Combined intraportal infusion of acetylcholine and adrenergic blockers augments net hepatic glucose uptake

MASAKAZU SHIOTA, PATRICIA J. JACKSON, PIETRO GALASSETTI, MELANIE SCOTT, DOSS W. NEAL, AND ALAN D. CHERRINGTON
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Shiota, Masakazu, Patricia J. Jackson, Pietro Galasetti, Melanie Scott, Doss W. Neal, and Alan D. Cherrington. Combined intraportal infusion of acetylcholine and adrenergic blockers augments net hepatic glucose uptake. Am. J. Physiol. Endocrinol. Metab. 278: E544–E552, 2000.—Portal glucose delivery in the conscious dog augments net hepatic glucose uptake (NHGU). To investigate the possible role of altered autonomic nervous activity in the effect of portal glucose delivery, the effects of adrenergic blockade and acetylcholine (ACh) on hepatic glucose metabolism were examined in 42-h-fasted conscious dogs. Each study consisted of an equilibration (-120 to -20 min), a control (-20 to 0 min), and a hyperglycemic-hyperinsulinemic period (0 to 300 min). During the last period, somatostatin (0.8 µg·kg⁻¹·min⁻¹) was infused along with intraportal insulin (1.2 mU·kg⁻¹·min⁻¹) and glucagon (0.5 ng·kg⁻¹·min⁻¹). Hepatic sinusoidal insulin was four times basal (73 ± 7 µU/ml) and glucagon was basal (55 ± 7 µg/ml). Glucose was infused peripherally (0–300 min) to create hyperglycemia (220 mg/dl). In test protocol, phentolamine and propranolol were infused intraportally at 0.2 µg and 0.1 µg·kg⁻¹·min⁻¹ from 120 min on. ACh was infused intraportally at 3 µg·kg⁻¹·min⁻¹ from 210 min on. In control protocol, saline was given in place of the blockers and ACh. Hyperglycemia-hyperinsulinemia switched the net hepatic glucose balance (mg·kg⁻¹·min⁻¹) from output (2.1 ± 0.3 and 1.1 ± 0.2) to uptake (2.8 ± 0.9 and 2.6 ± 0.2) and lactate balance (µmol·kg⁻¹·min⁻¹) from uptake (7.5 ± 2.2 and 6.7 ± 1.6) to output (3.7 ± 2.6 and 3.9 ± 1.6) by 120 min in the control and test protocols, respectively. Therefore, in the control protocol, NHGU tended to increase slightly (3.0 ± 0.6 mg·kg⁻¹·min⁻¹ by 300 min). In the test protocol, adrenergic blockade did not alter NHGU, but ACh infusion increased it to 4.4 ± 0.6 and 4.6 ± 0.6 mg·kg⁻¹·min⁻¹ by 220 and 300 min, respectively. These data are consistent with the hypothesis that alterations in nerve activity contribute to the increase in NHGU seen after portal glucose delivery.

hepatic blood flow; lactate

THE LIVER AND MUSCLES PLAY the major role in clearing alimentary-derived glucose from the plasma. It has been shown that both oral and portal glucose loadings result in greater rates of hepatic glucose uptake than does peripheral glucose loading. In the presence of hyperglycemia, the ability of portal glucose delivery to augment net hepatic glucose uptake (NHGU) has been observed at both basal and elevated insulin levels. Both oral and portal glucose loads create a negative arterial-portal glucose gradient, the magnitude of which correlates with the extent of NHGU (35). The increase in glucose uptake by the occurrence of negative arterial-portal glucose gradient was observed in the ex vivo isolated rat liver perfused via the artery and the portal vein (21). Therefore, a negative arterial-portal glucose gradient seems to create a "signal" that stimulates NHGU. The mechanisms involved in sensing the arterial-portal glucose gradient and transmitting an efferent signal to the hepatocytes remain unclear.

The liver is richly innervated by sympathetic and parasympathetic nerves. Both adrenergic and cholinergic nerve endings have been found in the livers of many species with terminations on and near the blood vessels, in and near the space of Disse, and on the hepatocyte cell membranes (review in Refs. 27, 39, and 47). In an earlier study with normal dogs, NHGU during portal glucose delivery was much greater (3.5 ± 0.8) than it was during peripheral glucose delivery (1.4 ± 0.7 mg·kg⁻¹·min⁻¹), despite similar hormonal levels and hepatic glucose loads (1). In dogs that had undergone surgical denervation of the liver, on the other hand, peripheral and portal glucose delivery resulted in identical rates of NHGU (2.1 ± 0.5 and 2.2 ± 0.7 mg·kg⁻¹·min⁻¹) (2). These data suggest that the effect of the portal signal on NHGU is mediated by the autonomic nervous system.

Consistent with this, Shimazu (39) showed that electrical stimulation of the peripheral end of a cut vagus nerve increased the rate of incorporation of glucose into glycogen approximately fivefold and enhanced glycogen deposition after glucose injection in anesthetized rabbits. After selective destruction of the sympathetic nerve terminals in the liver by injection of 6-hydroxydopamine to the cat, electrical stimulation of the remaining parasympathetic fibers to the liver resulted in a rapid decrease in net hepatic glucose output (27). In the presence of adrenergic blockade and insulin, stimulation of nerves located around the portal vein and the hepatic artery enhanced glucose uptake by the perfused rat liver (20). Furthermore, bethanechol chloride, a muscarinic cholinergic agonist, induced a 25% decrement in the plasma glucose concentration in humans during somatostatin infusion with insulin, glucagon, and growth hormone replacement at basal

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rates (9). In the isolated perfused rat liver supplied with no arterial-portal glucose gradient, infusion of ACh in the presence of insulin increased glucose uptake (41) and glycogen synthesis (33). The addition of ACh could stimulate glycogen synthesis from glucose and activate glycogen synthase in hepatocytes isolated from fed rats (4), suggesting the direct effect of ACh on hepatocytes.

In contrast, NHGU after an oral glucose load was significantly less in atropine-treated dogs (13). In the isolated rat liver perfused via artery and portal vein, the increase in glucose uptake caused by the occurrence of negative arterial-portal glucose gradient in the presence of insulin was also inhibited by atropine infusion (41). Taken together, these findings suggest that the occurrence of a negative arterial-portal glucose gradient may stimulate hepatic glucose uptake via changes in parasympathetic signaling.

There is a decrease in sympathetic tone together with an increase in parasympathetic tone after meal. To investigate the possible role of altered parasympathetic activity in mediating the effects of the portal signal in the presence of the decrease in sympathetic activity, we examined whether the combined intraportal infusion of adrenergic blockers and ACh could reproduce the effect of the portal signal on NHGU in the conscious dog.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were performed on ten 42-h-fasted mongrel dogs (17.7–27.1 kg, mean 22.4 ± 1.3 kg) of both sexes that had been fed a standard meat and chow diet (31% protein, 52% carbohydrate, 11% fat, and 6% fiber, based on dry weight; Kal-Kan, Vernon, CA, and Purina Lab Canine Diet No. 5006, Purina Mills, St. Louis, MO) once daily. The dogs were housed in a facility that met the guidelines of the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. At least 16 days before an experiment, a laparotomy was performed under general endotracheal anesthesia (15 mg/kg pentothal sodium presurgery and 1.0% isoflurane as an inhalation anesthetic during surgery), and catheters for blood sampling were placed into a femoral artery, the portal vein, and a hepatic vein as previously described (1, 2, 14–17, 39). Catheters for hormone and drug infusion were placed into a saphenic and a jejunal vein. The tips of the sphenic and jejunal vein catheters were placed 1 cm beyond the first site of coalescence of the catheterized vein with another vessel. On the day of the experiment, the catheters were exteriorized under local anesthesia (2% lidocaine, Abbott, North Chicago, IL), and their contents were aspirated and flushed with saline. Angiocaths (20-gauge, Abbott) were inserted into both cephalic veins for infusions of radioactive tracers and glucose and into a saphenous vein for the infusion of somatostatin.

On the day before the experiment, leukocyte count and hematocrit were determined. Dogs were used for an experiment only if they had 1) a leukocyte count <18,000/mm³, 2) a hematocrit >38%, 3) a good appetite, and 4) normal stools.

Experimental design. Experiments consisted of a tracer equilibration period (−120 to −20 min), a basal sampling period (−20 to 0 min), and a hyperglycemic-plus-hyperinsulinemic period (0 to 300 min). A primed (1.2 µCi/kg) and continuous (0.17 µCi/min) infusion of [3-H]glucose was started at −120 min. A 50% dextrose solution was infused via a leg vein at variable rates starting at 0 min to clamp plasma glucose at 220 mg/dl. Somatostatin was infused (0.8 µg·kg⁻¹·min⁻¹) to inhibit endogenous pancreatic insulin and glucagon secretion (0 to 300 min). Insulin (1.2 mU·kg⁻¹·min⁻¹) and glucagon (0.5 ng·kg⁻¹·min⁻¹) were infused at constant rates into the portal vein starting at 0 min. In one protocol,

Table 1. Hepatic blood flow, blood pressure, and heart rate before and during intraportal infusion of adrenergic blockers and acetylcholine or saline in the presence of hyperglycemia and hyperinsulinemia in 42-h-fasted conscious dogs given somatostatin and basal glucagon

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Period</th>
<th>90</th>
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<th>130</th>
<th>150</th>
<th>180</th>
<th>210</th>
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<tr>
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<td>18 ± 1</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
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</tr>
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<td>23 ± 2</td>
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<tr>
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<td>9 ± 1</td>
<td>8 ± 1</td>
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<tr>
<td>Hepatic</td>
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<tr>
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<td>117 ± 4</td>
<td>112 ± 5</td>
<td>110 ± 3</td>
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<td>105 ± 2</td>
<td>100 ± 2</td>
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<td>102 ± 4</td>
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<tr>
<td>Total</td>
<td>112 ± 3</td>
<td>118 ± 4</td>
<td>114 ± 4</td>
<td>116 ± 3</td>
<td>117 ± 2</td>
<td>115 ± 2</td>
<td>111 ± 4</td>
<td>109 ± 1</td>
<td>104 ± 1</td>
<td>111 ± 3</td>
<td>111 ± 3</td>
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<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>92 ± 11</td>
<td>95 ± 14</td>
<td>95 ± 14</td>
<td>97 ± 11</td>
<td>91 ± 13</td>
<td>87 ± 13</td>
<td>88 ± 11</td>
<td>84 ± 6</td>
<td>97 ± 9</td>
<td>97 ± 8</td>
<td>94 ± 10</td>
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<tr>
<td>Heart rate, beat/min</td>
<td>81 ± 10</td>
<td>85 ± 7</td>
<td>77 ± 7</td>
<td>80 ± 6</td>
<td>84 ± 9</td>
<td>86 ± 9</td>
<td>87 ± 10</td>
<td>89 ± 8</td>
<td>86 ± 10</td>
<td>95 ± 12</td>
<td>100 ± 3</td>
</tr>
</tbody>
</table>

Data for control period are means of values at −30 and 0 min. Data are means ± SE for 5 dogs. *Significantly different from corresponding values in control group (P < 0.05). †Significantly different from values at 210 min (P < 0.05).
phenolamine and propranolol were infused into the portal vein at constant rates of 0.2 and 0.1 μg·kg⁻¹·min⁻¹, respectively, from 120 to 300 min. ACh was infused into the portal vein at a constant rate of 3 μg·kg⁻¹·min⁻¹ from 210 to 300 min. In the second protocol, saline was infused in place of the blockers and ACh.

Analytical procedures. Plasma glucose concentrations were determined by use of the glucose oxidase method in a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Blood concentrations of lactate, glycerol, and alanine were determined according to the method of Lloyd et al. (28) in samples deproteinized with perchloric acid. Plasma concentrations of free fatty acids were determined by use of the Wako NEFA C test kit (Wako Pure Chemical, Osaka, Japan). To determine plasma glucose radioactivity, samples were deproteinized with barium hydroxide and zinc sulfate, and the supernatant was evaporated and reconstituted in 1 ml of water and 10 ml of liquid scintillation fluid (Ecolite (+), ICN Biomedicals, Irvine, CA) (14).

Liver samples were obtained at the end of the experiment by anesthetizing the dog with pentobarbital sodium, exposing the liver by laparotomy, and freeze-clamping ~5 g liver sections from two lobes in situ. The time elapsed from anesthesia to freeze-clamping was <2 min. The entire liver was then removed from the dog and weighed. The frozen samples were stored at −70°C for subsequent analysis. On the day of the assay, samples were powdered and homogenized, and glycogen concentrations were determined by acid hydrolysis and enzyme degradation with exo-1,4-α-amylglucosidase (12).

Immunoactive plasma insulin was measured by a double-antibody procedure (interassay coefficient of variation of 11%) (32). Immunoactive glucagon was measured in plasma samples containing 500 kalikrein-inactivating units per milliliter of aprotinin (TrasyloL, FBA Pharmaceuticals, New York, NY) by an RIA method (interassay coefficient of variation of 8%) (3). Plasma cortisol was measured with the Clinical Assays Gamma Coat RIA kit (interassay coefficient of variation of 6%; Clinical Assays, Travonol-Gentech Diagnostics, Cambridge, MA). Plasma epinephrine and norepinephrine were determined by high-pressure liquid chromatography as previously described (interassay coefficient variations of 8 and 14%, respectively) (10).

Materials. [3-3H]glucose (New England Nuclear, Boston, MA) was used as the glucose tracer. Insulin was obtained from Squibb-Novo (Princeton, NJ), and glucagon was obtained from Eli Lilly (Indianapolis, IN). Cyclic somatostatin was purchased from Bachem (Torrance, CA). The insulin, glucagon, and somatostatin infusates were prepared with

Table 2. Hepatic sinusoidal plasma insulin and glucagon levels before and during intraportal infusion of adrenergic blockers and acetylcholine or saline in the presence of hyperglycemia and hyperinsulinemia in 42-h-fasted conscious dogs given somatostatin and basal glucagon

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Period</th>
<th>Phentolamine and Propranolol</th>
<th>Acetylcholine or Saline</th>
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<tbody>
<tr>
<td>Control</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>7 ± 1</td>
<td>21 ± 2</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Portal</td>
<td>15 ± 2</td>
<td>93 ± 2</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Sinusoidal</td>
<td>13 ± 2</td>
<td>76 ± 3</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Test</td>
<td>18 ± 4</td>
<td>103 ± 15</td>
<td>98 ± 15</td>
</tr>
<tr>
<td>Sinusoidal</td>
<td>15 ± 2</td>
<td>80 ± 11</td>
<td>72 ± 11</td>
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</table>

Data for control period are means of values at −30 and 0 min. Data are means ± SE for 5 dogs. *Significantly different from corresponding values in control group (P < 0.05). †Significantly different from values at 210 min (P < 0.05).
normal saline and contained 3% (vol/vol) of the dog's own plasma.

Calculations. Hepatic arterial and portal blood flows were measured with Transonic (Ithaca, NY) flow probes. Net hepatic substrate balance was calculated by means of the formula \[ HF_t = (A F_a + P F_p) \], where \( A \), \( P \), and \( H \) are the arterial, portal vein, and hepatic vein substrate concentrations, and \( F_a \), \( F_p \), and \( F_t \) are hepatic arterial, hepatic portal vein, and hepatic total blood or plasma flows, respectively. Tracer-determined glucose production and glucose utilization were determined as shown previously (14, 20).

Statistical analysis. Data are expressed as means ± SE. Analysis of variance (ANOVA) with a repeated-measures design and the unpaired and paired t-tests were used for statistical analysis. Where significant changes were found with ANOVA, the Student-Newman-Keuls multiple-range test was used for post hoc analysis. A P value < 0.05 was accepted as significant (40).

RESULTS

Heart rate, arterial blood pressure, and hepatic blood flow. Hepatic blood flow, blood pressure, and heart rate were not changed significantly by the combination of hyperglycemia and hyperinsulinemia in either the control or the test groups (Table 1). In the test group, intraportal infusion of \( \alpha \)- and \( \beta \)-adrenergic blockers had no effect on hepatic blood flow, blood pressure, or heart rate. However, intraportal infusion of ACh in the presence of adrenergic blockade increased hepatic arterial blood flow from 9 to 21 ml·kg\(^{-1}\)·min\(^{-1}\), whereas it did not change portal blood flow. As a result, total hepatic blood flow increased from 27 to 37 ml·kg\(^{-1}\)·min\(^{-1}\) ± 10 min of ACh infusion. Arterial blood pressure fell slightly, from 111 to 102 mmHg, in response to ACh infusion. Heart rate did not change significantly when ACh was added to adrenergic blockade.

Hormone levels. There were no differences in the plasma levels of insulin, glucagon, cortisol, and catecholamines in the control period between the two groups (Table 2). Intraportal infusion of insulin at 1.2 mU·kg\(^{-1}\)·min\(^{-1}\) increased the liver sinusoidal insulin level about four- to fivefold in both groups. In the control group, arterial, portal vein, and sinusoidal insulin levels did not change during the 300-min hyperglycemic period. In the test group, the intraportal infusion of ACh in the presence of adrenergic blockade increased hepatic arterial flow to total liver blood flow increased. Intraportal infusion of glucagon at 0.5 ng·kg\(^{-1}\)·min\(^{-1}\) and immunoglobulin light-chain constant domain clearance (CI) (ml·kg\(^{-1}\)·min\(^{-1}\)) between the control and test groups (Table 3). In the control group, glucose infusion increased R\(_{gl} \) (mg·kg\(^{-1}\)·min\(^{-1}\)), R\(_{d} \) (mg·kg\(^{-1}\)·min\(^{-1}\)), and CI (mg·kg\(^{-1}\)·min\(^{-1}\)) from 2.5, 2.5, and 2.3 to 8.9, 2.6, and 2.0, respectively.

Fig. 1. Arterial plasma glucose levels and net hepatic glucose balance before and during portal infusion of adrenergic blockades and ACh or saline in presence of hyperglycemic hyperinsulinemia. *P < 0.05 vs. control group. +P < 0.05 vs. values at 210 min in same group.
Table 3. Peripheral GIR used to maintain hyperglycemia, hepatic glucose load, NHGB, net hepatic glucose fractional extraction, extrahepatic glucose utilization rate, and tracer-determined glucose Ra, Rd, and CI before and during infusion of adrenergic blockers and acetylcholine or saline in presence of hyperglycemic hyperinsulinemia in 42-h-fasted conscious dogs given somatostatin and basal glucagon.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Period</th>
<th>90</th>
<th>120</th>
<th>130</th>
<th>150</th>
<th>180</th>
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<td>Control</td>
<td>9.7±1.3</td>
<td>9.9±1.3</td>
<td>9.8±1.8</td>
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<td>10.0±1.2</td>
<td>10.5±1.4</td>
<td>10.6±1.4</td>
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</tr>
<tr>
<td>Test</td>
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<td>9.2±0.7</td>
<td>8.7±0.9</td>
<td>9.4±0.7</td>
<td>10.1±1.0</td>
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<td>13.2±1.2†</td>
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<td>58±9†</td>
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<td>10.0±1.6</td>
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<td>10.4±1.5</td>
<td>10.9±1.3</td>
</tr>
<tr>
<td>Control</td>
<td>2.3±0.2</td>
<td>9.9±1.1</td>
<td>9.8±1.1</td>
<td>10.5±1.0</td>
<td>10.8±0.7</td>
<td>11.7±0.7</td>
<td>11.7±0.7</td>
<td>13.5±1.2†</td>
</tr>
<tr>
<td>Test</td>
<td>2.2±0.2</td>
<td>4.6±0.5</td>
<td>4.5±0.5</td>
<td>4.8±0.4</td>
<td>4.9±0.3</td>
<td>5.4±0.3</td>
<td>5.4±0.3</td>
<td>6.2±0.6†</td>
</tr>
</tbody>
</table>

Data for control period are means of values at −30 and 0 min. Data are means ± SE for 5 dogs. GIR, glucose infusion rate; NHGB, net hepatic glucose balance; Ra, rate of appearance; Rd, rate of disappearance; Cl, clearance. Positive and negative values in NHGB represent output and uptake, respectively. *Significantly different from the corresponding values in control group (P < 0.05). †Significantly different from the values at 210 min (P < 0.05).

9.1, and 4.3 by 120 min, respectively, after which they rose slowly further to 11.0, 10.9, and 5.2 by 300 min. In the test group, glucose infusion increased Ra, Rd, and Cl from 2.3, 2.3, and 2.2 to 9.7, 9.8, and 4.5 by 120 min, respectively. These rates increased slightly during adrenergic blocker infusion (to 11.6, 11.7, and 5.4 by 210 min), but the increments were similar to those observed in the control group. Infusion of ACh in the presence of adrenergic blockade, on the other hand, initially increased Ra, Rd and Cl to 13.5, 13.5, and 6.2 (P < 0.05), but by the end of the experiment they were 14.9, 15.1, and 6.9, respectively. Extrahepatic glucose utilization rates were increased over the test period in both groups; on the other hand, there was no statistically significant increase by the infusion of adrenergic blockers and ACh in extrahepatic glucose utilization rates.

Blood lactate and net hepatic lactate balance. The hyperglycemic-hyperinsulinemic clamp switched the net hepatic lactate balance from uptake (7.5 ± 2.2 and 6.7 ± 1.6 mmol·kg⁻¹·min⁻¹) to output (3.7 ± 2.6 and 3.6 ± 1.6 mmol·kg⁻¹·min⁻¹) by 120 min in the control and test groups, respectively (Fig. 2). Lactate production decreased over time in the control studies (0.4 ± 1.7 by 210 min and 0.9 ± 1.9 mmol·kg⁻¹·min⁻¹ by 300 min). In response to adrenergic blockade, net hepatic lactate production remained unchanged (4.3 ± 2.0 mmol·kg⁻¹·min⁻¹), whereas in response to ACh infusion it rose to 10.1 ± 2.5 mmol·kg⁻¹·min⁻¹.

Arterial blood levels and net hepatic uptake of alanine and glycerol. The arterial blood alanine level and the net hepatic alanine uptake did not change during hyperglycemic hyperinsulinemia (Table 3). Neither intraportal infusion of saline in the control group nor the infusion of adrenergic blockers or ACh in the test group affected these parameters. In both the control and the test groups, the hyperglycemic-hyperinsulinemic clamp decreased arterial glycerol from 80 and 85 to 31 and 20 μM, and net hepatic glycerol uptake from 1.2 ± 0.2 and 1.4 ± 0.4 to 0.5 ± 0.2 and 0.6 ± 0.2 mmol·kg⁻¹·min⁻¹ by 120 min, respectively (Table 4). Intraportal infusion of saline in the control group and intraportal infusion of adrenergic blockers and ACh in the test group did not affect these parameters.

Plasma nonesterified free fatty acids and net hepatic nonesterified free fatty acid uptake. Hyperglycemic hyperinsulinemia decreased the plasma nonesterified fatty acids.
acid (NEFA) levels from 0.83 ± 0.16 and 0.92 ± 0.09 to 0.12 ± 0.05 and 0.12 ± 0.03 mM and net hepatic NEFA uptake from 2.5 ± 0.4 and 2.3 ± 0.8 to 0.27 ± 0.21 and 0.22 ± 0.10 µmol·kg⁻¹·min⁻¹ by 120 min in the control and test groups, respectively (Table 4). Intraportal infusion of saline in the control group and adrenergic blockers and ACh in the test group produced no effects.

**DISCUSSION**

The results of the present study demonstrate that ACh, at least in the presence of adrenergic blockade, can augment NHGU rapidly in the presence of hyperglycemia and hyperinsulinemia. This resulted in the need for peripheral glucose infusion to sustain the hyperglycemic clamp. The increase in NHGU was accompanied by an increase in net hepatic lactate output, which accounted for 30% of NHGU.

The liver is richly innervated by the sympathetic nervous system (27, 39, 47). Electrical stimulation of the sympathetic nerves increases glucose release by the liver (39). In our previous studies, intraportal infusion of phentolamine at 2 µg·kg⁻¹·min⁻¹ blocked the effect of a large rise in portal norepinephrine (137–3,351 pg/ml) on net hepatic glucose production (16, 17). Likewise, intraportal infusion of the β-adrenergic blocker, propranolol, at 1 µg·kg⁻¹·min⁻¹ blocked the effect of a large rise in epinephrine (29–746 pg/ml) on net hepatic glucose output (16, 17). In the present study, the α- and β-adrenergic blockers were infused at 0.2 and 0.1 µg·kg⁻¹·min⁻¹ before and during ACh infusion. At those doses they did not affect NHGU in the presence of combined hyperglycemia and hyperinsulinemia (Fig. 1). The doses of the blockers used in the present study should have been adequate to block the action of basal sympathetic nerve activity and the effects of the basal amounts of the catecholamines in the circulation. The blockers competitively inhibit the binding of catecholamines to their receptors, and because propranolol at 2 µg·kg⁻¹·min⁻¹ and phentolamine at 1 µg·kg⁻¹·min⁻¹ completely blocked the effect of 30-fold increases in the level of each catecholine, it seems likely that we had an effective blockade in the present study (16, 17). The fact that blockade of basal sympathetic tone did not augment NHGU suggests that a decrease in basal sympathetic tone is not a determinant of net hepatic glucose balance in the presence of hyperglycemia and hyperinsulinemia.

Although the intraportal infusion of ACh augmented NHGU by 65%, the net hepatic fractional extraction of glucose was increased by only 22% (Table 3) because of the increase (30%) that occurred in the hepatic glucose load. The latter results from the rise in hepatic arterial blood flow, because the liver sinusoidal glucose concentration rose by <2%. It remains unclear how much difference there is between the effects of increasing the hepatic glucose load by increasing blood flow with the glucose level constant and the effects of increasing the glucose level with blood flow constant. McGuinness et al. (30) showed that intraperitoneal administration of bacteria (Escherichia coli) in conscious dogs receiving total parenteral nutrition increased hepatic arterial blood flow (20.3 ± 4.6 ml·kg⁻¹·min⁻¹) but not hepatic portal blood flow (30.7 ± 5.1 ml·kg⁻¹·min⁻¹) compared with that in noninfected dogs (5.9 ± 1.3 and 26.5 ± 2.7 ml·kg⁻¹·min⁻¹, respectively). These changes in hepatic blood flows induced by the treatment with bacteria (30) are similar to the change caused by ACh infusion (Table 1) in the present study. Net hepatic glucose uptake and net fractional hepatic glucose extraction were decreased rather than increased by the infection (30). Therefore, it also remains unclear whether a portion of the effect of ACh on NHGU was due to the increase in hepatic arterial blood flow.

The hepatic insulin and glucagon loads were also increased by 30% during ACh infusion; however, the sinusoidal insulin and glucagon concentrations were similar both before and during ACh infusion (Table 2). One presumes that it is the concentration of a hormone at the liver that is the important determinant of its effect on liver glucose uptake. In any event, the changes in the hormone loads were small and the consequences of the increases in the glucagon and insulin loads would have been offsetting. It seems unlikely, therefore, that changes in insulin and glucagon mediated the increase in NHGU caused by ACh.
Lactate, alanine, and NEFA, and as µM for glycerol. NHB are described as µmol·kg⁻¹·min⁻¹. Positive and negative values in NHB represent output and uptake rates, respectively. Arterial blood or plasma levels of metabolites are described as mM for lactate, alanine, and NEFA, and as µM for glycerol. NHB are described as µmol·kg⁻¹·min⁻¹.

The increased hepatic arterial blood flow that we observed probably resulted from such a vasodilatory effect of this transmitter within the liver. Nitric oxide (NO) is an important regulator of basal hepatic arterial and sinusoidal resistance. The administration of an inhibitor of nitric oxide synthase increased hepatic arterial resistance and decreased hepatic arterial blood flow and total hepatic blood flow in anesthetized rats, pigs, and perfused rat livers (6, 23, 31). On the other hand, L-arginine and Sin-1 (NO donor) increased liver blood flow in isolated perfused rat livers (48). The effect of ACh to dilate the hepatic artery was abolished by an inhibition of nitric oxide synthase in the perfused rabbit liver (29). These results suggest that the ACh-induced increase in hepatic arterial blood flow was mediated by an increase in hepatic NO. This raises the question whether NO has a direct effect on hepatic glucose metabolism. It has been reported that the administration of NO inhibits gluconeogenesis in cultured hepatocytes and in vivo (11, 24). Borgs et al. (8) reported that the administration of NO or its precursor caused a transient increase in glycogenolysis in perfused livers of fed rats. There is no evidence to support an effect of NO on NHB; therefore, it remains unknown whether the increase in NHB caused by ACh was secondary to a rise in NO release.
The effect of the portal signal is characterized by a rapid induction of NHGU (36). When the portal signal was turned on in the presence of hyperglycemia and basal insulin, NHGU increased from 0.1 mg·kg
superscript-1·min
superscript-1 to 2.3 mg·kg
superscript-1·min
superscript-1 by 15 min and remained unchanged thereafter. Hyperglycemia and hyperinsulinemia, when induced in the absence of the portal signal, increased NHGU to only 0.7 mg·kg
superscript-1·min
superscript-1 by 15 min and did not reach maximum (2.9 mg·kg
superscript-1·min
superscript-1) until 90 min. On the other hand, when the portal signal was added to hyperglycemia and hyperinsulinemia, NHGU increased to 2.7 mg·kg
superscript-1·min
superscript-1 by 15 min and reached maximum (4.3 mg·kg
superscript-1·min
superscript-1) by 60 min. Thus the portal signal augments NHGU by 2.3 mg·kg
superscript-1·min
superscript-1 by 15 min and 1.4 mg·kg
superscript-1·min
superscript-1 during the steady state in the presence of hyperinsulinemia. The changes in NHGU brought about by intraportal ACh infusion (Fig. 1) are consistent with those induced by the portal signal in terms of duration and magnitude. This can be viewed as evidence to support the involvement of the parasympathetic nervous system in the liver’s response to portal glucose delivery. On the other hand, the portal signal does not alter hepatic arterial blood flow as does intraportal ACh infusion. This difference might be explained by the fact that, in the present study, ACh arrived at the hepatocytes via the vasculature, whereas it would normally be derived from the synapse. It could also be that we infused an excess of ACh and thereby activated both a vascular and a metabolic effect. In our previous study (20), net whole body nonhepatic glucose uptake and net hindlimb glucose uptake were lower than they were during peripheral glucose infusion (5.4 ± 1.1 mg·kg
superscript-1·min
superscript-1 and 14.8 ± 3.2 mg/min, respectively) than they were during peripheral glucose infusion (7.9 ± 1.3 mg·kg
superscript-1·min
superscript-1 and 20.4 ± 4.5 mg/min, respectively) despite comparable glucose loads and insulin levels. In this present study, during ACh infusion, when NHGU increased, net whole body nonhepatic glucose uptake did not change significantly. This suggests that the decrease in the net whole body nonhepatic glucose uptake by portal signal is not secondary to the altered hepatic glucose metabolism, and this in turn suggests that the portal signal might send an inhibitory signal to nonhepatic tissues independently from the stimulatory signal to the liver. Thus, although the data are consistent with the hypothesis that ACh plays an important role in mediating the effects of portal glucose delivery on NHGU, they do not prove that such is the case.

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