dogs before vagal cooling was similar to that seen in the SHAM dogs (230 ± 2 and 231 ± 2, respectively) (Fig. 2). The average glucose infusion rates (mg · kg^{-1} · min^{-1}) were 7.9 ± 1.6 and 6.8 ± 0.4 in the SHAM and COOL dogs, respectively. During nerve cooling, the arterial plasma glucose concentration was allowed to fall to an average of 194 ± 4 mg/dL (~16%) to hold the hepatic glucose load constant in the presence of the increase in HBF. During coil perfusion, the average glucose infusion rates were 10.0 ± 1.5 vs 7.5 ± 0.9 mg · kg^{-1} · min^{-1} in the SHAM and COOL dogs, respectively.

3.3. Arterial plasma and liver sinusoidal insulin, and glucagon, cortisol, and catecholamines

Arterial and liver sinusoidal plasma insulin levels were maintained at ~3 to ~4× basal throughout the experimental periods in both the SHAM and COOL groups.

Table 2
Arterial plasma and hepatic sinusoidal insulin and glucagon levels during the basal and experimental periods in both SHAM and COOL 42-hour fasted conscious dogs

	Basal			exp + saline				exp ± COOL				
	−40	−20	0	30	60	75	90	105	120	150	165	180
Arterial plasma insulin (μU/mL)												
SHAM	9 ± 1	8 ± 1	8 ± 1	22 ± 1	22 ± 1	22 ± 2	22 ± 1	23 ± 1	21 ± 2	23 ± 1	23 ± 2	24 ± 1
COOL	6 ± 1	6 ± 1	8 ± 2	24 ± 2	21 ± 2	22 ± 3	23 ± 3	26 ± 3	25 ± 2	24 ± 3	25 ± 3	27 ± 3
Hepatic sinusoidal insulin (μU/mL)												
SHAM	22 ± 4	13 ± 3	19 ± 5	76 ± 6	87 ± 10	81 ± 7	78 ± 6	82 ± 11	95 ± 6	77 ± 7	95 ± 4	75 ± 8
COOL	17 ± 4	18 ± 5	23 ± 7	94 ± 20	71 ± 12	84 ± 13	87 ± 15	96 ± 8	82 ± 7	83 ± 8	96 ± 9	97 ± 8*

	Basal		exp + saline			exp ± COOL			
	−40	0	30	60	90	105	120	150	180
Arterial plasma glucagon (pg/mL)									
SHAM	36 ± 7	35 ± 6	34 ± 5	40 ± 6	41 ± 4	39 ± 5	37 ± 5	35 ± 4	33 ± 6
COOL	37 ± 4	35 ± 6	44 ± 5	43 ± 4	40 ± 5	42 ± 4	37 ± 2	40 ± 3	40 ± 2
Hepatic sinusoidal glucagon (pg/mL)									
SHAM	41 ± 5	46 ± 6	53 ± 7	59 ± 10	56 ± 8	52 ± 6	55 ± 6	48 ± 5	47 ± 6
COOL	42 ± 6	43 ± 7	65 ± 7	59 ± 5	62 ± 4	59 ± 3	52 ± 5	55 ± 2	56 ± 4

	Basal	exp + saline	exp ± COOL
	−20	75	165
Plasma cortisol (μg/dL)			
SHAM	2.8 ± 0.9	3.4 ± 0.4	2.0 ± 0.5
COOL	4.2 ± 1.0	5.4 ± 1.2	5.9 ± 2.5

n = 6 in each group. There were no significant differences between the exp ± cool and exp + saline periods in each respective group in any of the parameters above.

* $P < .05$ vs SHAM dogs.

The arterial plasma and liver sinusoidal glucagon levels, on the other hand, were maintained at basal values throughout the experimental periods in both groups (Table 2).

Cortisol (Table 2) and catecholamine (data not shown) levels did not change from basal in either the SHAM group or the COOL group and were not statistically different from one another at any time point during the experiment.

3.4. Hepatic glucose load, net hepatic glucose balance, and net hepatic fractional extraction

By adjusting the glucose level to compensate for the increase in total HBF that occurred in response to cooling, the hepatic glucose load was kept constant over time in the 2 experimental periods and was not significantly different in the 2 groups. The average hepatic glucose load throughout the entire experiment was 46 ± 1 and 50 ± 2 mg · kg^{-1} · min^{-1} in the SHAM and COOL groups, respectively (Fig. 3).

With the onset of peripheral hyperglycemia, net hepatic glucose balance switched from output to uptake. Net hepatic glucose uptake, in the 90 minutes before the cooling period, averaged 2.2 ± 0.5 and 2.9 ± 0.8 mg · kg^{-1} · min^{-1} in the SHAM and COOL dogs, respectively. During cooling, the average NHGU was 3.0 ± 0.5 and 3.4 ± 0.6 in the SHAM and COOL dogs, respectively. Thus, NHGU was not affected by vagal cooling. Likewise, the fractional extraction of glucose by the liver was not altered by the decrease in parasympathetic signaling.

3.5. Blood levels and net hepatic balance of lactate

The liver exhibited net lactate uptake in the basal period in both groups. Hyperglycemia resulted in a switch to net hepatic lactate output that was similar in both groups and waned over time. The arterial blood lactate levels initially rose because of the change in net hepatic lactate balance and then fell over time (Table 3). Given our ability to measure net hepatic glucose and lactate balance, we were able to estimate the average glycogen synthetic rate. The hepatic glycogen synthetic rates before cooling (1.4 ± 0.5 and 2.2 ± 0.5 mg · kg^{-1} · min^{-1}) and during vagal sham cooling or cooling (2.5 ± 0.5 and 3.1 ± 0.6 mg · kg^{-1} · min^{-1}) were not significantly different in the SHAM and COOL dogs, respectively.

3.6. Nonhepatic glucose uptake and clearance

There was a slight drift up in nonhepatic glucose uptake over time in each group. Vagal cooling had no effect on this parameter in either group. Likewise, no

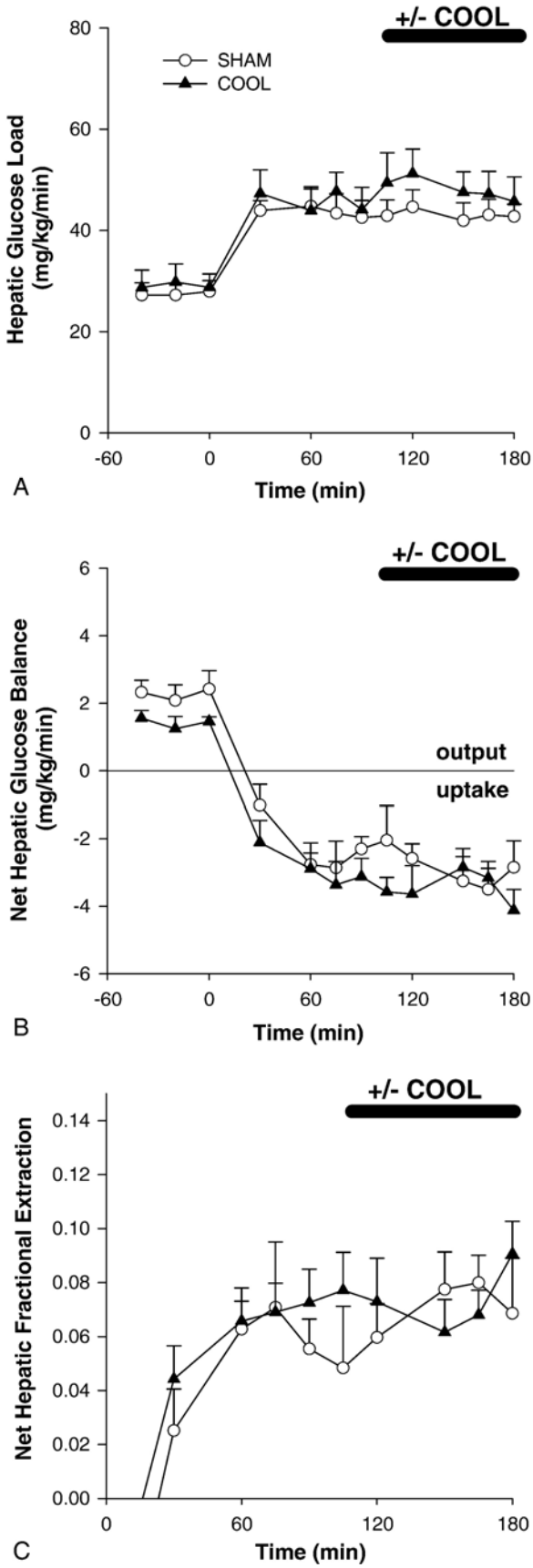

Fig. 3. Hepatic glucose load (A), net hepatic glucose balance (B), and net hepatic fractional extraction (C) in SHAM and COOL dogs maintained on a pancreatic clamp during the basal and experimental periods (n = 6 per group). Data are mean ± SEM.

Table 3
Arterial blood lactate levels and net hepatic lactate balance during the basal and experimental periods in both SHAM and COOL 42-hour fasted conscious dogs

	Basal			exp + saline				exp ± COOL				
	−40	−20	0	30	60	75	90	105	120	150	165	180
Arterial blood lactate (μmol/L)												
SHAM	365 ± 37	346 ± 43	324 ± 44	604 ± 55	871 ± 92	846 ± 85	784 ± 69	838 ± 73	845 ± 86	804 ± 85	779 ± 32	796 ± 132
COOL	403 ± 112	375 ± 104	400 ± 108	746 ± 148	1038 ± 147	1057 ± 139	1001 ± 92*	964 ± 56	910 ± 68	707 ± 165	658 ± 145†	698 ± 141†
Net hepatic lactate balance (μmol · kg^{-1} · min^{-1})												
SHAM	−7.0 ± 1.5	−5.9 ± 1.4	−6.0 ± 1.0	7.6 ± 2.4	10.8 ± 2.2	6.5 ± 1.5	5.6 ± 1.5	4.5 ± 1.6	4.6 ± 1.0	3.5 ± 2.3	2.8 ± 1.6	4.5 ± 2.4
COOL	−4.2 ± 0.4	−3.6 ± 0.6	−4.0 ± 0.5	6.9 ± 3.5	6.6 ± 2.5	5.5 ± 2.3	3.6 ± 1.5	1.8 ± 2.8	1.6 ± 1.2*	1.7 ± 1.1	1.2 ± 1.1†	0.7 ± 1.2†

n = 6 in each group.

* $P < .05$ vs SHAM dogs.

† $P < .05$ vs exp + saline period in the respective group.

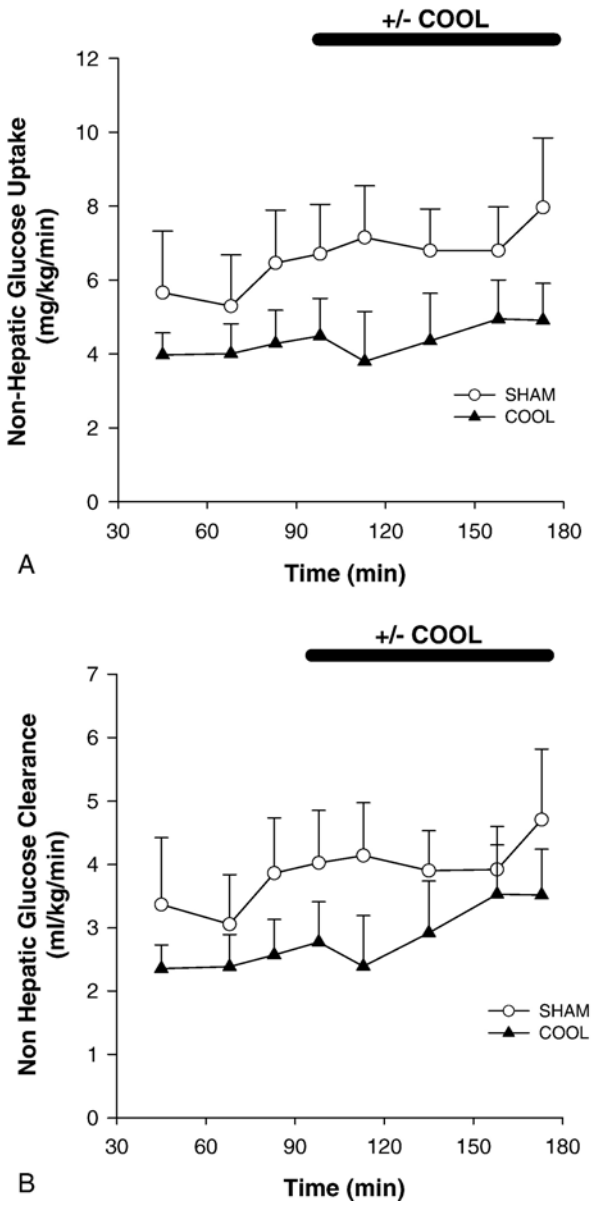

Fig. 4. Nonhepatic glucose uptake (A) and nonhepatic glucose clearance (B) in SHAM and COOL dogs maintained on a pancreatic clamp during the basal and experimental periods (n = 6 per group). Data are mean ± SEM.

changes were apparent in nonhepatic glucose clearance (Fig. 4).

4. Discussion

In the current studies, NHGU was stimulated by hyperinsulinemia and hyperglycemia brought about in the absence of portal glucose delivery. Vagal nerve activity was then blocked using a nerve cooling technique [16,44-47]. The efficacy of cooling was evident from the increase in heart rate and the third eyelid response. Blocking vagal nerve activity had no effect on either net hepatic or nonhepatic glucose uptake.

Because it has been suggested that the portal signal may be neurally mediated, it is important to understand the role of the parasympathetic and sympathetic nerves in controlling the response of the liver. Glucose-sensitive neurons in the portal vein [44] have a discharge rate that is inversely correlated with the portal vein glucose concentration. A decrease in afferent firing is accompanied by an increase in efferent firing in the pancreatic branch of the vagus nerve and decreases in the efferent firing of the hepatic branch of the splanchnic nerve and the adrenal nerve [48].

Sympathetic nerve fibers reach the liver through the celiac ganglia, celiac plexus, and the splanchnic nerves [15]. The sympathetic fibers form an anterior plexus around the hepatic artery. Alexander [49] showed that the hepatic artery receives only sympathetic fibers. The sympathetic efferents penetrate into the acinus, where they end with varicosities in the space of Disse close to the hepatocytes [50] and the hepatic stellate cells [51]. Stimulation of the sympathetic efferents results in an increase in glucose output by the liver through rapid activation of glycogen phosphorylase [8-11] as well as an increase in PEPCK activity, thus stimulating glycogenolysis and gluconeogenesis [52].

There have been several studies that have explored the role of the hepatic nerves in the mediation of the portal glucose signal. In a recent study by Cardin et al [34], NHGU was stimulated by hyperinsulinemia, hyperglycemia, and the portal glucose signal. When vagus nerve activity was blocked using the same method as used in the present study (vagal cooling), NHGU did not change. In an earlier study by Shiota et al [33], adrenergic blockade (portal vein phentolamine and propranolol infusion) and coincident cholinergic stimulation (portal vein acetylcholine infusion) brought about in the presence of hyperinsulinemia and hyperglycemia (produced by peripheral glucose infusion) increased NHGU by 1.8 mg · kg^{-1} · min^{-1} when compared to controls. These results were inconclusive, however, because portal vein administration of acetylcholine caused doubling of hepatic artery blood flow, leading to a significant rise in the glucose and insulin loads to the liver. Other studies have shown that delivery of glucose into the hepatic portal vein results in a fall in the firing rate of efferent fibers in the hepatic branch of the splanchnic nerve [48]. More recently we have shown that selective sympathetic denervation results in a greater increase in NHGU in response to hyperglycemia of peripheral origin than would otherwise be the case, suggesting that the sympathetic nerves exert a tonic inhibition of NHGU [29]. This raises the possibility that the portal glucose signal may bring about its effect in part at least by causing a diminution in this inhibitory sympathetic tone.

Total denervation of the liver resulted in an increase in NHGU in response to a hyperinsulinemia and a rise in glucose of peripheral origin [25]. This rise was not as large as that seen with a selective sympathetic denervation [29], suggesting that the input from the sympathetic efferents plays a more important role in control of NHGU than that

from the parasympathetic efferents. Thus, an increase in NHGU should again have been seen when vagal cooling occurred in the present study if our hypothesis were correct, and this did not occur.

Based on the aforementioned data, it thus appears that the vagus nerves do not play a role in the mediation of the portal glucose signal. However, several caveats must be considered. First, it is possible the afferent signal is transmitted through the vagal parasympathetic nerves but that the nerves were not adequately cooled. This seems unlikely, given that the heart rate increased significantly during cooling and Horner sign was present. In addition, it has been shown previously that this vagal cooling method is sufficient to halt vagal signaling in the dog [19] and cat [53] and that an injection of atropine is not able to increase the rate over the effect of cooling at this temperature [19]. The second caveat relates to the possibility that the increase in NHGU is due to a local reflex involving the parasympathetic nerves within the hepatoportal region [13,34]. We have previously demonstrated that sensing of the arterial-portal glucose gradient that generates the portal glucose signal is likely to be an intrahepatic event [54]. Moreover, data from the perfused liver preparation are consistent with the portal signal operating as a local response [13]. Another possibility is that the portal signal does not result from a local reflex, but the afferent limb follows a route other than the vagus nerve. In particular, spinal afferent nerve fibers provide a route for communication from the liver to the central nervous system, with the nucleus of the solitary tract, the parabrachial nucleus, and the paraventricular nucleus serving to integrate the peripheral signals [55]. Involvement of the spinal afferents has previously been suggested for the liver's response to hypoglycemia [56]. A nonvagal afferent pathway from the liver to the brain was implied by early neurophysiologic data [45,57]. If such an alternate route exists, then afferent signaling could have remained unimpaired during vagal cooling in the current investigation. In addition, the rise in arterial blood flow caused by vagal cooling, albeit small, might be associated with a signal that could itself alter NHGU, thus complicating data interpretation.

It is interesting to note that we observed a statistically significant effect of vagal blockade on hepatic arterial blood flow and, as a result, total HBF. The former increased by 50% in response to vagal cooling. Studies of the effect of the hepatic vagal nerves on HBF are limited and the results are controversial; Bobbioni et al [58] showed that there was an increase in HBF in rats when the vagus nerve was electrically stimulated. Most other studies, however, have reported no change in HBF after vagal nerve stimulation [30,59-61] or hepatic vagotomy [62]. It is unclear why there was an increase in hepatic arterial blood flow in response to vagal cooling in the present study because we did not observe such an effect in our earlier "cooling" studies [24,34,63].

In summary, vagal cooling to halt electrical transmission in the vagus nerves had no effect on NHGU under hyperglycemic and hyperinsulinemic conditions. Such is the case whether glucose was given via a peripheral or the portal vein. The observation makes it unlikely that the vagus nerves are involved in the mediation of at least the afferent limb of the portal glucose signal, although we cannot definitively rule out a role for the vagus in the efferent limb in the response.

Acknowledgments

This work was supported by the National Institutes of Health grant RO1 DK43706 and the Diabetes Research and Training Center grant SP-60-AM20593.

The authors appreciate the assistance and support from Sylvain Cardin and the Diabetes Research and Training Center Hormone Core in these studies.

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