Inhibition of glycogenolysis enhances gluconeogenic precursor uptake by the liver of conscious dogs

MASAKAZU SHIOTA, PATRICIA A. JACKSON, HILMAR BISCHOFF, MICHAEL McCaleb, MELANIE SCOTT, MICHAEL MONOAHAN, DOSS W. NEAL, AND ALAN D. CHERINGTON

Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0615; and Bayer Research Center, West Haven, Connecticut 06516-4175

Shiota, Masakazu, Patricia A. Jackson, Hilmar Bischoff, Michael McCaleb, Melanie Scott, Michael Monohan, Doss W. Neal, and Alan D. Cherrington. Inhibition of glycogenolysis enhances gluconeogenic precursor uptake by the liver of conscious dogs. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E868–E879, 1997.—We investigated the effect of inhibiting glycogenolysis on gluconeogenesis in 18-h-fasted conscious dogs with the use of intragastric administration of BAY R 3401, a glycogen phosphorylase inhibitor. Isotopic (13C-glucose and [U-13C]-alanine) and arteriovenous difference methods were used to assess glucose metabolism. Each study consisted of a 100-min equilibration, a 40-min control, and two 90-min test periods. Endogenous insulin and glucagon secretions were inhibited with somatostatin (0.8 µg·kg

1 ·min

2) and the two hormones were replaced intraportally (insulin: 0.25 mU·kg

2 ·min

2; glucagon: 0.6 ng·kg

1 ·min

2). Drug (10 mg/kg) or placebo was given after the control period. Insulin and glucagon were kept at basal levels in the first test period, after which glucagon infusion was increased to 2.4 ng·kg

1 ·min

2; BAY R 3401 decreased basal endogenous glucose production [rate of glucose production (Rg): 14 ± 1 to 7 ± 1 µmol·kg

1 ·min

2] and net hepatic glucose output (11 ± 1 to 3 ± 2 µmol·kg

1 ·min

2) during test 1. It increased the net hepatic uptake of gluconeogenic substrates from 9.0 ± 2.0 to 11.6 ± 0.6 µmol·kg

1 ·min

2. Basal glycogenolysis was decreased by drug (9.1 ± 0.7 to 1.5 ± 0.2 µmol glucosyl U·kg

1 ·min

1). Placebo had no effect on Rg or the uptake of gluconeogenic precursors by the liver. The rise in glucagon increased Rg by 22 ± 3 and by 8 ± 2 µmol·kg

1 ·min

1 (at 10 min) in placebo and drug, respectively. The rise in glucagon caused little change in the net hepatic uptake (µmol·kg

1 ·min

1) of gluconeogenic substrates in placebo (8.2 ± 0.6 to 9.0 ± 1.0) but increased it markedly (11.6 ± 0.6 to 15.4 ± 1.0) in drug. Glucagon increased glycogenolysis by 22.1 ± 2.5 and by 7.8 ± 1.6 µmol·kg

1 ·min

1 in placebo and drug, respectively. The amount of glycogen (µmol glucosyl U/kg) synthesized from gluconeogenic carbon was four times higher in drug (48.6 ± 9.7) than in placebo (11.3 ± 1.7). We conclude that BAY R 3401 caused a marked reduction in basal and glucagon-stimulated glycogenolysis. As a result of these changes, there was an increase in the net hepatic uptake of gluconeogenic precursors and in glycogen synthesis.

BAY R 3401: glucagon

THE LIVER PRODUCES GLUCOSE via glycogen breakdown and/or gluconeogenesis, and the relative contribution of each to total glucose production changes with altered nutritional and metabolic states. Several studies in dogs and humans have shown that increased delivery of gluconeogenic precursors, such as alanine (11, 39), glycerol (20, 38), or lactate (6, 8, 21), to the liver has no acute effect on the amount of glucose produced by that organ. Delivery of lactate (8), glycerol (38), or alanine (11) to postabsorptive dogs in the presence of fixed basal levels of insulin and glucagon increased both the hepatic uptake of these precursors and their conversion into glucose but did not change the total rate of glucose production appreciably. These data support the concept that when gluconeogenesis increases in the liver, gluconeogenesis decreases. Gluconeogenic precursors can alter hepatic glycogen metabolism not only by exerting regulatory effects on glycogen phosphorylase and synthase but also by serving as substrates for glycogen synthesis (for review, see Ref. 40). The above data suggest the existence of an autoregulatory mechanism within the liver such that the desired rate of hepatic glucose output can be maintained regardless of the gluconeogenic precursor supply.

Gluconeogenesis is a primary determinant of hepatic glucose production (for review, see Refs. 2 and 19). In postabsorptive dogs, physiological changes in plasma glucose have been shown to alter hepatic glucose production through changes in both gluconeogenesis and glycogenolysis (2, 3). The dose-response relationships between gluconeogenesis and hepatic glycogenolysis and gluconeogenesis appear to be similar (33), but the time courses of the responses are different. The gluconeogenic effect of glucagon is initially small, whereas the glycogenolytic effect is marked (2, 3, 35). Thereafter, the gluconeogenic effect of glucagon on the liver increases progressively while glucagon-induced glycogenolysis decreases (2, 3, 35). It is possible, therefore, that the increase in glycogenolysis caused by glucagon limits the initial gluconeogenic effects of the peptide and that the decline in glycogenolysis over time allows gluconeogenesis to increase.

BAY R 3401 is a novel compound that reduces blood glucose levels by inhibiting glycogen phosphorylase in the liver after oral administration (unpublished data).

THE LIVER PRODUCES GLUCOSE via glycogen breakdown and/or gluconeogenesis, and the relative contribution of each to total glucose production changes with altered nutritional and metabolic states. Several studies in dogs and humans have shown that increased delivery of gluconeogenic precursors, such as alanine (11, 39), glycerol (20, 38), or lactate (6, 8, 21), to the liver has no acute effect on the amount of glucose produced by that organ. Delivery of lactate (8), glycerol (38), or alanine (11) to postabsorptive dogs in the presence of fixed basal levels of insulin and glucagon increased both the hepatic uptake of these precursors and their conversion into glucose but did not change the total rate of glucose production appreciably. These data support the concept that when gluconeogenesis increases in the liver, gluconeogenesis decreases. Gluconeogenic precursors can alter hepatic glycogen metabolism not only by exerting regulatory effects on glycogen phosphorylase and synthase but also by serving as substrates for glycogen synthesis (for review, see Ref. 40). The above data suggest the existence of an autoregulatory mechanism within the liver such that the desired rate of hepatic glucose output can be maintained regardless of the gluconeogenic precursor supply.

Gluconeogenesis is a primary determinant of hepatic glucose production (for review, see Refs. 2 and 19). In postabsorptive dogs, physiological changes in plasma glucose have been shown to alter hepatic glucose production through changes in both gluconeogenesis and glycogenolysis (2, 3). The dose-response relationships between gluconeogenesis and hepatic glycogenolysis and gluconeogenesis appear to be similar (33), but the time courses of the responses are different. The gluconeogenic effect of glucagon is initially small, whereas the glycogenolytic effect is marked (2, 3, 35). Thereafter, the gluconeogenic effect of glucagon on the liver increases progressively while glucagon-induced glycogenolysis decreases (2, 3, 35). It is possible, therefore, that the increase in glycogenolysis caused by glucagon limits the initial gluconeogenic effects of the peptide and that the decline in glycogenolysis over time allows gluconeogenesis to increase.

BAY R 3401 is a novel compound that reduces blood glucose levels by inhibiting glycogen phosphorylase in the liver after oral administration (unpublished data). The active metabolite of the compound, BAY U 6751, inhibits the a- and b-forms of the enzyme with an in vitro 50% inhibitory concentration of 55 and 19 ng/ml, respectively. The aim of the present study, therefore, was to assess the effects of inhibiting glycogenolysis (with BAY R 3401) on basal and glucagon-stimulated gluconeogenesis in the conscious dog.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were performed on 10 overnight-fasted mongrel dogs (17.4–29.0 kg, mean ± SE 22.4 ± 1.1 kg) of either sex that had been fed a standard meat and chow diet (34% protein-46% carbohydrate-
14% fat-6% fiber based on dry wt; 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 American Association for the Accreditation of Laboratory Animal Care guidelines, 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 body wt pentobarbital 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, a hepatic vein, a jejunal vein, and splenic vein as previously described (7, 8, 11, 28, 29, 31, 33, 35, 37). The catheter for drug infusion was placed into the stomach as previously described (29). On the day of the experiment, the catheters were exteriorized under local anesthesia (2% lidocaine; Abbott, North Chicago, IL); their contents were aspirated, and they were flushed with saline. Angiocaths (20 gauge; Abbott) were inserted into both cephalic veins for infusion of indocyanine green, radioactive tracers, and glucose and into a saphenous vein for the infusion of somatostatin.

On the day before the experiment, the 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. Each experiment consisted of a 100-min tracer and dye equilibration period (−140 to −40 min), a 40-min control period (−40 to 0 min), and two 90-min experimental periods (0 to 180 min). A priming dose of [3-3H]glucose (41.7 µCi) was given at −140 min. Continuous infusions of [3-3H]glucose (0.34 µCi/min), [U-14C]alanine (0.67 µCi/min), and indocyanine green (0.1 mg·m⁻²·min⁻¹) were also started at −140 min and were continued throughout the experiment. At −140 min a peripheral infusion of somatostatin (0.8 µg·kg⁻¹·min⁻¹) was started to inhibit endogenous insulin and glucagon secretion. Intraportal replacement infusions of insulin (0.25 mU·kg⁻¹·min⁻¹) and glucagon (0.6 ng·kg⁻¹·min⁻¹) were started simultaneously with initiation of the somatostatin infusion. The plasma glucose level was then monitored every 5 min, and the rate of insulin infusion was adjusted until the plasma glucose level was stabilized at a euglycemic value. Once stabilization had been achieved, the insulin infusion rate was left unchanged. The final infusion rate of insulin used in placebo and drug were 0.26 and 0.24 mU·kg⁻¹·min⁻¹, respectively. Two experimental protocols were used. An intragastric bolus of a 0.5% methylcellulose-saline solution (50 ml) with (10 mg/kg) (drug group) or without (placebo group) BAY R 3401 was given at 0 min. After a 90-min test period, the infusion rate of glucagon was increased fourfold in both groups for another 90 min.

Analytic procedures. Plasma glucose concentrations and plasma glucose radioactivity (³H and ¹⁴C) were determined as previously described (4, 35). Plasma [¹⁴C]alanine and [¹⁴C]lactate specific activities were determined with the use of short-column, ion-exchange chromatography as previously described by Chiasson et al. (4). Blood concentrations of lactate, alanine, glycerol, ketones, glucose, and glutamate and plasma concentration of nonesterified fatty acids were determined according to the methods reported previously (7, 8, 28, 29, 35). Individual blood amino acid levels were assessed with the use of the method of Venkatakrishnan et al. (34) with an interassay coefficient of variation (CV) of 4%. Plasma arterial and hepatic vein indocyanine green concentrations were determined spectrophotometrically at 805 nm (23).

Liver samples were obtained at the end of the experiments by euthanizing the dog with pentobarbital sodium, exposing the liver by laparotomy, and freeze clamping ~5-g liver sections from each lobe. The time elapsed from euthanasia to freeze clamping was <4 min. The entire liver was then removed from the dog and weighed. The frozen liver 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 as described previously (18). Net incorporation of [³H] and [¹⁴C] into glycogen was determined after liquid scintillation counting of the processed samples.

Immunoreactive plasma insulin, glucagon, and cortisol concentrations were determined as previously described (35).

Materials. [3-³H]glucose (NEC, Boston, MA) was used as the glucose tracer (500 µCi/0.005 mg), and [U-¹⁴C]alanine (Amersham, Chicago, IL) was used as the labeled gluconeogenic precursor (171 mCi/mmol). Indocyanine green was purchased from Hynson, Westcott, and Dunning (Baltimore, MD) and was prepared in sterile water. 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 normal saline and contained 3% (vol/vol) of the dog’s own plasma. Cortisol radioimmunoassay kits were obtained from Micromedic Systems (Hosham, PA).

Calculations. Hepatic blood flow was assessed by measuring hepatic extraction of indocyanine green (23). Based on data from Greenway and Stark (17), the proportions of the hepatic blood supply provided by the hepatic artery and portal vein were assumed to be 28 and 72%, respectively. This ratio conforms to data that we obtained with Doppler flow probes during pancreatic clamps (31). These proportions were assumed to remain constant throughout all experiments, since treatment did not significantly affect hepatic blood flow. Net hepatic substrate balance was calculated using the formula (H − (0.28A + 0.72P)) · HF, where A, P, and H are the arterial, portal vein, and hepatic vein substrate concentrations, respectively, and HF is the hepatic blood or plasma flow. When blood levels of the substrate were measured, blood flow was used in the calculation, whereas plasma flow was used when plasma levels were measured. Tracer-determined glycogen production and glucose utilization were determined by the method of DeBodo et al. (10) and with the use of the two-compartment model of Mari (25). The data calculated by the two-compartment model were described. The [¹⁴C]glucose production rate was determined using the tracer technique as described by Chiasson et al. (4).

The hepatic gluconeogenic conversion rate of alanine to glucose and the efficiency of the hepatic gluconeogenesis was calculated by dividing the [¹⁴C]glucose production rate (disintegrations·min⁻¹·dpm⁻¹·kg⁻¹·min⁻¹) by the specific activity of alanine (dpm·µmol) entering the liver (using weighted arterial and portal specific activities) and by the rate of net hepatic [¹⁴C]alanine uptake, respectively. [¹⁴C]lactate was considered in the gluconeogenic calculations (both gluconeogenic conversion and efficiency) only when [¹⁴C]lactate was consumed by the liver. In the case of conversion, the specific activity of the precursor pool was estimated by taking into account the relative contribution of [¹⁴C]lactate uptake and [¹⁴C]alanine uptake by the liver. These contributions were used to determine the average specific activity of these precursors entering the liver. The conversion rate and efficiency are actually minimal estimates of gluconeogenesis because of dilution of the gluconeogenic precursor specific activity within the hepatic oxalacetate pool and the fact that they assess gluconeogenesis from only two precursors. To
Results

Hormone levels and hepatic blood flow. Portal and arterial glucagon levels were basal and unchanged in both protocols during the first two periods. They rose approximately threefold, however, when the glucagon infusion rate was increased (Fig. 1). Portal and arterial insulin levels remained basal and unchanged throughout the study with both placebo and drug administration (Fig. 1). Arterial cortisol (2.2 ± 0.2 in placebo and 2.0 ± 0.4 µg/ml in drug), norepinephrine (174 ± 37 in placebo and 122 ± 21 pg/ml in drug), and epinephrine (96 ± 36 in placebo and 90 ± 25 pg/ml in drug) levels did not change with either treatment. Hepatic blood flow was initially higher in drug group (31 ± 2 ml·kg⁻¹·min⁻¹) than in the placebo group (21 ± 2 ml·kg⁻¹·min⁻¹), but liver blood flow did not change over time or with treatment in either group.

Glucose kinetics. The plasma glucose level fell slightly in the placebo group before the rise in glucagon while net hepatic glucose output (NHGO) remained unchanged (Fig. 2). With drug administration, NHGO decreased markedly (31.5 mg·kg⁻¹·min⁻¹) by 90 min; P < 0.05), necessitating glucose infusion (Table 1) to maintain euglycemia. In the placebo group the rise in glucagon infusion increased NHGO by 4.0 ± 0.5 mg·kg⁻¹·min⁻¹ in 10 min (P < 0.05). NHGO then fell but remained elevated relative to the previous period. As a result of the increase in NHGO, the plasma glucose level rose from 90 ± 3 to 171 ± 18 mg/dl by the end of the study (P < 0.05). The rise in glucagon in the drug treatment group increased NHGO by 2.0 ± 0.6
tracer-determined endogenous glucose production ($R_a$), during the period of increased glucagon. Rates of glucose infusion necessary to maintain euglycemia in the drug treatment group to maintain euglycemia, even with intragastric administration of BAY R 3401 (drug) in 18-h-fasted conscious dogs. Animals received intragastric bolus injection of placebo and of BAY R 3401 (10 mg/kg) at 0 min. Net hepatic glucose outputs before placebo and drug administration were 1.4 ± 0.1 and 2.0 ± 0.1 mg·kg$^{-1}$·min$^{-1}$, respectively. Animals treated with BAY R 3401 received glucose peripherally (Table 2) from 0 min on to establish and maintain euglycemia. Values for arterial plasma glucose are means ± SE and for changes in net hepatic glucose balance are mean differences ± SE from mean value of net hepatic glucose balance in control period. *Significantly different from corresponding value in placebo group (P < 0.05). †Significantly different from control period in identical group (P < 0.05).

glucose outputs before placebo and drug administration were 1.4 ± 0.1 and 2.0 ± 0.1 mg·kg$^{-1}$·min$^{-1}$, respectively. Animals treated with BAY R 3401 received glucose peripherally (Table 2) from 0 min on to establish and maintain euglycemia. Values for arterial plasma glucose are means ± SE and for changes in net hepatic glucose balance are mean differences ± SE from mean value of net hepatic glucose balance in control period. *Significantly different from corresponding value in placebo group (P < 0.05). †Significantly different from control period in identical group (P < 0.05).

glucose outputs before placebo and drug administration were 1.4 ± 0.1 and 2.0 ± 0.1 mg·kg$^{-1}$·min$^{-1}$, respectively. Animals treated with BAY R 3401 received glucose peripherally (Table 2) from 0 min on to establish and maintain euglycemia. Values for arterial plasma glucose are means ± SE and for changes in net hepatic glucose balance are mean differences ± SE from mean value of net hepatic glucose balance in control period. *Significantly different from corresponding value in placebo group (P < 0.05). †Significantly different from control period in identical group (P < 0.05).

As shown in Fig. 3, treatment with placebo did not cause a change in either the arterial lactate level or net hepatic lactate balance. In the presence of placebo the rise in glucagon was associated with an increase in net hepatic lactate production (by 6.7 ± 6.5 µmol·kg$^{-1}$·min$^{-1}$ by 10 min), although this change, unlike the rise in the blood lactate level (P < 0.05), did not reach significance. In contrast, drug administration caused net hepatic lactate output to cease (although, once again, the change was not significant) and stimulated net hepatic lactate uptake of 3.5 ± 1.0 µmol·kg$^{-1}$·min$^{-1}$, despite a fall in the arterial lactate level (567 ± 154 to 374 ± 70 µM; P < 0.05) before glucagon infusion. The rise in glucagon in the treatment group was associated with a further increase in net hepatic lactate uptake to 7.3 ± 0.8 µmol·kg$^{-1}$·min$^{-1}$ (P < 0.05) and a further decrease in the arterial lactate level (to 290 ± 21 µM by 180 min; P < 0.05). The lactate level was reduced by >90% in response to the drug treatment, and the liver consumed rather than produced lactate (P < 0.05).

As shown in Fig. 4, treatment with placebo did not affect the arterial blood alanine level, net hepatic alanine uptake, or net hepatic alanine fractional extraction. In the presence of placebo the rise in glucagon did not change the arterial alanine level significantly, but it caused small increases in net hepatic alanine uptake (2.6 ± 0.5 to 3.4 ± 0.4 µmol·kg$^{-1}$·min$^{-1}$; P < 0.05) and the net hepatic fractional extraction of alanine (0.36 ± 0.04 to 0.50 ± 0.05; P < 0.05) by 180 min. With drug administration alanine metabolism did not change significantly until the glucagon concentration rose. The rise in glucagon induced a fall in the arterial alanine level (from 256 ± 40 to 168 ± 15 µM; P < 0.05) and increases in net hepatic alanine uptake (from 3.3 ± 0.6 to 3.8 ± 0.5 µmol·kg$^{-1}$·min$^{-1}$) and fractional extraction (from 0.39 ± 0.04 to 0.62 ± 0.06; P < 0.05). The

Table 1. Rate of glucose infusion necessary to maintain euglycemia with intragastric administration of BAY R 3401

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Drug</th>
<th>Drug + glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.14 ± 0.05</td>
<td>0.37 ± 0.27</td>
</tr>
<tr>
<td>60</td>
<td>0.68 ± 0.13</td>
<td>0.71 ± 0.40</td>
</tr>
<tr>
<td>90</td>
<td>1.16 ± 0.21</td>
<td>0.87 ± 0.42</td>
</tr>
<tr>
<td>120</td>
<td>1.00 ± 0.25</td>
<td>1.11 ± 0.35</td>
</tr>
</tbody>
</table>

Values are means ± SE in mg·kg$^{-1}$·min$^{-1}$. Data are rates before and during a 4-fold increase in intraportal glucagon brought about in presence of basal insulin with intragastric administration of BAY R 3401 in 18-h-fasted conscious dogs.

---

**Fig. 2.** Arterial plasma glucose levels and changes in net hepatic glucose balance before and during a 4-fold increase in intraportal glucagon brought about in presence of basal insulin with or without intragastric administration of BAY R 3401 (drug) in 18-h-fasted conscious dogs. Animals received intragastric bolus injection of vehicle in placebo and of BAY R 3401 (10 mg/kg) at 0 min. Net hepatic glucose outputs before placebo and drug administration were 1.4 ± 0.1 and 2.0 ± 0.1 mg·kg$^{-1}$·min$^{-1}$, respectively. Animals treated with BAY R 3401 received glucose peripherally (Table 2) from 0 min on to establish and maintain euglycemia. Values for arterial plasma glucose are means ± SE and for changes in net hepatic glucose balance are mean differences ± SE from mean value of net hepatic glucose balance in control period. *Significantly different from corresponding value in placebo group (P < 0.05). †Significantly different from control period in identical group (P < 0.05).
Table 2. Tracer-determined rates of endogenous glucose production, utilization, and clearance

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Control Period</th>
<th>Drug or placebo</th>
<th>Drug or placebo + glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R&lt;sub&gt;a&lt;/sub&gt;</td>
<td>R&lt;sub&gt;d&lt;/sub&gt;</td>
<td>Cl</td>
</tr>
<tr>
<td>30</td>
<td>2.04 ± 0.13</td>
<td>2.13 ± 0.15</td>
<td>2.19 ± 0.17</td>
</tr>
<tr>
<td>60</td>
<td>1.96 ± 0.13</td>
<td>2.14 ± 0.11</td>
<td>2.23 ± 0.12</td>
</tr>
<tr>
<td>90</td>
<td>2.07 ± 0.08</td>
<td>2.18 ± 0.17</td>
<td>2.34 ± 0.21</td>
</tr>
</tbody>
</table>

Data are means ± SE. Data for control period are means of values at −40, −20, and 0 min. Rates of glucose production (R<sub>a</sub>) and utilization (R<sub>d</sub>) and rate of glucose clearance (Cl) are in ml·kg<sup>−1</sup>·min<sup>−1</sup>. Data are rates before and during a 4-fold increase in intraportal glucagon brought about in presence of basal insulin with or without intragastric administration of BAY R 3401 in 18-h-fasted conscious dogs. *Significantly different from corresponding value in placebo group (P < 0.05). †Significantly different from control period (P < 0.05).

latter was twofold greater than that seen in the absence of drug.

Arterial glycerol levels and net hepatic balance. Arterial glycerol levels, net hepatic glycerol uptake, and net hepatic fractional glycerol uptake remained unchanged with placebo treatment and were unaffected by the fourfold rise in glucagon (Fig. 5). Drug administration caused an increase in net fractional extraction of glycerol by the liver from 0.56 ± 0.05 to 0.66 ± 0.05 by 90 min (P < 0.05), but neither the arterial glycerol level nor net hepatic glycerol uptake changed significantly. In the presence of drug the rise in glucagon resulted in increases in the net hepatic uptake (from 1.4 ± 0.3 to 1.8 ± 0.3 µmol·kg<sup>−1</sup>·min<sup>−1</sup>) and fractional extraction of glycerol by the liver (from 0.66 ± 0.06 to 0.81 ± 0.03) by 150 min. These changes were significantly greater than those seen in the absence of drug.

Arterial levels and net hepatic balances of the gluconeogenic amino acids. Arterial glutamine, glycine, serine, and threonine levels and their uptakes by the liver remained unchanged with placebo or drug treatment before glucagon infusion (Table 3). The rise in glucagon shifted net glutamine balance from production to uptake and resulted in a decrease in blood glutamine levels in both groups (P < 0.05). The blood levels of glutamine, glycine, serine, and threonine fell in response to glucagon in both groups. Their rates of uptake by the liver were not significantly changed but their net hepatic fractional extractions increased significantly (P < 0.05).

Gluconeogenic parameters. Net hepatic gluconeogenic substrate uptake, obtained by summing the net hepatic uptakes of lactate, glycerol, pyruvate, and all gluconeogenic amino acids, did not change in the placebo group before or during the period of increased glucagon (Fig. 6). Likewise, the conversion of gluconeogenic precursors to glucose and gluconeogenic efficiency did not increase in the placebo group (Fig. 7) before glucagon infusion, although gluconeogenic conversion did increase in response to the rise in glucagon. In contrast, drug administration increased net hepatic gluconeogenic precursor uptake by 50% (P < 0.05), and the rise in glucagon caused a further increase up to 15 µmol·kg<sup>−1</sup>·min<sup>−1</sup>. Gluconeogenic conversion and effi-
Efficiency did not change in response to drug administration, and although glucagon may have increased glucose conversion slightly, the change was not significant. The amounts of $^3$H and $^{14}$C incorporated into liver glycogen by the end of study were two and five times higher, respectively, in the presence of drug than placebo. Glycogen content at 180 min was significantly higher with drug (46.7 ± 0.5 mg/g liver) than placebo (30.9 ± 5.0 mg/g liver). Glycogen synthesis via both the direct and indirect pathways was also higher with drug (66.6 ± 5.5 and 48.6 ± 9.7 µmol glucosyl U/kg body wt) than placebo (41.1 ± 5.1 and 11.3 ± 1.7 µmol glucosyl U/kg body wt) ($P < 0.05$).

Arterial nonesterified fatty acid and ketone body levels and net hepatic balance. Arterial levels, net hepatic uptake, and hepatic fractional extraction of
nonesterified fatty acids (NEFA) did not change significantly with placebo or drug treatment before the rise in glucagon (Fig. 8). The glucagon increment did not alter NEFA levels or uptake in the placebo group. In the drug treatment group, the rise in glucagon increased the net hepatic uptake and fractional extraction of NEFA slightly (P < 0.05). The arterial level and hepatic production of ketone bodies were not affected by placebo or drug treatment (Fig. 9). The rise in glucagon increased ketone body production in the presence of drug but not placebo (Fig. 9).

**DISCUSSION**

The relative contributions of glycolysis and gluconeogenesis to basal glucose production in the present study were the same as those noted in previous studies (2, 3, 7, 35). Gluconeogenic efficiency increased slightly with time in the placebo group (Fig. 7; Table 4), but this observation agrees with our earlier data (7). Whether it represents a transition of the animal into a more fasted state or the attainment of tracer equilibration in the metabolite pools of the liver remains unclear (7). The magnitudes of the increased glucose output, lactate production, and gluconeogenic efficiency induced by hyperglucagonemia in the placebo group (Figs. 2, 3, 7) were similar to those observed in previous studies (33, 35). The early glucagon-stimulated increase in glucose production, and net uptake of gluconeogenic precursors and gluconeogenesis was responsible for at least 80% of the overall increase in glucose production (88% at 100 min and 77% at 180 min) (Table 4). A time-dependent decrease in the glycogenolytic response to glucagon has been reported previously (3), and its cause has been discussed (3). It can thus be concluded that the placebo (intragastric administration of vehicle) produced no metabolic effects of its own.

Drug treatment, on the other hand, decreased basal hepatic glucose production by 70% and limited the glucagon-induced increase in glucose production by...
76%. Whereas plasma glucose level was maintained at basal levels during the period of increased glucagon in the drug-treated group, the rise in glucagon elevated plasma glucose level in the placebo group (Fig. 2). Because hyperglycemia has been shown to limit net hepatic glycogenolysis (2), the effect of drug treatment on the glycogenolytic action of glucagon is even more pronounced than is evident from a comparison of the glucose production rates in the two groups.

It is also evident that drug treatment increased the net uptake of gluconeogenic precursors by the liver (Fig. 6; Table 4). As a result, the maximal contribution of gluconeogenesis to glucose production increased to 80% before the rise in glucagon and was 91% by the end of the glucagon infusion period. Tracer-determined whole body glucose production reflects both hepatic and renal glucose production (1, 28). In the overnight-fasted dog, the kidney has been shown to produce glucose at rates equal to 13% (28) or 24% (1) of tracer-determined whole body glucose production. With the assumption that renal glucose production, which represents gluconeogenesis, did not change during the treatment with the drug, glucose production at the end of the experimental period was probably entirely due to a combination of renal and hepatic gluconeogenesis. Thus the decreased basal and glucagon-induced glucose production observed in the presence of BAY R 3401 was probably the result of an almost complete inhibition of glycogenolysis.

As noted above, the decrease in glycogenolysis brought about by drug treatment increased the basal rate of gluconeogenic precursor uptake by the liver. It also resulted in an increase in the ability of glucagon to stimulate net hepatic gluconeogenic precursor uptake (Fig. 6). The latter parameter provides a maximal estimate of gluconeogenesis that closely approximates the directly determined gluconeogenic rate (15). The increase in the net hepatic uptake of gluconeogenic precursors induced by decreasing glycogenolysis was not accompanied by increases in the rates of gluconeogenic conversion or efficiency (Fig. 7). Because glycogen synthesis operates simultaneously with glycogen breakdown (9, 24), an inhibition of glycogenolysis can cause an increase in the retention of [14C] from gluconeogenic precursors in glycogen. If this were the case, true gluconeogenic efficiency would be underestimated by an amount equal to the rate of [14C]glucose deposition in glycogen. Glycogen content at the end of the study...
was significantly higher in the drug treatment group than in the placebo group, and the amount of $^{14}$C retained in glycogen after drug administration was four times that after placebo treatment. With the assumption that $^{14}$C incorporation into glycogen occurred constantly during the 180 min after the control period, the rates of $^{14}$C incorporation into glycogen in placebo and drug were equivalent to 3 and 15% of the hepatic $[^{14}$C$]$glucose production rate, respectively. Even if one considers the combination of $[^{14}$C$]$glucose production plus $[^{14}$C$]$glucose deposition in glycogen, the reduction of glycogenolysis did not enhance gluconeogenic efficiency. With the assumption that the $^{14}$C was incorporated into glycogen only during the rise in glucagon, the rates of $^{14}$C incorporation into glycogen in the placebo and in the drug treatment group corresponded to 8 and 28% of the $[^{14}$C$]$glucose production rate during this period. If the $[^{14}$C$]$glucose production and $[^{14}$C$]$glucose deposition in glycogen are again added, gluconeogenic efficiency increased 17% in placebo and 50% in drug
In conclusion, the present results show that BAY R 3401 decreased glycogenolysis in the conscious dog. Furthermore, decreased glycogenolysis per se aug-

Table 4. Minimal and maximal gluconeogenic rates

<table>
<thead>
<tr>
<th>Drug or placebo</th>
<th>Drug or placebo + glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Period</td>
<td>Time, min</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Maximum GNG rate</td>
<td>4.28 ± 0.55 3.94 ± 0.74 4.28 ± 0.62 4.09 ± 1.04 4.07 ± 0.94 4.39 ± 0.61 4.43 ± 0.26 4.51 ± 0.51</td>
</tr>
<tr>
<td>GNG efficiency</td>
<td>0.30 ± 0.04 0.37 ± 0.07 0.35 ± 0.05 0.34 ± 0.06 0.46 ± 0.09 0.36 ± 0.07 0.43 ± 0.09 0.41 ± 0.08</td>
</tr>
<tr>
<td>Minimum GNG rate</td>
<td>1.28 ± 0.30 1.46 ± 0.21 1.50 ± 0.36 1.41 ± 0.36 1.87 ± 0.66 1.58 ± 0.24 1.90 ± 0.40 1.84 ± 0.47</td>
</tr>
<tr>
<td>Endogenous GNG rate</td>
<td>11.33 ± 0.72 10.88 ± 0.72 11.50 ± 0.44 11.72 ± 0.50 32.17 ± 4.20† 29.94 ± 3.67† 22.06 ± 2.28† 19.89 ± 1.94†</td>
</tr>
<tr>
<td>Max GNG cont (endo Rg)</td>
<td>38 ± 5 35 ± 5 37 ± 4 34 ± 7 12 ± 2 15 ± 2 21 ± 2 23 ± 3</td>
</tr>
<tr>
<td>BAY R 3401</td>
<td>Maximum GNG rate</td>
</tr>
<tr>
<td>GNG efficiency</td>
<td>0.25 ± 0.05 0.24 ± 0.05 0.23 ± 0.03 0.22 ± 0.03 0.32 ± 0.06 0.28 ± 0.04 0.29 ± 0.03 0.30 ± 0.05</td>
</tr>
<tr>
<td>Minimum GNG rate</td>
<td>1.38 ± 0.44 1.49 ± 0.27 1.44 ± 0.30 1.23 ± 0.16 1.57 ± 0.28 1.94 ± 0.33 1.96 ± 0.35 1.98 ± 0.17</td>
</tr>
<tr>
<td>Endogenous GNG rate</td>
<td>13.94 ± 0.94 11.39 ± 0.83 9.11 ± 1.11† 7.28 ± 1.17† 15.17 ± 2.94* 12.22 ± 2.67† 9.67 ± 1.28† 8.44 ± 0.72†</td>
</tr>
<tr>
<td>Max GNG cont (endo Rg)</td>
<td>32 ± 6 55 ± 6† 68 ± 8† 80 ± 6† 39 ± 6† 57 ± 6† 79 ± 7† 91 ± 6†</td>
</tr>
</tbody>
</table>

Data are means ± SE in μmol glucosyl U·kg⁻¹·min⁻¹. Data for control period are means of values at ~40, ~20, and 0 min. Rates are before and during a 4-fold increase in intraportal glucagon in presence of basal insulin with or without intragastric administration of BAY R 3401 in 18-h-fasted conscious dogs. Max GNG cont (endo Rg), maximal contribution of gluconeogenesis (endogenous Rg). *Significantly different from corresponding value in placebo group (P < 0.05). †Significantly different from control period (P < 0.05).

during the rise in glucagon. In this case, the effect of glucagon to increase gluconeogenic efficiency would have increased significantly (P < 0.05) when glycogenolysis was inhibited. Thus we cannot rule out a change in gluconeogenic efficiency in response to the inhibition of glycogenolysis, but even if it occurred it was not very large.

The drug failed to increase the fractional extraction of gluconeogenic precursors (Figs. 3–5). The rates of amino acid uptake by the liver depend on their concentration gradient across the membrane as well as on activity or number of the amino acid transporter(s). Because there is rapid equilibration between the plasma lactate pool and the intracellular lactate and pyruvate pools, the increase in net hepatic lactate uptake (Fig. 3) probably reflected a decrease in the intracellular pyruvate levels. Decreased concentration of intracellular pyruvate within the liver might accelerate the transamination of alanine and, as the result, therefore, contribute to the driving force for hepatic amino acid uptake (12). Because fatty acids have been shown in vitro to inhibit glycolysis and increase gluconeogenesis from lactate, (30) and because fatty acid oxidation has been reported to support the gluconeogenic effect of glucagon (19), increased fatty acid uptake and the oxidation might serve to augment the glucagon-induced uptake of gluconeogenic precursors.

The rise in glucagon shifted net hepatic glutamine balance from slight net production to net uptake (Table 3), as observed in a previous study (18). In contrast to alanine uptake, decreasing glycogenolysis did not affect basal hepatic glutamine balance or glucagon-stimulated net hepatic uptake of this amino acid (Table 3). The regulation of hepatic glutamine metabolism may differ from the regulation of alanine metabolism, because the transporter that mediates the uptake of glutamine (system N) is different from that of alanine (system A) (5). In addition, glucagon stimulates intracel-

lular glutamine degradation via the activation of glutaminase.

Glucagon causes increased net hepatic NEFA uptake and net hepatic ketone production in drug-treated animals, whereas this peptide had no such effect in the control group, in line with previous studies (22, 35). The glucagon-induced increases in NEFA uptake and ketogenesis in the drug-treated group were not due to an increased NEFA availability, as indicated by unchanged plasma NEFA levels and increased fractional extraction of free fatty acids (FFA) (Fig. 8). A study by Goresky et al. (16) in the liver of anesthetized dogs (16) in which the rate constants for transport and metabolism of tracer [1-14C]palmitate were assessed by means of the multiple-indicator dilution technique suggested that net NEFA uptake is not solely determined by the FFA load but that intracellular factors are equally important. Wasserman et al. (37) showed in conscious dogs that glucagon exerted a ketogenic effect during prolonged exercise and suggested that the effect was on the ketogenic process within the liver. In vitro studies in perfused liver or hepatocytes have shown that glucagon stimulates triglyceride degradation, fatty acid oxidation, and ketogenesis and that it inhibits fatty acid synthesis (13, 26). Because lactate has an inhibitory effect on ketogenesis in the liver (13), on the other hand, the dramatically reduced blood lactate levels are associated with a reduction in the direct inhibitory effects of hyperglycemia and lactate on hepatic ketogenesis. Consequently, the effects of glucagon on inhibition of hepatic fatty acid synthesis and stimulation of hepatic ketogenesis are more evident, and the resultant decrease in the intracellular concentration of fatty acids might in turn promote an increase in FFA uptake.

In conclusion, the present results show that BAY R 3401 decreased glycogenolysis in the conscious dog. Furthermore, decreased glycogenolysis per se aug-
mented gluconeogenesis by increasing net hepatic glu-
coneogenic precursor uptake and by increasing FFA
metabolism within the liver. This suggests the exis-
tence of a reciprocity between glycogenolysis and gluco-
neogenesis and fatty acid metabolism in maintaining
total glucose output by the liver. The augmentation of
the gluconeogenic effect of glucagon when glycogeno-
dalysis was inhibited suggests that the increase in
glycogenolysis, which always follows a rise in glucagon,
delays and/or represses the gluconeogenic effect of this
peptide within the hepatocytes.

We thank Jon Hastings and the members of the Vanderbilt
Diabetes Research and Training Center Core Labs (Wanda Sneed,
Eric Allen, Pamela Venson, Pat Donahue, Annapurna Ven-
katakamaran, and Paul Flakoll) for technical support.

This research was supported by Miles Laboratories (Bayer) Grant.
Part of this work was presented at the 55th Annual Meeting of the
Address for reprint requests: M. Shiota, Molecular Physiology and
Biophysics, Vanderbilt Univ. School of Medicine, 710E Medical
Research Bldg. 1, 215 Ave. South and Garland Ave., Nashville, TN
37232–0615.

Received 5 November 1996; accepted in final form 12 J une 1997.

REFERENCES

1. Cersosimo, E., R. L. J udd, and J. M. Miles. Insulin regulation of renal glu-
2. Cherrington, A. D., R. W. Stevenson, K. E. Stein, M. A. Davis, S. R. Myers, B. A. Adkins, N. N. Abumrad, and P. E. Williams. Insulin, glycogen, and glucose as regulators of hepatic
3. Cherrington, A. D., P. E. Williams, G. I. Shulman, and W. W. Lacy. Differential time course of glucagon’s effect on glycogenoly-
5. Christensen, H. N. Role of amino acid transport and counter-
7. Connolly, C. C., K. E. Steiner, R. W. Stevenson, D. W. Neal, P. E. Williams, K. G. M. M. Alberti, and A. D. Cherrington. Regulation of glucose metabolism by noradrenaline in conscious
8. Connolly, C. C., R. W. Stevenson, D. W. Neal, D. H. Wasserman,
  and A. D. Cherrington. The effects of lactate loading on alanine and glucose homeostasis in the conscious dog. Metabolism
  King, and E. J. Barrett. Simultaneous synthesis and degradation
10. DeBodo, R. C., R. Steele, N. Altszuler, A. Dunn, and J. S. Bishop. On the hormonal regulation of carbohydrate metabo-
11. Diamond, M. P., R. C. Roblings, K. E. Steiner, P. E. Williams,
  W. W. Lacy, and A. D. Cherrington. Effect of alanine concentra-
  tion independent of changes in insulin and glucagon on alanine
  and glucose homeostasis in the conscious dog. Metabolism 37: