A Cyclic Guanosine Monophosphate–Dependent Pathway Can Regulate Net Hepatic Glucose Uptake in Vivo

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We previously showed that hepatic nitric oxide regulates net hepatic glucose uptake (NHGU), an effect that can be eliminated by inhibiting hepatic soluble guanylate cyclase (sGC), suggesting that the sGC pathway is involved in the regulation of NHGU. The aim of the current study was to determine whether hepatic cyclic guanosine monophosphate (cGMP) reduces NHGU. Studies were performed on conscious dogs with transhepatic catheters. A hyperglycemic-hyperinsulinemic clamp was established in the presence of portal vein glucose infusion. 8-Br-cGMP (50 μg/kg/min) was delivered intraportally, and either the glucose load to the liver (CGMP/GLC; $n = 5$) or the glucose concentration entering the liver (CGMP/GCC; $n = 5$) was clamped at 2× basal. In the control group, saline was given intraportally (SAL; $n = 10$), and the hepatic glucose concentration and load were doubled. 8-Br-cGMP increased portal blood flow, necessitating the two approaches to glucose clamping in the cGMP groups. NHGU (mg/kg/min) was 5.8 ± 0.5, 2.7 ± 0.5, and 4.8 ± 0.3, whereas the fractional extraction of glucose was 11.0 ± 1, 5.5 ± 1, and 8.5 ± 1% during the last hour of the study in SAL, CGMP/GLC, and CGMP/GCC, respectively. The reduction of NHGU in response to 8-Br-cGMP was associated with increased AMP-activated protein kinase phosphorylation. These data indicate that changes in liver cGMP can regulate NHGU under postprandial conditions. ***Diabetes*** **61:2433–2441, 2012**

Excessive postprandial hyperglycemia results in part from a dysregulation in hepatic glucose uptake and is a distinguishing characteristic of type 2 diabetes. The study of glucose uptake and utilization by the liver and extrahepatic tissues after food ingestion in vivo is therefore of great importance, particularly as it relates to the development of new pharmaceutical agents for the treatment of type 2 diabetes.

Earlier we showed that the elevation of hepatic nitric oxide (NO) by intraportal infusion of the NO donor 3-morpholinosydnonimine (SIN-1) reduced net hepatic glucose uptake (NHGU) in the presence of portal glucose delivery, hyperglycemia, and hyperinsulinemia. These data suggested that hepatic NO can regulate NHGU through a direct effect on the liver (1). NO activates soluble guanylate cyclase (sGC) and increases the concentration of cyclic guanosine monophosphate (cGMP) in the liver (2). Using the sGC inhibitor [^{1}H]-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) in a loss-of-function experiment, we showed that NO regulates NHGU, at least partially, if not completely through the sGC pathway (3). Given our recent observation that the hepatic concentrations of nitrate and nitrite, indices of NO levels, decline in response to food consumption in the dog (Z.A. and A.D.C., unpublished observations), it is possible that a reduction in NO/sGC is involved in the ability of portal glucose delivery to promote NHGU. In line with our observations, a study carried out by Ming et al. (4) in anesthetized cats showed that bolus delivery of SIN-1 intraportally potentiated norepinephrine-induced glucose fluxes from the liver, and this potentiation was blocked by inhibition of guanylate cyclase. Given that sGC catalyzes the conversion of guanosine-5′-triphosphate to the second messenger molecule cGMP, it seems possible that NHGU can be regulated by hepatic cGMP.

ODQ is a highly potent and specific sGC inhibitor, and its inhibitory effect on sGC activity is most likely due to a change in the oxidation state of the sGC heme (5). However, at high concentrations, ODQ has been suggested to interfere with other hemoproteins, such as hemoglobin (5), myoglobin (6), and cytochrome P450 enzymes (7). Furthermore, in a recent in vitro experiment, ODQ was found to promote cell death and inhibit migration of prostate cancer cells at the dose of 1 μmol/L and to inhibit growth at the dose of 10 μmol/L independently from its effects on cGMP levels (8). Thus, the potential nonspecific actions of ODQ complicate the interpretation of results in our previous study, although it seems unlikely that off-target effects explain our earlier results, as we used a very low rate of ODQ infusion. To clarify this issue, we have now infused 8-Br-cGMP, a potent and specific cell membrane-permeable cGMP analog (9), to determine the effect of hepatic cGMP on NHGU, in a gain-of-function study (glucose concentration entering the liver [CGMP/GCC group]). To resolve the potential impact of the cGMP-induced change in hepatic blood flow and thus the hepatic glucose load (HGL) on NHGU (10), we clamped the glucose concentration at twofold basal in one protocol (CGMP/GCC), whereas in the other (glucose load to the liver clamped [CGMP/GLC]), we clamped the HGL at twofold basal by lowering the glucose level (to compensate for the impact of the increase in flow on the HGL). The aim of the current study, therefore, was to determine the effect of cGMP on NHGU under hyperinsulinemic, hyperglycemic conditions in the conscious dog in vivo.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Studies were carried out on healthy conscious 42-h–fasted mongrel dogs (21.7 ± 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 (11,12). All animals were maintained on a diet of meat (Pedigree, Franklin, TN) and chow (Purina Laboratory Canine Diet No. 5006; Purina Mills, St. Louis, MO) comprised 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight. The animals were housed in a facility that met

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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, and sampling catheters were inserted in the hepatic vein, the portal vein, and a femoral artery as described in detail elsewhere (11). Catheters for intraportal infusion were also placed in a splenic and a jejunal vein, and ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the portal vein and the hepatic artery (11). The criteria for study were as previously described (3).

Experimental design. As described in Fig. 1, each experiment consisted of a 90-min equilibration period (−120 to −30 min), a 30-min basal period (−30–0 min), and a 240-min experimental period (0–240 min), which was divided into period 1 (P1; 0–90 min), period 2 (P2; 90–180 min), and period 3 (P3; 180–240 min). At 0 min, a constant infusion of somatostatin (0.8 μg/kg/min; Bachem, Torrance, CA) was begun via the left saphenous vein to suppress endogenous insulin and glucagon secretion. At the same time, basal glucagon (0.57 ng/kg/min; Glucagen; Novo Nordisk, Bagsvaerd, Denmark) and fourfold basal insulin (1.2 mU/kg/min; Eli Lilly, Indianapolis, IN) infusions were started through the splenic and jejunal catheters and maintained for the duration of the study. Glucose (20% dextrose) was given intraportally at 4 mg/kg/min, and *p*-aminohippuric acid (PAH) was mixed with it at a concentration allowing a delivery rate of 0.4 mg/kg/min. In addition, at time 0, a primed continuous infusion of 50% dextrose was started via the right cephalic vein so that the blood glucose could be quickly be clamped at the desired hyperglycemic level (~176 mg/dL). During P2 and P3, saline was infused intraportally in the control group (SAL; n = 10), whereas 8-Br-cGMP (Tocris Cookson, Ellisville, MO) was infused intraportally at 50 μg/kg/min in the CGMP/GCC (n = 5) and CGMP/GLC (n = 5) groups. The peripheral glucose infusion rate was adjusted as needed in P2 to maintain a similar blood glucose level to that seen in P1. 8-Br-cGMP raised the hepatic portal blood flow during P2. Thus, during P2, the HGL increased. P3 was included to account for this confounding factor. During P3, the hyperglycemic clamp (2× basal) was continued in the SAL and CGMP/GCC groups, but in CGMP/GLC, blood glucose was lowered to ~130 mg/dL in order to clamp the HGL rather than plasma glucose at 2× basal.

After completion of each experiment, the animal was killed, and biopsies (~1 gm) from three liver lobes were immediately freeze clamped with precooled Wallenburger tongs and stored at −70°C for poststudy assays.

Processing and analysis of samples. The collection and immediate processing of blood samples have been described previously (13). Glucose was measured in a glucose analyzer (Beckman Instruments, Fullerton, CA; Analox Instruments, London, U.K.). Plasma insulin and glucagon concentrations were determined by radioimmunoassay, as previously described (14). Cortisol, catecholamines, lactate, glycerol, and nonesterified fatty acid (NEFA) concentrations were also measured as described elsewhere (15). Electrophoretic separation, blotting, immunodetection, and quantification of proteins were performed as described previously (3). The activities of hepatic glycogen synthase and phosphorylase were measured as described elsewhere (3). Hepatic adenine nucleotide levels were measured by high-performance liquid chromatography as described previously (16).

Calculations and data analysis. To assess the mixing of the portal infusate in the laminar flow of the portal vein, the recovery of PAH in the portal and hepatic veins was assessed as described elsewhere (1). In the SAL group, 12 dogs were studied, and 10 were included because they exhibited adequate mixing, as defined previously (10); 5 out of 6 dogs were included in the CGMP/GCC group, and all 5 dogs were included in the CGMP/GLC group. In the animals that were retained, the ratio of PAH recovery in both the portal and the hepatic veins to the PAH infusion rate was 0.9 ± 0.1 in each group (with a ratio of 1.0 representing perfect mixing).

An indirect (I) method was used to assess NHGU in order to minimize the potential errors that arise due to any imperfect mixing of the infused glucose into the blood. Thus, the $Load_{in}$ was calculated as:

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

where G_A is the arterial blood glucose concentration, HBF is total hepatic blood flow, GIR_{PO} is the portal glucose infusion rate, and GUG is the uptake of glucose by the gastrointestinal tract, calculated as previously described (17).

The load of a substrate exiting 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:

$$NHGB = Load_{out} - Load_{in}(I).$$

NHGB was also calculated using a direct calculation that has been previously described (18). The results obtained did not differ significantly from those obtained using the indirect calculation, but only the data calculated with the indirect calculation are reported. The average nonhepatic glucose uptake between two time points (T1 and T2) was calculated by subtracting the rate of NHGU and the change in the glucose mass from the total glucose infusion rate (1). Net hepatic carbon retention was calculated as the sum of the net balances of glucose and lactate, once the latter was converted to glucose equivalents (1). The calculation of net hepatic carbon retention to estimate hepatic glycogen accretion has been described and validated previously (18). Net fractional glucose extraction by the liver was calculated as the ratio of NHGB to $Load_{in}$. The net hepatic balances of lactate, glycerol, and NEFA were

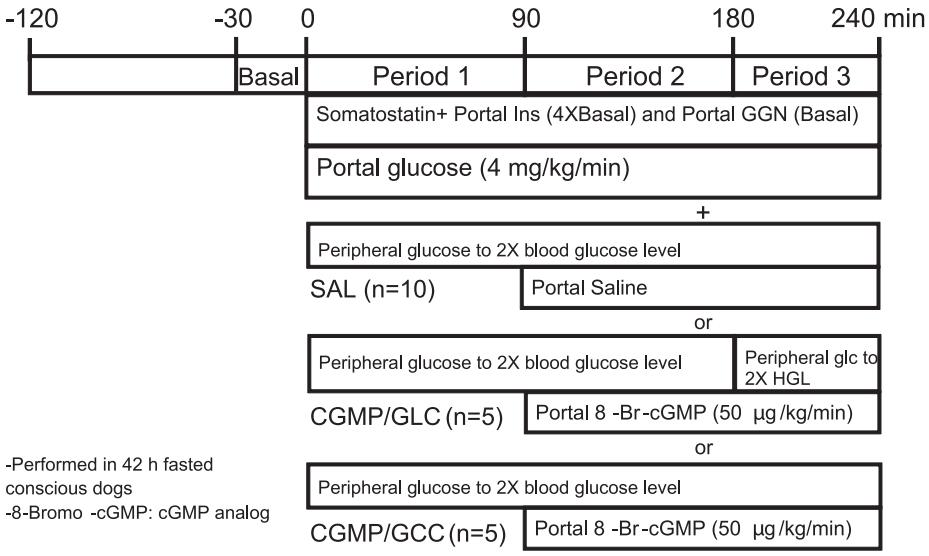

FIG. 1. Schematic representation of the study. The protocol comprises the basal (−30–0 min) and experimental periods (P1, 0–90 min; P2, 90–180 min; P3, 180–240 min). Somatostatin was infused peripherally, and insulin (fourfold basal) and glucagon (basal) were given intraportally, whereas glucose was delivered intraportally (4 mg/kg/min) and peripherally at a variable rate to increase the HGL twofold basal or arterial blood glucose twofold basal during P1, P2, and P3. The SAL group (n = 10) received intraportal saline during P2 and P3. Ten subjects received intraportal 8-Br-cGMP at 50 μg/kg/min during P2 and P3, five had lowered arterial glucose to match the HGL to that in P1 (CGMP/GLC, n = 5), and five had arterial glucose clamped to that in P1 (CGMP/GCC, n = 5).

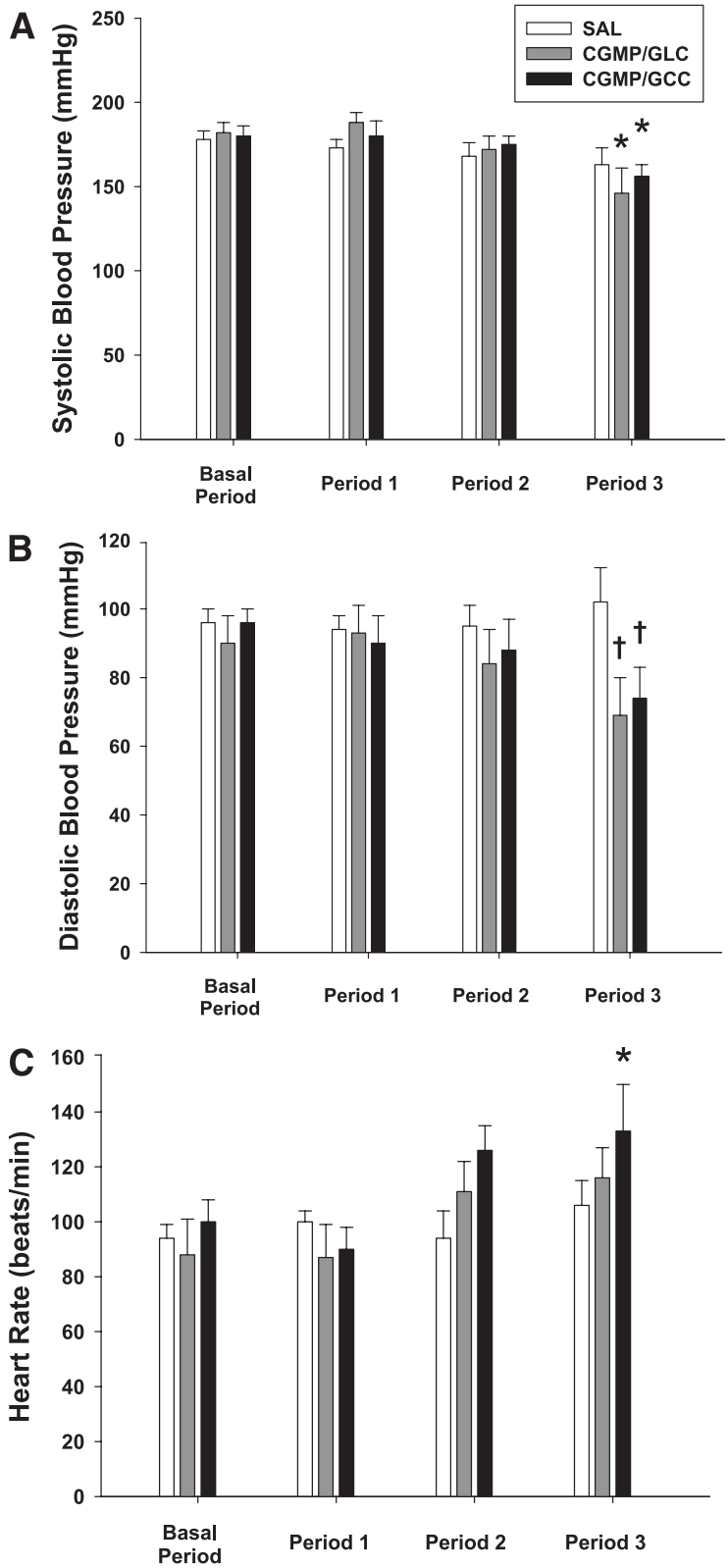

FIG. 2. Systolic (*A*) and diastolic (*B*) blood pressure and heart rate (*C*) during the basal and experimental periods. See Fig. 1 for description of study conditions. Data are means ± SEM; $n = 8$ in the SAL group, $n = 5$ in the group that received 8-Br-cGMP in the portal vein in which arterial glucose was maintained at ~130 mg/dL in P3 (CGMP/GLC), and $n = 5$ in the group that received 8-Br-cGMP in the portal vein and arterial glucose was maintained at ~175 mg/dL in P3 (CGMP/GCC). *Significant statistical difference ($P < 0.05$) from basal period within the group; †significant statistical difference ($P < 0.05$) from SAL group and from basal period within the group.

calculated directly as for glucose. The hepatic sinusoidal insulin and glucagon concentrations were calculated as previously described (19).

For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using previously determined (20,21) 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 into the erythrocyte.

Statistical analysis. All data are presented as means ± SEM. Time-course data were analyzed with two-way repeated-measures ANOVA, and one-way ANOVA was used for any comparisons of other mean data. Post hoc analysis was carried out using the Student-Newman-Keuls method. Statistical significance was accepted at $P < 0.05$.

RESULTS

Hemodynamic response. Blood pressure did not change significantly over the course of the experiment in response to saline infusion (Fig. 2*A* and *B*). The systolic blood pressure in the CGMP/GLC and CGMP/GCC groups was similar to that seen in SAL in the basal period and P1 but tended to decrease in P2 and was significantly reduced from the basal values during P3 (Fig. 2*A*). Diastolic blood pressure also tended to fall in P2 and fell significantly during P3 in response to intraportal 8-Br-cGMP infusion (Fig. 2*B*). The average heart rate did not change over time in SAL, but it tended to increase during P2 and P3 in the CGMP/GLC and CGMP/GCC groups (Fig. 2*C*).

Hepatic blood flow, blood glucose concentrations, and HGL. Portal vein blood flow decreased by ~20% in all groups during P1 in response to somatostatin infusion (Table 1). There was a concomitant and offsetting (~20%) increase in hepatic arterial flow. As a result, total hepatic blood flow tended to be slightly reduced during P1 in all groups. Hepatic portal blood flow increased by ~70% in response to 8-Br-cGMP. Consequently, total hepatic blood flow remained near basal during P2 and P3 in the SAL group, but it increased by just over −50% in CGMP/GLC and CGMP/GCC.

The arterial blood glucose level increased in all groups from a basal value of 85 ± 2 to 176 ± 3 mg/dL during P1 and P2 (Fig. 3*A*). It was sustained at that level during P3 in the SAL and CGMP/GCC groups, whereas it was allowed to drop to 130 ± 6 mg/dL during P3 in the CGMP/GLC group. The HGL increased to 2× basal during P1, P2, and P3 in the SAL group and during P1 in CGMP/GLC and CGMP/GCC groups. During P2, it increased by an additional ~50% in the CGMP/GLC and CGMP/GCC groups, because of the increase in portal vein blood flow. It remained elevated during P3 in the CGMP/GCC group, but returned to 2× basal in the CGMP/GLC group as a result of the reduction in the blood glucose concentration (Fig. 3*B*).

Hormone concentrations. The arterial and hepatic sinusoidal insulin levels increased three- to fourfold during P1 in all groups (Table 2). Compared with P1, the elevated hepatic sinusoidal insulin levels declined by ~20% during 8-Br-cGMP infusion (P2 and P3) due to the increase in hepatic portal blood flow (Table 1). Arterial plasma glucagon concentrations remained near basal throughout the study in all groups. In contrast, hepatic sinusoidal glucagon concentrations also fell in proportion to the change of hepatic blood flow (Table 2). Plasma catecholamines remained unchanged in the SAL control group (data not shown). Norepinephrine (pg/ml) rose from 101 ± 12 in P1 to 234 ± 24 and 209 ± 31 ($P < 0.05$) in P2 and P3, respectively, whereas epinephrine (pg/ml) tended to rise from 139 ± 27 in P1 to 150 ± 36 and 175 ± 68 (NS) in response to the hypotension induced by 8-Br-cGMP (two groups averaged). The mean plasma cortisol concentrations remained unchanged throughout in all groups (Table 2).

NHGB and net hepatic fractional glucose extraction. All groups exhibited a similar rate of net hepatic glucose output during the basal period (Fig. 3*C*). Coincident with the start of the experimental period (4× basal insulin, basal glucagon, 2× basal blood glucose, and portal glucose delivery), all groups switched from net output to net uptake of glucose (4.8 ± 0.4, 4.3 ± 0.7, and 4.4 ± 0.4 mg/kg/min in SAL, CGMP/GLC, and CGMP/GCC groups, respectively). NHGU was 5.4 ± 0.5 in SAL during P2, whereas it averaged 4.8 ± 0.6 mg/kg/min in the two cGMP groups despite the increase in the HGL (Fig. 2*C*). During P3, NHGU rose to 5.8 ± 0.5 mg/kg/min in SAL, whereas it was 4.8 ± 0.3 mg/kg/min in the CGMP/GCC group, but only 2.7 ± 0.5 mg/kg/min ($P < 0.05$ vs. SAL) in the CGMP/GLC group. In P1, the livers in all groups were taking up about 10% of the presented glucose. This stayed constant over time in SAL, but fell in 8-Br-cGMP

TABLE 1
Average hepatic arterial, portal, and total hepatic blood flow during the basal and experimental periods in conscious 42-h–fasted dogs given saline, or 8-Br-cGMP (50 μg/kg/min) infused into the portal vein

Group	Basal period	Experimental period		
		P1	P2	P3
Average hepatic arterial blood flow (mL/kg/min)				
SAL	6.2 ± 0.6	9.5 ± 0.9*	10.1 ± 1.1*	10.8 ± 1.4*
CGMP/GLC	4.8 ± 0.8	5.4 ± 0.6†	5.8 ± 0.7†	6.0 ± 0.6†
CGMP/GCC	4.9 ± 0.7	6.1 ± 0.9†	6.1 ± 0.7†	6.1 ± 0.6†
Average hepatic portal blood flow (mL/kg/min)				
SAL	22.2 ± 2.0	18.4 ± 1.5	19.0 ± 1.6	19.2 ± 1.8
CGMP/GLC	25.7 ± 3.4	19.5 ± 2.7	33.2 ± 4.0†	31.0 ± 3.3†
CGMP/GCC	19.9 ± 2.7	15.5 ± 2.4	27.4 ± 3.8†	27.6 ± 4.5†
Average total hepatic blood flow (mL/kg/min)				
SAL	28.3 ± 2.2	27.9 ± 2.1	29.1 ± 2.4	30.0 ± 2.7
CGMP/GLC	30.5 ± 3.4	24.8 ± 2.6	39.0 ± 4.0†	36.9 ± 3.6
CGMP/GCC	24.7 ± 2.9	21.6 ± 2.6	33.5 ± 3.6	33.7 ± 4.4

Data are means ± SEM; $n = 10$ in the SAL group, $n = 5$ in the group that received 8-Br-cGMP in the portal vein in which arterial glucose was maintained at ~130 mg/dL in period 3 (CGMP/GLC), and $n = 5$ in the group that received 8-Br-cGMP in the portal vein and arterial glucose was maintained at ~175 mg/dL in period 3 (CGMP/GCC). *Significant statistical difference ($P < 0.05$) from basal period within the group. †Significant statistical difference ($P < 0.05$) from SAL group.

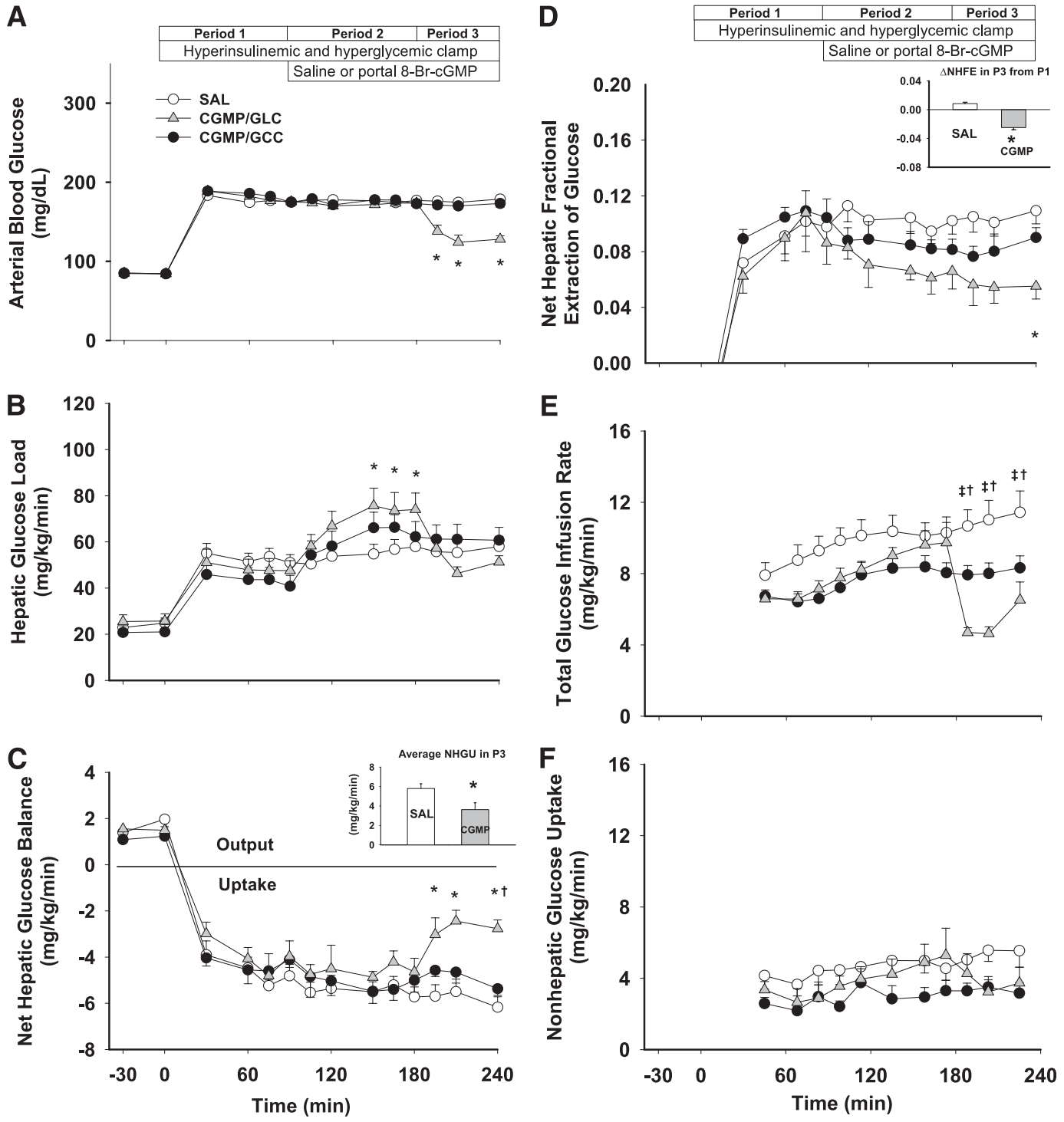

FIG. 3. Arterial blood glucose (*A*), HGL (*B*), NHGU (*C*), net hepatic fractional extraction (NHFE) of glucose (*D*), total glucose infusion rate (*E*), and nonhepatic glucose uptake (*F*) in 42-h–fasted conscious dogs during the basal and experimental periods. See Fig. 1 for description of study conditions. Data are means ± SEM. Average NHGU in P3 data expressed as histogram represent averaged values for the P3 (last hour) in the SAL group (n = 10) and the 8-Br-cGMP–treated group (n = 10). ΔNHFE in P3 from P1 data expressed as histogram represent the change of averaged values for P3 from P1 in the saline group (n = 10) and the 8-Br-cGMP–treated group (n = 10). *P < 0.05 compared with SAL group; †P < 0.05 compared with CGMP/GCC group; ‡P < 0.05 compared with CGMP/GLC group.

groups so that by the end of the experiment, it was reduced by ~35% (both 8-Br-cGMP groups combined) (Fig. 3*D*).

Glucose infusion rates and nonhepatic glucose uptake. The glucose infusion rate increased modestly over time in all groups except in the CGMP/GLC group during P3 when the glucose infusion was reduced to clamp the total HGL to that in P1 (Fig. 3*E*). Nonhepatic glucose uptake did not differ significantly among groups at any time (Fig. 3*F*), but it fell in the CGMP/GLC group when the plasma glucose level was reduced.

TABLE 2
Hormone concentrations during the basal and experimental periods in conscious 42-h–fasted dogs given saline or 8-Br-cGMP (50 μg/kg/min) infused into the portal vein

Group	Basal period	Experimental period P1	P2	P3
Arterial plasma insulin (μU/mL)				
SAL	8 ± 1	25 ± 3*	25 ± 2*	25 ± 2*
CGMP/GLC	8 ± 2	21 ± 2*	25 ± 2*	25 ± 2*
CGMP/GCC	7 ± 1	20 ± 1*	25 ± 2*	25 ± 3*
Hepatic sinusoidal insulin (μU/mL)				
SAL	21 ± 4	79 ± 8*	85 ± 10*	80 ± 8*
CGMP/GLC	18 ± 3	95 ± 14*	80 ± 10*	81 ± 9*
CGMP/GCC	21 ± 4	94 ± 11*	67 ± 7*	65 ± 6*
Arterial plasma glucagon (pg/mL)				
SAL	45 ± 7	50 ± 4	46 ± 6	46 ± 6
CGMP/GLC	43 ± 11	42 ± 6	39 ± 4	38 ± 5
CGMP/GCC	42 ± 11	46 ± 7	44 ± 4	38 ± 4
Hepatic sinusoidal glucagon (pg/mL)				
SAL	58 ± 7	69 ± 6	65 ± 6	62 ± 7
CGMP/GLC	48 ± 9	55 ± 5	47 ± 7	45 ± 9
CGMP/GCC	53 ± 10	58 ± 7	52 ± 5	50 ± 5
Arterial cortisol (μg/dL)				
SAL	3 ± 1	5 ± 1	3 ± 1	4 ± 1
CGMP/GLC	3 ± 1	4 ± 1	5 ± 1	6 ± 1
CGMP/GCC	2 ± 1	3 ± 1	4 ± 1	4 ± 1

Data are means ± SEM; n = 10 in the SAL group, n = 5 in the group that received 8-Br-cGMP in the portal vein and arterial glucose was maintained at ~130 mg/dL in period 3 (CGMP/GLC), and n = 5 in the group that received 8-Br-cGMP in the portal vein and arterial glucose was maintained at ~175 mg/dL in period 3 (CGMP/GCC). *Significant statistical difference ($P < 0.05$) from basal period within the group.

Lactate metabolism and net hepatic carbon retention. The arterial blood lactate concentrations rose in all groups during P1 and remained elevated in P2 and P3 (Supplementary Table 1). Net hepatic lactate balance changed from net uptake to net output during P1 in all groups (Supplementary Table 1). Net hepatic lactate output continued throughout the study. Net hepatic carbon retention (in mg glucose equivalents/kg/min) did not differ among groups during P1 but decreased during P3 in response to 8-Br-cGMP relative to the control group (2.3 ± 0.4 in CGMP/GLC [$P <$ 0.05 vs. SAL], 4.0 ± 0.2 in CGMP/GCC, and 5.2 ± 0.3 in SAL group).

Glycogen synthase and phosphorylase in the liver. Compared with the SAL group, intraportal infusion of 8-Br-cGMP (CGMP/GLC and CGMP/GCC) was associated with a decrease in hepatic glycogen synthase activity (glycogen synthase ratio in the presence of low and high Glc6P 0.03 ± 0.01 in CGMP vs. 0.11 ± 0.01 in SAL; $P < 0.05$), but no change of glycogen phosphorylase activity (GPh ratio ± AMP 0.13 ± 0.01 in CGMP vs. 0.11 ± 0.06 in SAL).

Glycerol and NEFA metabolism. Arterial blood glycerol concentrations and net hepatic glycerol uptake were reduced by 60–65% in response to hyperglycemia and hyperinsulinemia and remained suppressed in all groups during P1 (Supplementary Table 1). The suppression of glycerol was, however, partially reversed during P2 and P3 in response to 8-Br-cGMP but not saline. Arterial plasma NEFA concentrations and net hepatic NEFA uptake changed in a pattern similar to glycerol, decreasing ~75% during P1 and remaining suppressed in P2 and P3 in all groups (Supplementary Table 1).

AMP-activated protein kinase signaling and adenine nucleotide levels in the liver. Compared with the SAL group, intraportal infusion of 8-Br-cGMP was associated with an increase of ~90% in the phosphorylation of Thr-172 in AMP-activated protein kinase (AMPK) as well as an increase (~65%) in the phosphorylation of Ser-79 in acetyl-CoA carboxylase (ACC) in the liver (Fig. 4). In contrast, the phosphorylation of Ser-485 in AMPK did not differ between groups (Supplementary Fig. 1). Adenine nucleotide levels in the liver between the SAL, CGMP/GLC, and CGMP/GCC were not different (Supplementary Table 1).

DISCUSSION

Our earlier experiments have shown that an increase in hepatic NO decreases hepatic glucose uptake (1). Most recently, we showed that the inhibition of hepatic sGC by intraportal infusion of its inhibitor ODQ enhanced NHGU and hepatic glycogen accumulation. Further, adding SIN-1

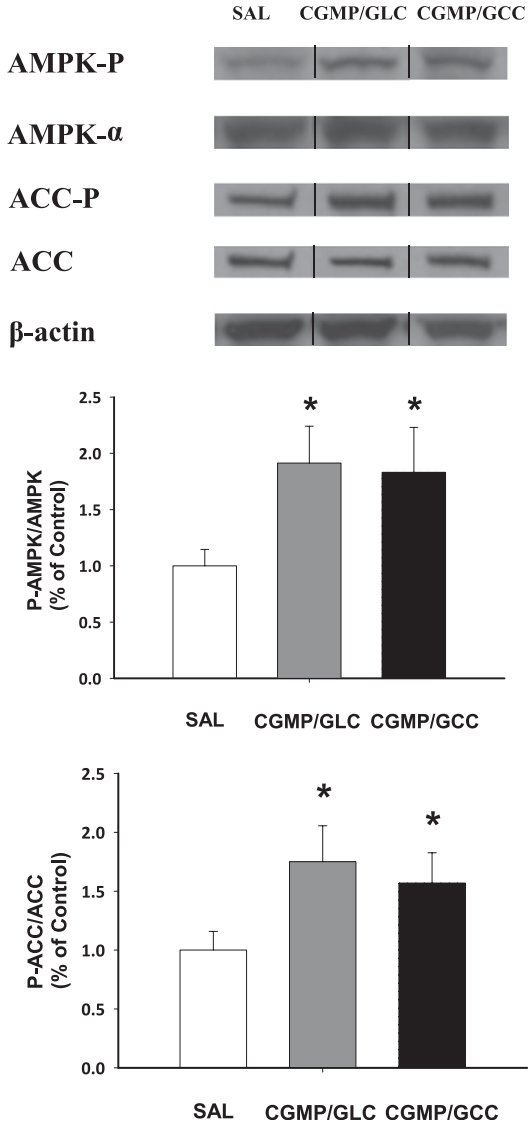

FIG. 4. Phosphorylation of AMPK at Thr172 and ACC at Ser79 in the liver biopsies at the end of the experiments. See Fig.1 for description of study conditions. Data are means ± SEM. The blots shown are representative of three to five blots obtained from independent experiments. *$P < 0.05$ compared with the SAL group.

did not override the increase in NHGU, suggesting that NO regulates NHGU through the sGC pathway (3). It is not clear, however, whether cGMP, the downstream messenger molecule of sGC, is involved in the regulation of NHGU. Moreover, despite the specificity of ODQ, one always has to be concerned about off-target effects of any drug. It is thus important to carry out a gain-of-function experiment to confirm the role of sGC in the regulation of NHGU. In the current study, we found that intraportal infusion of a cGMP analog, 8-Br-cGMP reduced NHGU in the presence of hyperinsulinemia, hyperglycemia, basal glucagon, and portal glucose delivery in 42-h–fasted dogs. Further, these responses were associated with increased phosphorylation and activation of AMPK in the liver. We thus show for the first time that hepatic cGMP, the downstream product of sGC, regulates NHGU, possibly through the modulation of AMPK. This finding further supports the important role of sGC in the regulation of NHGU in vivo.

In the current study, intraportal infusion of 8-Br-cGMP reduced the ability of the liver to take up glucose. During the last hour of the experiment, both systolic and diastolic blood pressure decreased by ~20%, undoubtedly due to the vasodilatory effects of 8-Br-cGMP, which led to an increase sympathetic tone. The fact that there was an increase in sympathetic drive in response to 8-Br-cGMP infusion is supported by our findings that the heart rate rose secondary to hypotension, the plasma norepinephrine and epinephrine levels were slightly elevated, and lipolysis increased modestly, as indicated by an increase in the arterial blood glycerol level. Increased sympathetic input to the liver, if it occurred, would have been expected to reduce NHGU (22). However, in our previous experiments (1), when SIN-1 was given via the hepatic portal vein or a leg vein, it brought about a similar hemodynamic response to that seen in the current study, but there was no decrease in NHGU or hepatic glycogen synthesis. This suggests that in the current study, increased sympathetic input to the liver even, if it did occur, was not the mechanism responsible for the reduction of NHGU.

At the same time, 8-Br-cGMP significantly increased hepatic blood flow. As the liver is a low-resistance organ, hepatic arterial flow is mainly determined by the resistance at the hepatic artery, whereas hepatic portal flow is mainly determined by resistance at gastrointestinal vessels. The fact that hepatic arterial blood flow remained unchanged in the current study was probably due to the offsetting effects of the decrease in blood pressure and the decrease in hepatic arterial resistance in response to 8-Br-cGMP. Portal blood flow, in contrast, increased by ~70% likely because the reduction of the resistance of the gastrointestinal vessels had a greater effect than the drop in blood pressure. As a result of this vascular change, total hepatic blood flow increased by ~50%, the HGL increased, and the hepatic sinusoidal insulin and glucagon levels declined. The latter occurred because, per unit time, the hormones were infused into an increased blood volume. It remains unknown whether there is a difference between the effects of an increase in the HGL resulting from increased hepatic blood flow with the glucose level held constant and those resulting from an increase in glucose level with hepatic blood flow held constant (23). To resolve the potential impact of the change in flow on hepatic glucose kinetics, we lowered the arterial glucose levels from ~176 to ~130 mg/dL in the CGMP/GLC group during P3 to match the HGL seen in P1. As a result, the HGL returned to 2× basal, and NHGU was significantly reduced (~50%; $P < 0.05$). In the CGMP/GCC group, the arterial glucose levels remained at ~176 mg/dL, and the HGL increased due to the change of the blood flow, and yet NHGU still tended to be reduced by 20% relative to NHGU in SAL. If one looks at the fractional extraction of glucose by the liver, it is clear that it dropped significantly in response to 8-Br-cGMP in both groups.

It should also be noted that in P3, there was a ~20% reduction in the hepatic sinusoidal insulin and glucagon levels as a result of the increase in hepatic blood flow caused by 8-Br-cGMP. One may postulate that this reduction of sinusoidal insulin could be partially responsible for the reduction of NHGU. However, the changes in the sinusoidal hormone concentrations were small, and the consequences of the decreases in the glucagon and insulin levels would have been offsetting. Data from a previous study indicated that a change in sinusoidal insulin of the magnitude seen in the current study would only be expected to decrease NHGU by ~0.3 mg/kg/min (17). It seems unlikely, therefore, that the small fall in hepatic sinusoidal insulin mediated the decrease in NHGU caused by 8-Br-cGMP. Thus, despite the dynamic change in hepatic blood flow associated with 8-Br-cGMP administration, the data support the conclusion that the increase in cGMP in the liver decreased NHGU.

It has been shown that there is cross-talk between cAMP and cGMP signaling pathways (24). Thus, it is conceivable that 8-Br-cGMP infusion in the current study activated cAMP-dependent protein kinase (PKA) in the liver, which could have been responsible for the change in NHGU. However, the activity of hepatic glycogen phosphorylase, one of the markers of PKA action, did not change in response to 8-Br-cGMP, suggesting this was not the case. Further, the effect of a potential activation of PKA on glycogen metabolism and NHGU under the present anabolic condition in the presence of hyperglycemia and hyperinsulinemia would have been overcome by the dephosphorylation/inactivation of both phosphorylase kinase and glycogen phosphorylase (25). In addition, the phosphorylation of Ser-485 in AMPK did not differ between groups, supporting the concept that the PKA pathway was not activated by 8-Br-cGMP. Furthermore, this finding also indicates that the small rise in catecholamines that occurred in response to 8-Br-cGMP was not the cause of the change of AMPK phosphorylation because catecholamines would have been expected to work through PKA.

NO can activate AMPK in the endothelium and muscle via a sGC/cGMP-dependent pathway (26,27), a process that may involve calcium/calmodulin-dependent protein kinase kinase (27). A number of studies have suggested that AMPK plays a role in hepatic glucose metabolism and could be a therapeutic target for people with type 2 diabetes (28,29). Activation of AMPK in mouse liver can inhibit hepatic gluconeogenesis and decrease glycogen content (30). Iglesias et al. (31) demonstrated that an intraperitoneal injection of AICAR resulted in a marked increment in net hepatic glycogen breakdown in rats; likewise, Pencek et al. (32,33) showed that intraportal AICAR infusion in 18-h–fasted conscious dogs caused an increase in glycogenolysis and hepatic glucose output. It should be noted, however, that AICAR may have effects on the liver in addition to activating AMPK, such as inhibition of fructose 1,6-bisphosphatase (34) and depletion of ATP (35). Nevertheless, the association between activation of AMPK and reduced hepatic glucose uptake and storage in the current study is consistent with other data. We recently showed that inhibiting hepatic sGC in dogs was also associated with a reduction in the

AMPK and ACC phosphorylation in the liver (3), suggesting that elevated hepatic NO/cGMP may limit NHGU through the activation of AMPK. This hypothesis is supported by the finding that AMPK can inactivate glycogen synthase (36), and our recent observation that increased hepatic AMPK phosphorylation is associated with suppressed glycogen synthase activity and reduced glycogen synthesis in the liver (37). It has been shown that activation of AMPK leads to phosphorylation of site 2 in glycogen synthase, thereby downregulating its activity (38,39). These data are in line with our current observation that 8-Br-cGMP increased the phosphorylation of Thr-172 in hepatic AMPK by 90% without a change in its protein level, in association with reduced hepatic glycogen synthase activity. The exact role AMPK plays in the regulation of NHGU by hepatic NO/cGMP needs further investigation. Furthermore, we have demonstrated that hepatic AMP/ATP ratio was not changed in response to 8-Br-cGMP, suggesting that the altered AMPK activity was not due to the change of energy status in the liver. Further investigation is required to determine the exact mechanism by which NO/cGMP regulates AMPK in the liver, as well as to delineate the role of AMPK in the regulation of NHGU.

In conclusion, the present data demonstrate that intraportal infusion of 8-Br-cGMP decreases NHGU under hyperglycemic hyperinsulinemic conditions in the presence of portal glucose delivery in conscious 42-h–fasted dogs. These data support a role for the sGC/cGMP pathway in the regulation of glucose uptake by the liver. The exact molecular mechanism by which NO/sGC/cGMP brings about this hepatic effect remains to be established, but it appears to involve an activation of hepatic AMPK.

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Z.A. researched data, wrote the manuscript, and reviewed and edited the manuscript. J.J.W., M.C.M., and J.M.I. researched data and reviewed and edited the manuscript. B.F. and M.S. researched data. P.J.R. contributed to discussion and reviewed and edited the manuscript. A.D.C. researched data, contributed to discussion, and reviewed and edited the manuscript. A.D.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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