α- and β-Cell Responses to Small Changes in Plasma Glucose in the Conscious Dog

Nancy Flattem, Kayano Igawa, Masakazu Shiota, Maya G. Emshwiller, Doss W. Neal, and Alan D. Cherrington

The responses of the pancreatic α- and β-cells to small changes in glucose were examined in overnight-fasted conscious dogs. Each study consisted of an equilibration (–140 to –40 min), a control (–40 to 0 min), and a test period (0 to 180 min), during which BAY R3401 (10 mg/kg), a glycogen phosphorylase inhibitor, was administered orally, either alone to create mild hypoglycemia or with peripheral glucose infusion to maintain euglycemia or create mild hyperglycemia. Drug administration in the hypoglycemic group decreased net hepatic glucose output (NHGO) from 8.9 ± 1.7 (basal) to 6.0 ± 1.7 and 5.8 ± 1.0 μmol · kg^{-1} · min^{-1} by 30 and 90 min. As a result, the arterial plasma glucose level decreased from 5.8 ± 0.2 (basal) to 5.2 ± 0.3 and 4.4 ± 0.3 mmol/l by 30 and 90 min, respectively (P < 0.01). Arterial plasma insulin levels and the hepatic portal-arterial difference in plasma insulin decreased (P < 0.01) from 78 ± 18 and 90 ± 24 to 24 ± 6 and 12 ± 12 pmol/l over the first 30 min of the test period and decreased to 18 ± 6 and 0 pmol/l by 90 min, respectively. The arterial glucagon levels and the hepatic portal-arterial difference in plasma glucagon increased from 43 ± 5 and 4 ± 2 to 51 ± 5 and 10 ± 5 ng/l by 30 min (P < 0.05) and to 79 ± 16 and 31 ± 15 ng/l by 90 min (P < 0.05), respectively. In euglycemic dogs, the arterial plasma glucose level remained at 5.9 ± 0.1 mmol/l, and the NHGO decreased from 10 ± 0.6 to –3.3 ± 0.6 μmol · kg^{-1} · min^{-1} (180 min). The insulin and glucagon levels and the hepatic portal-arterial differences remained constant. In hyperglycemic dogs, the arterial plasma glucose level increased from 5.9 ± 0.2 to 6.2 ± 0.2 mmol/l by 30 min, and the NHGO decreased from 10 ± 1.7 to 0 μmol · kg^{-1} · min^{-1} by 30 min. The arterial plasma insulin levels and the hepatic portal-arterial difference in plasma insulin increased from 60 ± 18 and 78 ± 24 to 126 ± 30 and 192 ± 42 pmol/l by 30 min, after which they averaged 138 ± 24 and 282 ± 30 pmol/l, respectively. The arterial plasma glucagon levels and the hepatic portal-arterial difference in plasma glucagon decreased slightly from 41 ± 7 and 4 ± 3 to 34 ± 7 and 3 ± 2 ng/l during the test period. These data show that the α- and β-cells of the pancreas respond as a coupled unit to very small decreases in the plasma glucose level. *Diabetes* 50:367–375, 2001

From the Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee.

Address correspondence and reprint requests to Masakazu Shiota, DVM, PhD, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, 710 Medical Research Bldg. 1, Nashville, TN 37232. E-mail: masakazu.shiota@mcmail.vanderbilt.edu.

Received for publication 12 January 2000 and accepted in revised form 6 October 2000.

ANOVA, analysis of variance; FFA, free fatty acid; NEFA, nonesterified fatty acid; NHGO, net hepatic glucose output.

Glucagon secretion increases in response to a decrease in the plasma glucose concentration and decreases in response to a rise in the plasma glucose level. Furthermore, insulin has been postulated to exert a paracrine influence on glucagon secretion when its release is modified in response to changes in the plasma glucose concentration. To date, studies have not provided a complete understanding of the relationship between a decrement in the plasma glucose level and glucagon or insulin secretion, because the insulin level itself has been elevated to decrease the glucose level, and insulin per se can affect not only its own secretion (1), but also the release of other counterregulatory hormones, including glucagon (2–11). Hyperinsulinemia, when paired with euglycemia, is known to increase the plasma concentrations of cortisol (5,6) and norepinephrine (7,8), but to decrease the plasma glucagon level (3,5,9). This decrease is thought to occur via a direct inhibitory effect on the α-cell (10,11). In addition, Davis et al. (2,12) showed that elevation of plasma insulin increases the sympatho-adrenal response to hypoglycemia in dogs and humans. The use of exogenous insulin to lower plasma glucose, therefore, does not permit assessment of the effects of a decrease in plasma glucose per se, in the absence of hyperinsulinemia, on pancreatic hormone secretion. As a result, the responses of α- and β-cells in vivo to a fall in blood glucose unaccompanied by an increase in insulin are poorly defined.

High concentrations of plasma glucose are known to decrease glucagon secretion from the α-cell and to potently increase insulin secretion from the β-cell. This increase may explain the observed decrease in glucagon secretion (10,11). Regardless, it is of interest to compare the ability of increases in plasma glucose and insulin to the ability of decreases in plasma glucose and insulin to alter glucagon secretion.

BAY R 3401 is a novel compound that can reduce blood glucose levels by inhibiting glycogen phosphorylase and thereby reducing hepatic glucose production (14). The use of this drug, in combination with the glucose clamp technique, allowed us to create mild hypoglycemia and hyperglycemia so that we could examine the relationship between the glucose level and the α- and β-cell function in the conscious dog.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Experiments were performed on 15 overnight-fasted mongrel dogs (mean weight 22.4 ± 1.1, range 17.4–29.0 kg) of either sex, which had been fed a standard laboratory diet (34% protein, 46% carbohydrate, 14% 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 American Association for the

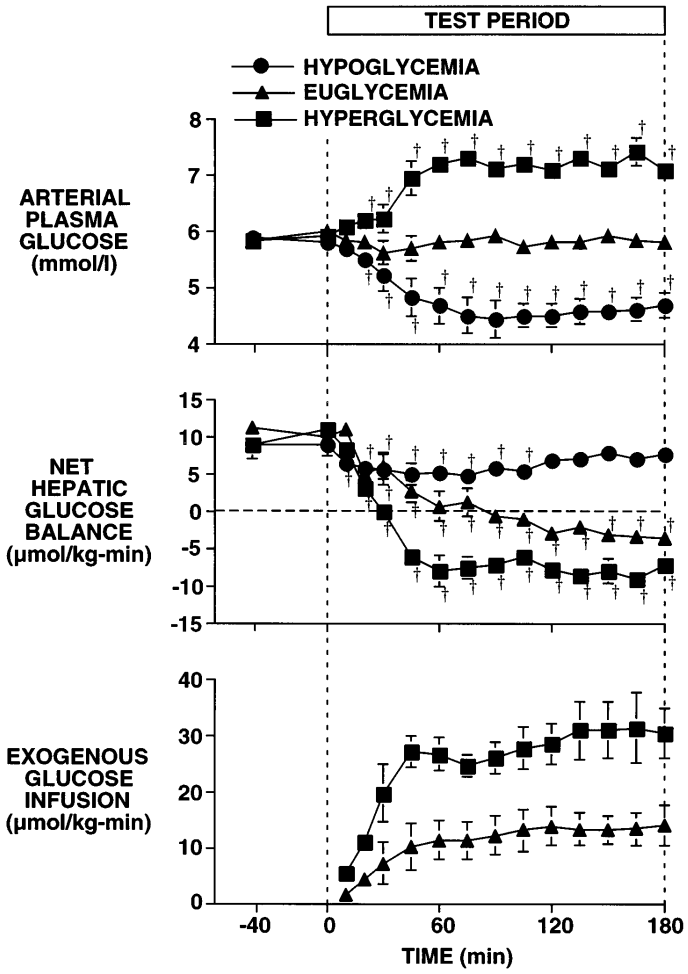

FIG. 1. Arterial plasma glucose levels, net hepatic glucose balances, and exogenous glucose infusion rates before and during intragastric administration of BAY R 3401 in the presence or absence of peripheral glucose infusion to maintain euglycemia or mild hyperglycemia in 18-h–fasted conscious dogs. Dogs received intragastric bolus injection of BAY R 3401 (10 mg/kg) at 0 min. Data are means ± SE. Each group consisted of five dogs. †Significantly different from control period in identical group ($P < 0.05$).

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 endotrachial anesthesia (15 mg/kg body wt pentothal sodium presurgery and 1.0% isoflurane as an inhalation anesthetic during surgery), and catheters were placed into a femoral artery, the portal, a hepatic, a jejunal, and a splenic vein for blood sampling, as previously described (2–4,7,14,15). 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 the right and left cephalic veins for infusion of glucose.

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/mm^3, *2*) a hematocrit >35%, *3*) a good appetite, and *4*) normal stools.

Experimental design. Each experiment consisted of a 100-min tracer equilibration period (–140 to –40 min), a 40-min control period (–40 to 0 min), and a 180-min experimental period (0–180 min). Three experimental protocols were used. A 0.5% methyl cellulose/saline solution (50 ml) with BAY R 3401 (10 mg/kg) was given by mouth at 0 min. The plasma glucose level was then monitored every 5 min. In one protocol, the glucose level was allowed to drop. In another protocol, the rate of glucose infusion was adjusted so that the plasma glucose level could be stabilized at a euglycemic value, and in a third protocol, the rate was adjusted to induce mild hyperglycemia.

Analytical procedures. Plasma glucose concentrations were determined using the glucose oxidase method in a Beckman glucose analyzer (Fullerton, CA) (16). Blood concentrations of lactate, glycerol, ketones, and alanine were determined according to the method of Lloyd et al. (17), as adapted to the Monarch 2000 centrifugal analyzer (Lexington, MA), in samples deproteinized with perchloric acid (3%). The plasma free fatty acid (FFA) concentrations were determined using the Wako nonesterified fatty acid (NEFA) C-test (Wako, Osaka, Japan). Plasma insulin, glucagon, cortisol, epinephrine, and norepinephrine levels were determined as previously described (14), with interassay coefficients of variation of 8, 9, 8, 10, and 5%, respectively.

Calculations. Hepatic, arterial, and portal vein blood flows were assessed using Transonic flow probes. Net hepatic substrate balance was calculated using the formula $H(F_a + F_p) - (AF_a + PF_p)$, where A, P, and H are the arterial, portal vein, and hepatic vein substrate concentrations, respectively, and F_a and F_p are the hepatic arterial and hepatic portal blood or plasma flows, respectively. The maximum gluconeogenic rate is equal to the sum of the net hepatic uptake rates of lactate, glycerol, and the gluconeogenic amino acids (alanine, glycine, serine, threonine, glutamine, and glutamate). The net glycogenolytic rate was obtained by subtracting the maximum gluconeogenic rate from the sum of net hepatic glucose output (NHGO), net hepatic lactate output, and glucose oxidation. The glucose oxidation rate was assumed to be 1.7 μmol · kg^{-1} · min^{-1} based on previous data (18).

Statistical analysis. Data are means ± SE. A one-way analysis of variance (ANOVA) for repeated measures was used to analyze changes in plasma substrate and hormone concentrations over time and rates of hormone secretion and net hepatic substrate balances. A two-way ANOVA for repeated measures was used to analyze time-course differences between the two groups. When significant changes were obtained, post hoc comparisons were made using a paired Student's t test (19).

RESULTS

Hepatic blood flow. The hepatic arterial (4.7 ± 0.3, 5.4 ± 0.6, and 5.1 ± 0.8 ml · kg^{-1} · min^{-1} in the euglycemic, hypoglycemic, and hyperglycemic groups, respectively) and portal (19.2 ± 1.2, 20.6 ± 2.2, and 22.5 ± 3.2 ml · kg^{-1} · min^{-1} in the euglycemic, hypoglycemic, and hyperglycemic groups) blood flows did not change over time or with treatment (data not shown).

Plasma glucose level and net hepatic glucose balance. In the hypoglycemic group, BAY R 3401 administration decreased NHGO from 8.9 ± 1.7 to 5.2 ± 1.1 μmol · kg^{-1} · min^{-1} by 60 min. Thereafter, NHGO gradually returned to its original rate (Fig. 1). Consequently, the arterial plasma glucose level decreased from 5.8 ± 0.2 to 5.2 ± 0.3 mmol/l by 30 min and to a minimum of 4.4 ± 0.4 mmol/l by 90 min. By the end of the experiment, the arterial plasma glucose level had increased slightly to 4.7 ± 0.2 mmol/l. In the euglycemic group, the plasma glucose level was maintained at a basal value by exogenous glucose infusion at 13.9 ± 3.3 μmol · kg^{-1} · min^{-1}. In the euglycemic group, NHGO decreased from 10.0 ± 0.6 to 0.6 ± 1.7 μmol · kg^{-1} · min^{-1} by 60 min, and by the end of the experiment the liver had begun to take up glucose (–3.3 ± 0.6 μmol · kg^{-1} · min^{-1}). In the hyperglycemic group, as a result of glucose infusion (28.9 ± 4.4 μmol · kg^{-1} · min^{-1}), the plasma glucose level increased from 5.9 ± 0.1 to 6.2 ± 0.2 mmol/l by 30 min and to a plateau of 7.1 ± 0.1 mmol/l by 90 min. NHGO decreased from 10.0 ± 1.7 to –8.3 ± 1.7 μmol · kg^{-1} · min^{-1} by 60 min, after which it remained constant.

Hormone levels. As shown in Fig. 2, the arterial and hepatic portal plasma insulin levels in the hypoglycemic group decreased from 78 ± 18 and 168 ± 36 to 24 ± 6 and 36 ± 12 pmol/l during the first 30 min of the test period and to 18 ± 6 and 24 ± 6 pmol/l by 90 min, respectively. Thereafter, the arterial and hepatic portal plasma insulin levels increased slightly to 36 ± 6 and 66 ± 12 pmol/l, respectively. The hepatic portal-arterial difference in plasma insulin decreased from 90 ± 24 to 12 ± 12pmol/l by 30 min and to 0 by 90 min, after which it increased slightly to 30 ± 18 pmol/l. The insulin

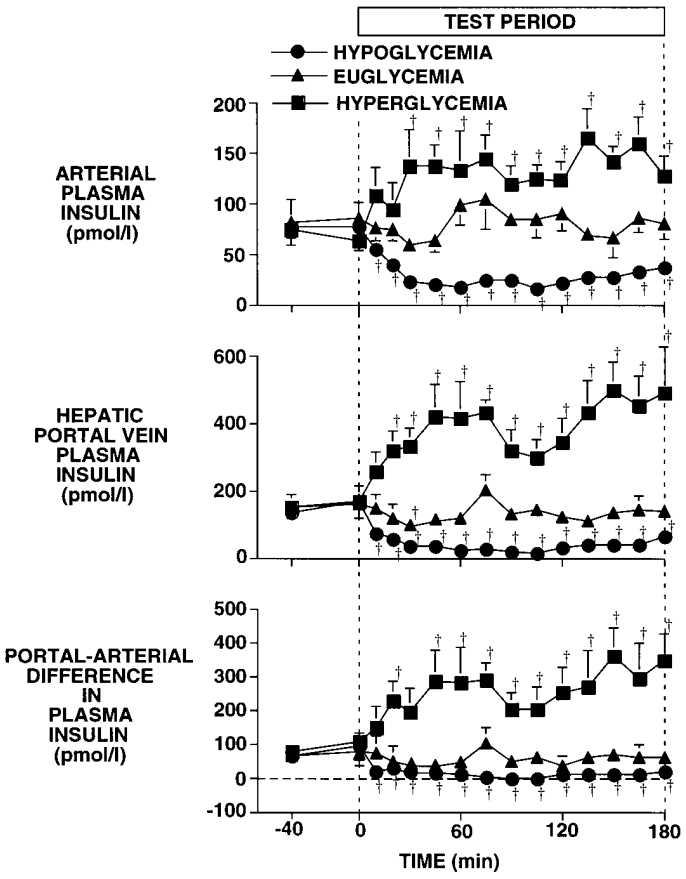

FIG. 2. Arterial and hepatic portal plasma insulin levels and the portal-arterial difference in plasma insulin before and during intragastric administration of BAY R 3401 in the presence or absence of peripheral glucose infusion to maintain euglycemia or mild hyperglycemia in 18-h–fasted conscious dogs. Dogs received intragastric bolus injection of BAY R 3401 (10 mg/kg) at 0 min. Data are means ± SE. Each group consisted of five dogs. †Significantly different from control period in identical group ($P < 0.05$).

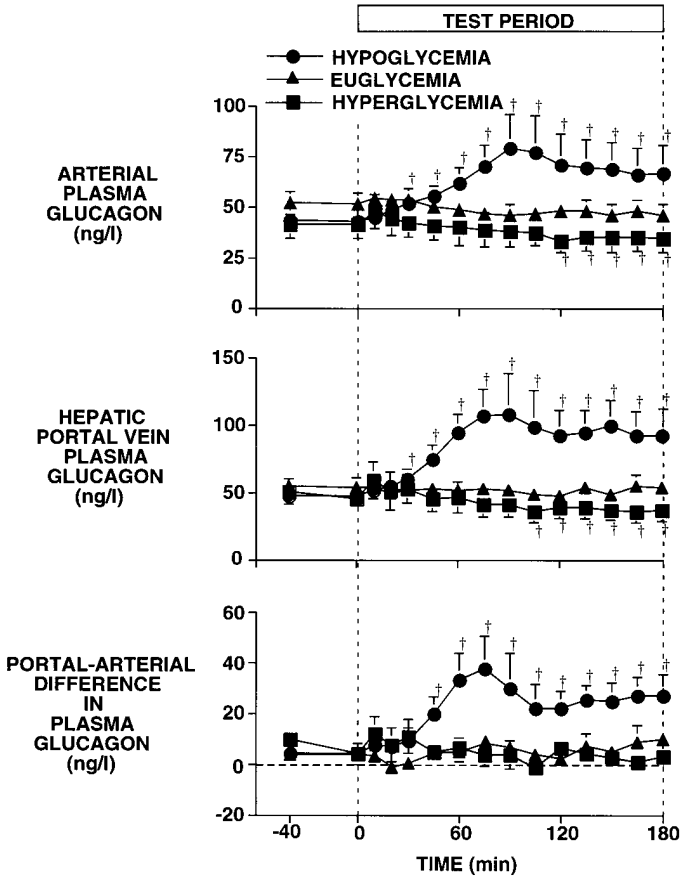

FIG. 3. Arterial and hepatic portal glucagon levels and the portal-arterial difference in plasma glucagon before and during intragastric administration of BAY R 3401 in the presence or absence of peripheral glucose infusion to maintain euglycemia or mild hyperglycemia in 18-h–fasted conscious dogs. Dogs received intragastric bolus injection of BAY R 3401 (10 mg/kg) at 0 min. Data are means ± SE. Each group consisted of five dogs. †Significantly different from control period in identical group ($P < 0.05$).

secretion rate, which was assessed by multiplying the hepatic portal-arterial difference in plasma insulin concentration by hepatic portal plasma flow rate, decreased from 1.2 ± 0.3 to 0.2 ± 0.1 pmol · kg^{-1} · min^{-1} by 30 min ($P < 0.05$) and to 0 by 90 min ($P < 0.05$), after which it increased slightly to 0.4 ± 0.2 pmol · kg^{-1} · min^{-1} by 30 min. The arterial and hepatic portal glucagon levels increased from 43 ± 5 and 48 ± 6 to 51 ± 5 and 61 ± 7 by 30 min and to 79 ± 16 and 109 ± 30 ng/l by 90 min, after which they decreased slightly (Fig. 3). The hepatic portal-arterial difference in plasma glucagon increased from 4 ± 2 (basal) to 10 ± 5 by 30 min and to 31 ± 15 by 90 min, after which it remained at 26 ± 13 ng/l. The glucagon secretion rate increased from 51 ± 26 (basal) to 128 ± 63 pg · kg^{-1} · min^{-1} by 30 min ($P < 0.05$) and to 397 ± 195 pg · kg^{-1} · min^{-1} by 90 min ($P < 0.05$), after which it decreased to 333 ± 166 pg · kg^{-1} · min^{-1}.

In the euglycemic dogs, the arterial plasma insulin and glucagon levels did not change significantly. Similarly, the portal levels and the hepatic portal-arterial differences were unchanged (Figs. 2 and 3). The insulin and glucagon secretion rates remained at 1.1 ± 0.3 pmol · kg^{-1} · min^{-1} and 92 ± 26 pg · kg^{-1} · min^{-1}, respectively. In the hyperglycemic dogs, on the other hand, the arterial and hepatic portal plasma insulin levels increased from 60 ± 18 and 150 ± 24 to 126 ± 30 and 348 ± 54 pmol/l by 30 min and averaged 138 ± 24 and 420 ± 78 pmol/l during the rest of the test period. The hepatic portal-arterial difference in plasma insulin increased from 78 ± 24 to 192 ± 42 pmol/l by 30 min and thereafter averaged 282 ± 30 pmol/l (Fig. 2). The insulin secretion rate increased from 1.1 ± 0.3 to 2.7 ± 0.4 pmol · kg^{-1} · min^{-1} by 30 min ($P < 0.05$), after which it averaged 4.0 ± 0.4 pmol · kg^{-1} · min^{-1}. The arterial and hepatic portal vein glucagon levels changed during the test period from 41 ± 7 and 48 ± 8 to 34 ± 7 and 36 ± 8 ng/l, respectively. The hepatic portal-arterial difference in plasma glucagon was stable between the basal and the test periods (4 ± 3 and 3 ± 2 ng/l, respectively). The glucagon secretion rate did not decrease significantly (from 56 ± 18 to 42 ± 27 pg · kg^{-1} · min^{-1}) during hyperglycemia.

As shown in Table 1, the arterial norepinephrine, epinephrine, and cortisol levels did not change significantly during mild-hypoglycemia, euglycemia, or modest hyperglycemia.

Arterial levels and net hepatic balances of plasma NEFAs and blood ketones, glycerol, lactate, and alanine. The arterial level of NEFAs and net hepatic NEFA uptake did not change significantly with the maintenance of euglycemia, but increased markedly in response to mild hypoglycemia and

TABLE 1
Arterial levels of plasma norepinephrine, epinephrine, and cortisol before and after oral administration of BAY R 3401 in the presence of noninsulin-induced hypoglycemia, euglycemia, or hyperglycemia in 18-h–fasted conscious dogs

Group	Control period	BAY R 3401 with or without peripheral glucose infusion (min)						
		10	20	30	60	90	120	180
Hypoglycemia								
Plasma norepinephrine (pmol/l)	906 ± 161	917 ± 158	830 ± 158	988 ± 126	933 ± 93	895 ± 115	966 ± 120	1,015 ± 235
Plasma epinephrine (pmol/l)	423 ± 68	513 ± 98	437 ± 98	475 ± 71	458 ± 87	458 ± 60	431 ± 82	453 ± 93
Plasma cortisol (nmol/l)	42 ± 8	49 ± 11	55 ± 11	58 ± 17	48 ± 9	46 ± 8	54 ± 10	57 ± 14
Euglycemia								
Plasma norepinephrine (pmol/l)	811 ± 189	873 ± 142	882 ± 142	726 ± 169	737 ± 142	824 ± 186	759 ± 180	786 ± 164
Plasma epinephrine (pmol/l)	420 ± 180	409 ± 87	284 ± 87	295 ± 115	273 ± 33	273 ± 175	218 ± 104	442 ± 196
Plasma cortisol (nmol/l)	58 ± 14	60 ± 13	57 ± 13	55 ± 21	36 ± 6	48 ± 9	55 ± 15	73 ± 35
Hyperglycemia								
Plasma norepinephrine (pmol/l)	693 ± 60	633 ± 38	650 ± 38	759 ± 126	737 ± 71	650 ± 98	671 ± 109	688 ± 71
Plasma epinephrine (pmol/l)	271 ± 41	224 ± 38	235 ± 38	224 ± 11	240 ± 16	262 ± 55	262 ± 55	246 ± 27
Plasma cortisol (nmol/l)	47 ± 15	66 ± 11	52 ± 11	41 ± 6	52 ± 14	72 ± 11	63 ± 8	77 ± 14

Data are means ± SE of five dogs.

decreased in response to mild hyperglycemia (Table 2). Arterial blood ketone levels and net hepatic ketone output were not affected by the drug treatment in the presence of euglycemia (Table 2). Mild hypoglycemia was associated with a rise in ketone levels and net hepatic ketone production, whereas both ketone levels and net hepatic ketone production were decreased by mild hyperglycemia. Arterial blood glycerol levels and net hepatic glycerol uptake did not change in the presence of euglycemia (Table 2). Mild hypoglycemia, on the other hand, caused the arterial blood glycerol level and net hepatic glycerol uptake to increase rapidly. In contrast, mild hyperglycemia caused the arterial glycerol level and net hepatic glycerol uptake to gradually decrease. In the presence of euglycemia, hypoglycemia, and hyperglycemia, arterial blood lactate levels decreased, whereas net hepatic lactate uptake gradually increased. Arterial blood alanine levels decreased in all groups, whereas net hepatic alanine uptake increased in the hypoglycemic group but did not change in the euglycemic and hyperglycemic groups.

Glycogenolysis and gluconeogenesis. When BAY R 3401 caused mild hypoglycemia by inhibiting phosphorylase, net glycogenolysis decreased from 7.39 ± 0.78 to 1.83 ± 1.83 μmol · kg^{-1} · min^{-1} by 30 min, after which it was effectively 0 (Table 3). On the other hand, gluconeogenesis began to increase at 30 min and by the end of the experiment had reached 8.7 ± 1.2 μmol · kg^{-1} · min^{-1}. From 60 to 180 min, the gluconeogenic rate was almost equivalent to the NHGO. In the presence of euglycemia, net glycogenolysis decreased from 8.2 ± 1.5 to –7.3 ± 1.4 μmol · kg^{-1} · min^{-1} by the end of test period, indicating net glycogen synthesis. The gluconeogenic rate did not change. In the presence of hyperglycemia, net glycogenolysis decreased rapidly and reached –3.9 ± 1.2 μmol · kg^{-1} · min^{-1} by 30 min. Over the last 90 min of the study, net glycogen synthesis averaged 10.1 ± 1.2 μmol · kg^{-1} · min^{-1}. Gluconeogenesis did not change significantly in the presence of hyperglycemia.

DISCUSSION

β-Cell response. Clearly, the plasma glucose concentration is a potent determinant of insulin secretion from the β-cell in vivo. In vitro studies using cultured islets have shown that the glucose dose-response curve for insulin secretion is sigmoidal and that the threshold and half-maximal responses occur around 5 and 10 mmol/l, respectively (20–24). Such data suggest that the β-cell should respond clearly to an increment in plasma glucose, such as the one induced in the present study. Thus, the sensitive response of the β-cells to increased glucose was expected. Amino acids, FFAs, and ketones have also been shown to stimulate insulin secretion (22). Thus, it is important to note that the plasma concentrations of these substances decreased in response to hyperglycemia to such an extent that they could not have contributed to the increase in insulin secretion. In fact, they may have opposed it.

It has been shown in the human that hyperinsulinemia induced by exogenous insulin infusion in the presence of euglycemia decreases the plasma C-peptide concentration (1), indicating that hyperinsulinemia per se suppresses insulin secretion. It was also evident from earlier work that under hyperinsulinemic clamp conditions, the plasma C-peptide concentration was suppressed further, as the plasma glucose concentration decreased from 5.1 to 4.9 mmol/l (25) or from 4.7 to 4.4 mmol/l (1). This suggests that insulin secretion is very sensitively suppressed in response to a decreasing plasma glucose level, at least in the presence of hyperinsulinemia. The results of the present study show that a small decrease in glucose can cause a marked reduction in insulin secretion, even in the absence of hyperinsulinemia. In fact, there was a significant 70% decrease in insulin secretion in response to a decrease of only 0.15 mmol/l in the plasma glucose level. Interestingly, the basal insulin secretion rates evident in the present study (1.1 ± 0.3 pmol · kg^{-1} · min^{-1}) closely correspond to the basal insulin infusion rates required to replace basal insulin secretion when the pancreatic clamp technique is used (1.1–1.5 pmol · kg^{-1} · min^{-1}), attesting to their validity.

Although the basal plasma glucose concentration is clearly a major determinant of insulin secretion in vivo, studies on isolated islets showed that insulin secretion did not decrease when the glucose concentration in the medium was lowered from 4–5 to 0 mmol/l (22,23). Other studies (24) also showed that insulin secretion from cultured islets decreased by only

TABLE 2
Arterial levels and net hepatic balances of plasma NEFAs, blood ketones, glycerol, lactate, and alanine before and after oral administration of BAY R 3401 in the presence of noninsulin-induced hypoglycemia, euglycemia, or hyperglycemia in 18-h–fasted conscious dogs

Group	Control period	BAY R 3401 with or without peripheral glucose infusion (min)						
		10	20	30	60	90	120	180
NEFA								
Hypoglycemia								
Plasma level (mmol/l)	0.67 ± 0.04	0.68 ± 0.07	0.80 ± 0.07*	0.92 ± 0.14*	1.29 ± 0.23*	1.39 ± 0.20*	1.49 ± 0.15*	1.52 ± 0.09*
NHB (μmol · kg^{-1} · min^{-1})	–2.51 ± 0.32	–2.51 ± 0.26	–2.85 ± 0.26	–3.22 ± 0.37*	–4.43 ± 0.90*	–5.32 ± 0.71*	–6.12 ± 0.74*	–6.69 ± 1.05*
Euglycemia								
Plasma level (mmol/l)	0.79 ± 0.09	0.73 ± 0.08	0.81 ± 0.08	0.80 ± 0.10	0.84 ± 0.13	0.78 ± 0.12	0.82 ± 0.06	0.91 ± 0.06
NHB (μmol · kg^{-1} · min^{-1})	–1.83 ± 0.29	–1.51 ± 0.35	–1.43 ± 0.33	–1.96 ± 0.33	–2.06 ± 0.51	–2.18 ± 0.39	–2.32 ± 0.25	–2.39 ± 0.24
Hyperglycemia								
Plasma level (mmol/l)	0.81 ± 0.10	0.74 ± 0.11	0.65 ± 0.09	0.63 ± 0.08	0.51 ± 0.07*	0.48 ± 0.05*	0.42 ± 0.04*	0.34 ± 0.05*
NHB (μmol · kg^{-1} · min^{-1})	–2.35 ± 0.31	–2.15 ± 0.25	–1.89 ± 0.26	–1.80 ± 0.21	–1.42 ± 0.20*	–1.32 ± 0.18*	–1.20 ± 0.15*	–0.91 ± 0.08*
Ketones								
Hypoglycemia								
Blood level (μmol/l)	116 ± 18	121 ± 13	109 ± 13	113 ± 17	124 ± 18	161 ± 21*	213 ± 27*	253 ± 42*
NHB (μmol · kg^{-1} · min^{-1})	1.20 ± 0.27	1.08 ± 0.26	1.17 ± 0.26	1.99 ± 0.64	2.58 ± 0.48*	3.25 ± 0.63*	4.50 ± 1.36*	7.95 ± 2.36*
Euglycemia								
Blood level (μmol/l)	135 ± 20	125 ± 20	121 ± 20	135 ± 14	133 ± 15	116 ± 11	110 ± 12	130 ± 21
NHB (μmol · kg^{-1} · min^{-1})	1.81 ± 0.66	1.45 ± 0.35	1.60 ± 0.35	1.68 ± 0.17	2.03 ± 0.16	1.41 ± 0.13	1.80 ± 0.38	2.10 ± 0.46
Hyperglycemia								
Blood level (μmol/l)	134 ± 19	104 ± 10	80 ± 10*	66 ± 8*	45 ± 7*	37 ± 4*	32 ± 4*	5 ± 4*
NHB (μmol · kg^{-1} · min^{-1})	2.06 ± 0.41	1.73 ± 0.35	1.24 ± 0.25	1.60 ± 0.32	0.83 ± 0.17*	0.70 ± 0.14*	0.67 ± 0.13*	0.11 ± 0.02*
Glycerol								
Hypoglycemia								
Blood level (μmol/l)	63 ± 4	66 ± 5	77 ± 5*	98 ± 12*	107 ± 15*	107 ± 12*	119 ± 5*	124 ± 11*
NHB (μmol · kg^{-1} · min^{-1})	–1.12 ± 0.11	–1.11 ± 0.11	–1.63 ± 0.11*	–2.34 ± 0.56*	–2.39 ± 0.51*	–2.31 ± 0.43*	–2.68 ± 0.27*	–2.91 ± 0.50*
Euglycemia								
Blood level (μmol/l)	85 ± 5	74 ± 7	98 ± 7	73 ± 10	96 ± 7	92 ± 12	70 ± 8	78 ± 13
NHB (μmol · kg^{-1} · min^{-1})	–1.41 ± 0.14	–1.29 ± 0.15	–1.26 ± 0.15	–1.35 ± 0.17	–1.68 ± 0.10	–1.63 ± 0.16	–1.49 ± 0.19	–1.45 ± 0.21
Hyperglycemia								
Blood level (μmol//l)	81 ± 6	75 ± 13	54 ± 13*	54 ± 5*	57 ± 9*	55 ± 6*	61 ± 15*	58 ± 6*
NHB (μmol · kg^{-1} · min^{-1})	–2.01 ± 0.25	–1.35 ± 0.56	–1.75 ± –0.28	–1.60 ± 0.23	–1.02 ± 0.21*	–1.19 ± 0.18*	–1.20 ± 0.25*	–1.22 ± 0.13*
Lactate								
Hypoglycemia								
Blood level (μmol/l)	632 ± 113	595 ± 65	522 ± 65*	573 ± 53	479 ± 56*	461 ± 44*	467 ± 54*	452 ± 42*
NHB (μmol · kg^{-1} · min^{-1})	–1.18 ± 2.76	–2.08 ± 2.67	–2.44 ± 2.67	–4.21 ± 1.78	–4.37 ± 0.83*	–5.80 ± 1.39*	–7.62 ± 1.24*	–8.73 ± 1.36*
Euglycemia								
Blood level (μmol/l)	636 ± 81	647 ± 124	615 ± 124	536 ± 98*	564 ± 91*	548 ± 69*	469 ± 71*	430 ± 73*
NHB (μmol · kg^{-1} · min^{-1})	–3.05 ± 2.69	–1.27 ± 4.15	–3.71 ± 4.15	–3.40 ± 3.56	–1.02 ± 2.23	–2.44 ± 0.89	–4.20 ± 0.27	–5.47 ± 0.42
Hyperglycemia								
Blood level (μmol/l)	975 ± 183	963 ± 159	903 ± 159	808 ± 137*	702 ± 109*	640 ± 112*	560 ± 102*	544 ± 121*
NHB (μmol · kg^{-1} · min^{-1})	–0.59 ± 1.21	–2.95 ± 2.81	–1.75 ± 2.11	–3.68 ± 0.91	–4.12 ± 0.15	–3.39 ± 1.84	–3.06 ± 1.63	–2.35 ± 0.92
Alanine								
Hypoglycemia								
Blood level (μmol/l)	317 ± 21	312 ± 21	301 ± 21	292 ± 13	270 ± 29*	227 ± 27*	206 ± 26*	161 ± 26*
NHB (μmol · kg^{-1} · min^{-1})	–1.99 ± 0.21	–1.67 ± 0.23	–2.37 ± 0.23	–2.71 ± 0.22*	–2.97 ± 0.52*	–2.73 ± 0.36*	–2.97 ± 0.47*	–2.74 ± 0.54*
Euglycemia								
Blood level (μmol/l)	299 ± 25	290 ± 25	257 ± 25	253 ± 28	246 ± 25*	245 ± 27*	232 ± 35*	204 ± 34*
NHB (μmol · kg^{-1} · min^{-1})	–2.31 ± 0.32	–2.12 ± 0.46	–1.94 ± 0.46	–2.33 ± 0.34	–1.85 ± 0.27	–2.02 ± 0.51	–2.20 ± 0.37	–1.99 ± 0.63
Hyperglycemia								
Blood level (μmol/l)	422 ± 52	430 ± 49	417 ± 49	380 ± 44*	327 ± 34*	279 ± 30*	245 ± 26*	205 ± 17*
NHB (μmol · kg^{-1} · min^{-1})	–2.61 ± 0.20	–2.30 ± 0.15	–3.21 ± 0.14	–3.03 ± 0.12	–3.23 ± 0.09	–2.04 ± 0.18	–2.47 ± 0.18	–2.61 ± 0.20

Data are means ± SE of five dogs. *Significantly changed from the values during control period in same group. Positive and negative values in net hepatic balance (NHB) represent output and uptake rates, respectively.

50% when the glucose concentration in the medium decreased from 5 to 2.5 mmol/l. Thus, the question arises as to whether the small decrease in the plasma glucose concentration that we induced was directly responsible for the complete suppression of insulin secretion that we observed, or whether it was attributable to some other signal present in vivo. Epinephrine and norepinephrine are known to inhibit insulin secretion through an α_2-adrenergic mechanism (26,27). Epinephrine is unlikely to have contributed to the suppression of insulin secretion in the present study, however, because its concentration in plasma did not change. On the other hand, it is known from other studies that activation of the sympathetic nerves to the islets of Langerhans induces the release of norepinephrine (28) and galanin (29), both of which inhibit insulin release (30). Therefore, it is theoretically possible that an increase in sympathetic discharge could have played a role in the decrease in insulin secretion that we observed in response to hypoglycemia. However, this would

TABLE 3
Hepatic gluconeogenic flux and net glycogenolytic rates before and after oral administration of BAY R 3401 in the presence of hypoglycemia, euglycemia, or hyperglycemia in 18-h–fasted conscious dogs

Group	Control period	BAY R 3401 with or without peripheral glucose infusion (min)						
		10	20	30	60	90	120	180
Gluconeogenic flux rate (μmol glucose · kg^{-1} · min^{-1})								
Hypoglycemia	3.11 ± 1.50	3.89 ± 1.39	4.39 ± 1.39	6.00 ± 0.89*	6.38 ± 0.61*	6.78 ± 1.39*	8.11 ± 1.11*	8.72 ± 1.22*
Euglycemia	4.50 ± 1.11	3.38 ± 1.72	4.39 ± 1.78	4.72 ± 1.38	3.22 ± 1.50	4.88 ± 0.89	5.00 ± 0.61	5.50 ± 0.50
Hyperglycemia	3.89 ± 0.88	4.39 ± 1.78	5.00 ± 1.28	5.17 ± 0.72	4.66 ± 0.27	4.28 ± 1.11	4.61 ± 1.00	4.38 ± 0.61
Net hepatic glycogenolytic rate (μmol glucose · kg^{-1} · min^{-1})								
Hypoglycemia	7.39 ± 0.78	7.05 ± 0.38	4.27 ± 0.38*	1.83 ± 1.83*	0.66 ± 0.39*	0.44 ± 0.78*	0.67 ± 1.22*	0.78 ± 1.22*
Euglycemia	8.17 ± 1.50	7.78 ± 1.22	4.17 ± 0.28*	3.06 ± 0.72*	–0.78 ± 1.22*	–3.72 ± 0.56*	–6.11 ± 1.39*	–7.33 ± 1.44*
Hyperglycemia	8.44 ± 1.61	6.39 ± 1.28	–0.28 ± 1.39*	–3.94 ± 1.22*	–12.0 ± 1.33*	–9.61 ± 1.17*	–10.61 ± 1.06*	–9.72 ± 1.39*

Data are means ± SE of five dogs. *Significantly changed from the values during control period in the same group. Positive and negative values in net hepatic balance represent output and uptake rates, respectively. Maximum gluconeogenic rates are the sum of the uptake rates of lactate, glycerol, and amino acids. The glycogenolytic rates are obtained by subtracting the maximum gluconeogenic rate from the sum of net hepatic glucose output, lactate production, and glucose oxidation.

require that a change in plasma glucose of 0.15 mmol/l significantly alter sympathetic input to the pancreas , and this seems unlikely. In addition, we failed to detect any change in the arterial plasma norepinephrine level, even thought although the latter is clearly only a crude index of sympathetic nerve activity.

Likewise, it is unlikely that the parasympathetic nervous system was involved. In the present study, the plasma pancreatic polypeptide concentration increased slightly (104 ± 27 to 141 ± 31 ng/l by 60 min) in association with the decreasing plasma glucose level. The secretion of this polypeptide from the F-cell is known to increase in response to an increased firing rate of the parasympathetic nervous system (31). Thus, it seems likely that there was a small increase in parasympathetic signaling to the β-cell in response to mild hypoglycemia. Because enhanced parasympathetic signaling increases insulin secretion, insulin release would have been increased rather than decreased.

As previously described, amino acids, FFAs, and ketones have been shown to stimulate insulin secretion (22). In the presence of hypoglycemia, plasma FFAs and ketone concentrations markedly increased (200%), whereas blood amino acid concentrations decreased modestly (50%). Therefore, it is unlikely that changes in the plasma concentrations of these substrates contributed to the decrease in insulin secretion that occurred in response to mild hypoglycemia, because their effects would have offset one another or resulted in a net increase in the stimulus for insulin release.

In summary, the increase in insulin secretion that occurred in response to mild hyperglycemia was undoubtedly mediated by changes in the plasma glucose level . On the other hand, the cause of the marked decrease in insulin secretion that occurred in response to non–insulin-induced hypoglycemia is less apparent. The β-cell is either very sensitive to reduction in plasma glucose or there is some unknown regulatory signal that is critical to the response.

α-Cell response. Glucagon secretion from α-cells is modulated by many factors, including the autonomic nervous system, blood substrate concentrations, and the plasma insulin level. Because the arterial plasma glucagon level and glucagon secretion decreased so little (if at all) in response to hyperglycemia, it is evident that hyperglycemia per se, even in the presence of modestly increased plasma insulin, had little effect on basal glucagon secretion. On the other hand, a decrease in plasma glucose from 5.8 to 5.2 mmol/l triggered a significant increase in arterial glucagon and glucagon secretion (>200% of basal) at a plasma glucose value well above the well-recognized threshold of 3.8 mmol/l observed in the presence of insulin-induced hypoglycemia in humans (1,12,25,32,33) and dogs (2–4,15). However, because basal plasma glucose levels in overnight-fasted humans (~4.7 mmol/l) (1–6) are usually lower than those in overnight-fasted dogs (~5.8 mmol/l), the extent of the decrease in plasma glucose required to initiate an increase in glucagon secretion in the present study (0.6 mmol/l) was only about half (~1.2 mmol/l) of that reported to initiate glucagon release in response to insulin-induced hypoglycemia in humans (1,25). Importantly, however, in a previous study (3), we showed that in overnight-fasted dogs, decreasing plasma glucose from 5.9 to 4.3 mmol/l in the presence of hyperinsulinemia (480 pmol/l) failed to trigger glucagon secretion. Thus, in the presence of hyperinsulinemia, a decrease of 1.6 mmol/l in the plasma glucose level failed to cause the plasma glucagon concentration to increase, whereas in the absence of an increase in insulin, a decrease of 1.4 mmol/l (from 5.8 to 4.4) caused a sixfold increase in glucagon secretion. These findings lead to the hypothesis that the α-cell response to hypoglycemia is diminished by hyperinsulinemia.

It should be noted that the measured basal glucagon secretion rate was only 10–20% of the basal glucagon infusion rate required to re-create basal glucagon levels during a pancreatic clamp. This suggests that streaming in the portal vein or the regional presence of α-cells in the pancreas prevented a quantitatively precise assessment of the pancreatic glucagon secretion. Nevertheless, the approach we used allowed an evaluation of alterations in pancreatic glucagon release to be made parallel with the changes we observed in arterial and portal plasma glucagon levels. Altogether, these observations make it clear that the α-cell response to hypoglycemia is more sensitive than previously thought.

Marked decrements in the plasma glucose concentration can increase glucagon secretion from the perfused pancreas,

so hypoglycemia per se is thought to directly stimulate glucagon secretion from the pancreatic α-cell (34). However, it is unclear whether a small decrement in blood glucose, such as the one that occurred in the present study, can directly stimulate glucagon secretion. It should be remembered that insulin secretion decreased markedly in response to hypoglycemia. Anatomical and physiological studies (35–38) suggest that blood in the islet flows from β-cells to α-cells, thereby exposing α-cells to high concentrations of intraislet insulin. It is possible, therefore, that the decrease in insulin secretion from β-cells lowered the level of the insulin concentration to which α-cells were exposed and, in turn, caused an increase in glucagon release. The administration of anti-insulin serum to perfused rat pancreas markedly increased glucagon secretion and abolished the ability of an increase in the glucose concentration to decrease glucagon secretion (39–41). It has also been shown that the destruction of islet β-cells prevents the increment in glucagon secretion seen in response to low glucose media in vitro (42). Therefore, it is likely that a decrease in intraislet insulin contributed in some way to the increased glucagon secretion that we observed in response to mild hypoglycemia.

There are three major autonomic inputs to the α-cell: sympathetic nerves, parasympathetic nerves, and the circulating neurohormone epinephrine (31,43). Epinephrine can stimulate glucagon secretion in vivo (44) and in vitro (45). Electrical stimulation of pancreatic sympathetic nerve (46), local infusion of the classical sympathetic neurotransmitter norepinephrine (47), or a pancreatic sympathetic neuropeptide, such as galanin (48), all increase glucagon secretion. Similarly, electrical stimulation of parasympathetic nerves (49,50), local infusion of the classical parasympathetic neurotransmitter acetylcholine (51), or a pancreatic parasympathetic neuropeptide, such as vasoactive intestinal peptide (52,53), also stimulates glucagon release from the α-cell. During the noninsulin-induced hypoglycemia generated in the present study, epinephrine could not have contributed to the stimulation of glucagon secretion, because its concentration in plasma did not change. In addition, there was no systemic activation of the sympathetic nervous system, as evidenced from the observation that the arterial norepinephrine levels did not rise. However, perfusion of the pancreas in vitro with low glucose media was associated with a local release of norepinephrine (28). α-Adrenergic blockade during such perfusion nearly abolished the glucagon response to glucopenia (54), suggesting that the effect of a decrease in glucose may be mediated by an α-adrenergic action of norepinephrine released intrapancreatically. It is not clear whether such a mechanism might be involved in the present findings. As noted earlier, the firing of the pancreatic parasympathetic nerves probably increased in the present study because a slight increase in the arterial pancreatic polypeptide levels occurred in response to hypoglycemia. Because increased parasympathetic signaling enhances glucagon release, it is possible that the parasympathetic nervous system might have contributed to the increase in glucagon secretion seen in the present study.

It is known that amino acids have stimulatory effects and that FFAs and ketones have inhibitory effects on glucagon secretion from the α-cell in the pancreas (22,41,55). As shown in Table 1, blood amino acid concentrations decreased, and plasma NEFA and ketone concentrations increased with the decrement in plasma glucose. Therefore, changes in blood metabolite levels could not have contributed to the rise in glucagon secretion seen in response to noninsulin-induced hypoglycemia and, in fact, probably opposed it.

In summary, glucagon secretion decreased little, if at all, in response to mild hyperglycemia; thus, the increases in plasma glucose and insulin that occurred in the hyperglycemic group had little effect on glucagon secretion. On the other hand, glucagon secretion markedly increased in response to a noninsulin-induced lowering of the blood glucose level. The increase in glucagon secretion may have resulted from decreases in the plasma glucose concentration, intraislet insulin levels, and/or increases in the discharge of intrapancreatic sympathetic and parasympathetic nerves. Regardless of the mechanism by which it occurs, the sensitivity of the α-cell to a decrease in glucose is greater than previously thought. As a result, the present data lead to the hypothesis that hyperinsulinemia desensitizes the α-cell to low blood glucose levels.

Lipolysis. Arterial plasma NEFA and blood glycerol levels increased as the arterial glucose level decreased during noninsulin-induced hypoglycemia. In contrast, the arterial plasma NEFA and blood glycerol levels decreased as the arterial glucose level was increased by exogenous glucose infusion (Table 1). The increase and the decrease in lipolysis were secondary to hypoglycemia and hyperglycemia, respectively, and not secondary to a direct effect of the drug on adipose tissue, because there was no significant change in the blood glycerol level in the euglycemic control group. Lipolysis in adipose tissue is regulated by numerous factors (56). Because the NEFA levels started to increase in response to hypoglycemia before the increase in arterial plasma glucagon level, and because the latter has only mild lipolytic effect (57–60), the increase in arterial plasma glucgon level is not likely to have contributed to the increase in lipolysis. Insulin, on the other hand, has a potent inhibitory effect on lipolysis (61), and the plasma insulin level decreased in response to hypoglycemia before the increase in NEFA levels (Fig. 2 and Table 1); thus, insulin is likely to have contributed to the increase in lipolysis. During hyperglycemia, plasma insulin and glucose increased before the decrease in arterial plasma NEFA levels, again suggesting a cause and effect relationship. There were no changes in plasma norepinephrine or epinephrine in response to hypo- or hyperglycemia, thereby ruling out their involvement. Therefore, it is likely that the activation and inactivation of lipolysis in response to hypo- and hyperglycemia, respectively, were mediated by the decrease or the increase in both plasma insulin and glucose.

Glucose production. Basal hepatic glucose production after an 18-h fast in dogs is mainly dependent on glycogenolysis (~60%). In the euglycemic dogs, NHGO was completely suppressed by the inhibition of phosphorylase, but the liver still took up gluconeogenic precursors at 5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (glucose equivalents). Because net glycogen flux is determined by the balance between glycogen phosphorylase activity and glycogen synthase activity (62), the inhibition of phosphorylase must have shifted the net balance toward synthesis. At the end of the experiment, net hepatic glucose uptake was 3.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, and net hepatic gluconeogenic precursor uptake, when expressed as glucose equivalents, was 5.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Because glycogenolysis was suppressed, net glycogen synthesis occurred, and the gluco-

neogenic precursors taken up by the liver were incorporated into glycogen. The net glycogen synthetic rate was ~7.3 μmol · kg^{-1} · min^{-1}. The shift of net hepatic glucose balance from output to uptake, and the shift of net glycogen metabolism from glycogenolysis to glycogen synthesis occurred in the absence of changes in the plasma levels of regulatory hormones or substrates, suggesting an important role for the dynamic balance between glycogen phosphorylase activity and glycogen synthase activity in determining net hepatic glucose balance. On the other hand, gluconeogenic flux did not change significantly in the presence of basal levels of plasma glucose, insulin, and glucagon, even though the contribution of gluconeogenically derived glucose 6-phosphate to NHGO decreased to zero (it was diverted to glycogen).

In the hypoglycemic dogs, inhibition of phosphorylase decreased net hepatic glucose production only transiently (Fig. 1). Although net hepatic glycogenolysis was almost completely suppressed, glycogen synthesis did not occur. During hypoglycemia, the plasma concentrations of glucose and insulin, both of which activate glycogen synthase, decreased (63); the plasma concentration of glucagon, which inhibits glycogen synthase, increased (63). It is likely, therefore, that glycogen synthase activity decreased, and as a result, the inhibition of phosphorylase activity did not result in net hepatic glycogen synthesis. It is also possible that the combined effect of the increase in glucagon and the decrements in insulin and glucose were able to partially overcome the effect of the drug on phosphorylase.

The net hepatic uptake of gluconeogenic precursors, which provides an estimate of gluconeogenic flux that closely approximates the directly determined gluconeogenic rate (64), increased from 3.3 to 8.9 μmol · kg^{-1} · min^{-1} when expressed in glucose equivalents. By the end of the study, gluconeogenesis was almost equal to NHGO (Table 1). Thus, the question arises as to the mechanism by which gluconeogenic flux increased. Because an increase in lipolysis led to increases in glycerol and NEFA uptake by the liver, the increase in lipolysis may have been critical for the increase in gluconeogenesis. An increase in lactate release from nonhepatic tissues also occurred. This increase was evident because, in both the control period and at the end of study, the blood lactate level was in a steady state, and yet the uptake rate of lactate by the liver was sevenfold higher at the end of the study than in the control period. In addition, the hepatic sinusoidal insulin level decreased by 90%, whereas the hepatic sinusoidal glucagon level doubled. Despite these dramatic changes, the gluconeogenic flux rate increased by only 5.6 μmol · kg^{-1} · min^{-1} during the hypoglycemic period.

In the hyperglycemic dogs, the suppression of glycogenolysis occurred rapidly (20 min); thereafter, net hepatic glycogen synthesis took place at a greater rate (10.6 μmol · kg^{-1} · min^{-1}) than was evident in the euglycemic dogs. Because insulin increased in response to hyperglycemia, it is likely that glycogen synthase activity was increased in this group. The gluconeogenic rate did not decrease during hyperglycemia, despite a marked increase in plasma insulin and a slight decrease in plasma glucagon, as well as decreases in plasma NEFA and glycerol levels. These findings confirm data that suggested physiological increases in plasma insulin have little effect on gluconeogenic flux (65,66).

The present study shows *1*) in vivo β-cells respond very sensitively to small changes in plasma glucose; *2*) glucagon secretion is sensitively increased in response to a small decrease in plasma glucose (0.6 mmol/l), but is not altered by hyperglycemia; *3*) mild hyperglycemia does not inhibit gluconeogenesis significantly, despite an increase in plasma insulin and decreases in both the plasma NEFA level and the gluconeogenic precursor load reaching the liver; and *4*) mild hypoglycemia increases gluconeogenesis in the presence of a decrease in plasma insulin, an increase in plasma glucagon, and an increase in the plasma NEFA level. In conclusion, these data establish the exceptional sensitivity of the α-/β-cell couple to a small decrease in plasma glucose. Also, these data suggest that the use of hyperinsulinemia to establish the relationship between α-cell function and the glucose level has caused a marked underestimatation of the role of hyperinsulinemia in defense of low blood glucose levels.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health (DK-43706 and DK-20593).

We thank Jon Hastings and the members of the Vanderbilt Diabetes Research and Training Center Core Labs (Wanda Snead, Eric Allen, and Angelina Penaloza) for technical support.

Part of this work was presented at the 57th Annual Meeting of the American Diabetes Association, Boston, Massachusetts, 8–11 June 1997.

REFERENCES

1. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Ciofetta M, Modarelli F, DiVincenzo A, Annibale B, Lepore M, Lalli C, Del Sindaco P, Brunetti P, Bolli GB: Relative roles of insulin and hypoglycemia on induction of neuroendocrine response to, symptoms of, and deterioration of cognitive function in hypoglycemia in male and female humans. *Diabetologia* 37:797–807, 1994
2. Davis SN, Dobbins R, Tarumi C, Colburn C, Neal D, Cherrington AD: Effects of differing insulin levels on response to equivalent hypoglycemia in conscious dogs. *Am J Physiol* 263:E688–E695, 1992
3. Davis SN, Dobbins R, Tarumi C, Jacobs J, Neal D, Cherrington AD: Paradoxical insulin-induced increase in gluconeogenesis in response to prolonged hypoglycemia in conscious dogs. *Am J Physiol* 268:E521–E530, 1995
4. Jackson PA, Pagliassotti MJ, Shiota M, Neal DW, Cardin S, Cherrington AD: Effects of vagal blockade on the counterregulatory response to insulin-induced hypoglycemia in the dog. *Am J Physiol* 273:E1178–E1785, 1997
5. Frizzell RT, Hendrick GK, Biggers DW, Lacy DB, Donahue DP, Green DR, Carr RK, Williams PE, Stevenson RW, Cherrington AD: Role of gluconeogenesis in sustaining glucose production during hypoglycemia caused by continuous insulin infusion in conscious dogs. *Diabetes* 37:749–759, 1988
6. Grunstein H, Jamus D, Storlein L, Smythe G, Kraegen EW: Hyperinsulinemia suppresses glucose utilization in specific brain regions: in vivo studies using the euglycemic clamp in the rat. *Endocrinology* 166:604–610, 1985
7. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL: Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87:2246–2252, 1991
8. Rowe JW, Young JB, Minaker KL, Stevens AL, Palotta J, Landsberg L: Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 30:219–225, 1981
9. Koopmans SJ, Mandarino L, DeFronzo RA: Time course of insulin action on tissue-specific intracellular glucose metabolism in normal rats. *Am J Physiol* 274:E642–E650, 1998
10. Ito K, Maruyama H, Hirose H, Kido K, Koyama K, Kataoka K, Saruta T: Exogenous insulin dose-dependently suppresses glucopenia-induced glucagon secretion from perfused rat pancreas. *Metabolism* 44:358–362, 1995
11. Liljenquist JE, Horwitz DL, Jennings AS, Chiasson J-L, Keller U, Rubenstein AH: Inhibition of insulin secretion by exogenous insulin in normal man as demonstrated by C-peptide assay. *Diabetes* 27:563–568, 1978
12. Davis SN, Cherrington AD, Goldstein RE, Jacobs J, Price L: Effects of insulin on the counterregulatory response to equivalent hypoglycemia in normal females. *Am J Physiol* 265:E680–E689, 1993
13. Gauthier C, Vranic M, Hetenyi G Jr: Importance of glucagon in regulatory rather than emergency responses to hypoglycemia. *Am J Physiol* 238:E131–E140, 1980
14. Shiota M, Jackson PA, Bischoff H, McCaleb M, Scott M, Monohan M, Neal DW,

Cherrington AD: Inhibition of glycogenolysis enhances gluconeogenic precursor uptake by the liver of conscious dogs. *Am J Physiol* 273:E868–E879, 1997
15. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT: Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 38:7–16, 1989
16. Kadish AH, Litle RL, Sternberg JC: A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. *Clin Chem* 14:116–131, 1968
17. Lloyd B, Burrin J, Smythe P, Albert KGMM: Enzymatic fluorometric continuous flow assays for glucose, lactate, pyruvate, alanine, and 3-hydrobutyrate. *Clin Chem* 34:1724–1729, 1978
18. Jacot E, DeFronzo RA, Jequier E, Maeder E, Feller J-P: The effect of hyperglycemia, hyperinsulinemia, and route of glucose administration on glucose oxidation and glucose storage. *Metabolism* 31:922–930, 1982
19. Snedecor GW, Cochran WG: *Statistical Methods*. 6th ed. Ames, IA, Iowa State University Press, 1967
20. Chen C, Hosokawa H, Bumbalo LM, Leady JL: Regulatory effects of glucose on the catalytic activity and cellular content of glucokinase in the pancreatic β-cell: study using cultured rat islets. *J Clin Invest* 94:1616–1620, 1994
21. Efrat S, Leiser M, Wu Y-J, Fusco-DeMane D, Emran OA, Surana M, Jetton TL, Magnuson MA, Weir G, Fleisher N: Ribozyme-mediated attenuation of pancreatic β-cell glucokinase expression in transgenic mice results in impaired glucose-induced insulin secretion. *Proc Natl Acad Sci U S A* 91:2051–2055, 1994
22. Gerich JE, Charles MA, Grodsky GM: Regulation of pancreatic insulin and glucagon secretion. *Ann Rev Physiol* 38:353–389, 1976
23. Ishihara H, Tashiro F, Ikuta K, Asano T, Katagiri H, Inukai K, Kikuchi M, Yazaki Y, Oka Y, Miyazaki J: Inhibition of pancreatic β-cell glucokinase by antisense RNA expression in transgenic mice: mouse strain-dependent alteration of glucose tolerance. *FEBS Lett* 371:329–332, 1995
24. Chen C, Hosokawa H, Bumbalo LM, Leahy JL: Regulatory effects of glucose on the catalytic activity and cellular content of glucokinase in the pancreatic β-cell: study using cultured rat islets. *J Clin Invest* 94:1616–1620, 1994
25. Schwartz NS, Clutter WE, Shah SD, Cryer PE: Glycemic thresholds for activation of glucose counterregulatory systems are higher than the thresholds for symptoms. *J Clin Invest* 79:777–781, 1987
26. Porte D Jr, Robertson RP: Control of insulin secretion by catecholamines, stress, and the sympathetic nervous system. *Fed Proc* 32:1792–1796, 1973
27. Strubbe JH, Steffens AB: Neural control of insulin secretion. *Horm Metab Res* 25:507–512, 1993
28. Christensen NJ, Iversen J: Release of large amounts of noradrenaline from the isolated perfused canine pancreas during glucose deprivation. *Diabetologia* 9:396–399, 1973
29. Dunning BE, Havel PJ, Veith RC, Taborsky Jr GJ: Pancreatic and extrapancreatic galanin release during sympathetic neural activation. *Am J Physiol* 258:E436–E444, 1990
30. Ahren B, Ostenson CG, Efendic S: Other islet peptides. In *The Endocrine Pancreas*. Samols E., Ed. New York, Raven Press, 1991, p. 153–173
31. Havel PJ, Taborsky GJ Jr: The contribution of the autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress. *Endocrinol Rev* 10:332–350, 1989
32. Cohen N, Rossetti L, Shlimovich P, Halberstam M, Hu M, Shamoon H: Counterregulation of hypoglycemia: skeletal muscle glycogen metabolism during three hours of physiological hyperinsulinemia in humans. *Diabetes* 44:423–430, 1995
33. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J,Cryer PE, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991
34. Gerich J, Charles M, Grodsky G: Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. *J Clin Invest* 54:833–841, 1974
35. Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 31:883–889, 1982
36. Samols E, Weir GC, Bonner-Weir S: Intraislet insulin-glucagon-somatostatin relationships. In *Glucagon*. Vol. II. Lebebre PJ, Ed. Berlin, Springer, 1983, p. 133–173
37. Samols E, Stagner JI: Intra-islet regulation. *Am J Med* 85:31–35, 1988
38. Stagner JI, Samols E: Retrograde perfusion as a method for testing the relative effects of glucose on the α-cell. *J Clin Invest* 77:1034–1037, 1986
39. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296–2299, 1984
40. Maruyama H, Tominaga M, Bolli G, Orci L, Unger RH: The alpha cell response to glucose change during perfusion of anti-insulin serum in pancreas isolated from normal rats. *Diabetologia* 28:836–840, 1985
41. Pagliara AS, Stillings SN, Haymond MW, Hover BA, Matschinsky FM: Insulin and glucose as modulators of the amino acid-induced glucagon release in the isolated pancreas of alloxan and streptozotocin diabetic rats. *J Clin Invest* 55:244–255, 1975
42. Tominaga M, Maruyama H, Vasko MR, Baetens D, Orci L, Unger RH: Morphologic and functional changes in sympathetic nerve relationship with pancreatic α-cells after destruction of β-cells in rats. *Diabetes* 36:365–373, 1987
43. Taborsky GJ Jr, Ahren B, Havel PJ: Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired α-cell response in type 1 diabetes. *Diabetes* 47:995–1005, 1998
44. Samols E, Weir GC: Adrenergic modulation of pancreatic A, B, and D cells: α-adrenergic suppression and β-adrenergic stimulation of somatostatin secretion, α-adrenergic stimulation of glucagon secretion in the perfused dog pancreas. *J Clin Invest* 63:230–239, 1979
45. Gerich JE, Karan JH, Forsham PH: Stimulation of glucagon secretion by epinephrine in man. *J Clin Endocrinol Metab* 37:479–481, 1973
46. Marliss EB, Girardier L, Seydoux J, Wollheim CB, Kanazawa Y, Orci L, Renold AE, Porte D Jr: Glucagon release induced by pancreatic nerve stimulation in the dog. *J Clin Invest* 52:1246–1259, 1973
47. Ahren B, Veith RC, Taborsky GJ Jr: Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: effects on basal release of insulin and glucagon. *Endocrinology* 121:323–331, 1987
48. Dunning BE, Ahren B, Veith RC, Böttcher G, Sundler F, Taborsky GJ Jr: Galanin: a novel pancreatic neuropeptide. *Am J Physiol* 251:E127–E133, 1986
49. Bloom SR, Edwards AV: Pancreatic endocrine responses to stimulation of the peripheral ends of the vagus nerves in conscious calves. *J Physiol (Lond)* 315:31–41, 1981
50. Kaneko A, Miki E, Kosaka K: Effects of vagal simulation on glucagon and insulin secretion. *Endocrinology* 95:1005–1010, 1974
51. Kaneko A, Kajinuma H, Kosaka K, Nakao K: Stimulation of insulin secretion by parasympathomimetic agents. *Endocrinology* 83:651–658, 1968
52. Havel PJ, Dunning BE, Verchere CB, Baskin DG, O'Dorisio T: Evidence that vasoactive intestinal polypeptide is a parasympathetic neurotransmitter in the endocrine pancreas in dogs. *Regul Pept* 71:163–170, 1997
53. Lindkaer S, Jensen J, Fahrenkrug J, Holst JJ, Nielsen OV, DeMuckadell OBS: Secretory effects of VIP on isolated perfused porcine pancreas. *Am J Physiol* 235:E387–E391, 1978
54. Hisatomi A, Maruyama H, Orci L, Vasko M, Unger RH: Adrenergically mediated intrapancreatic control of the glucagon response to glucopenia in the isolated rat pancreas. *J Clin Invest* 75:420–426, 1985
55. Pipeleers DG, Schuit FC, Van Schravendijk CF, Van de Winkel M: Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* 117:817–823, 1985
56. Coppack SW, Jensen MD, Miles JM: In vivo regulation of lipolysis in humans. *J Lipid Res* 35:177–193, 1994
57. Carlson MG, Snead WL, Campbell PJ: Regulation of free fatty acid metabolism by glucagon. *J Clin Endocrinol Metab* 77:11–15, 1993
58. Gerich JE, Lorenzi M, Bier DM, Tsalifian E, Schneider V, Karam JH, Forsham PH: Effect of physiologic levels of glucagon and growth hormone on human carbohydrate and lipid metabolism. *J Clin Invest* 57:875–884, 1976
59. Liljenquist JE, Bomboy JD, Lewis SB, Sinclair-Smith BC, Felts PW, Lacy WW, Crofford OB, Liddle GW: Effects of glucagon on lipolysis and ketogenesis in normal and diabetic man. *J Clin Invest* 53:190–197, 1974
60. Schade DS, Eaton RP: Modulation of fatty acid metabolism by glucagon in man: effects in normal subjects. *Diabetes* 24:502–509, 1975
61. Nurjhan N, Campbell PJ, Kennedy FP, Miles JM, Gerich JE: Insulin dose-response characteristics for suppression of glycerol release and conversion to glucose in humans. *Diabetes* 35:1326–1331, 1986
62. David M, Petit WA, Laughlin MR, Shulman RG, King JE, Barrett EJ: Simultaneous synthesis and degradation of rat liver glycogen. *J Clin Invest* 86:612–617, 1990
63. Hems DA, Whitton PD: Control of hepatic glycogenolysis. *Physiol Rev* 60:1–50, 1980
64. Goldstein R, Palmer B, Liu R, Massillon D: The effects of chronic hypercortisolemia on gluconeogenesis assessed using two independent methods in vivo (Abstract). *Diabetes* 44 (Suppl. 1):55A, 1995
65. Cherrington AD, Stevenson RW, Steiner KE, Connolly CC, Wada M: Acute hormonal regulation of gluconeogenesis in the conscious dog. In *New Concepts in the Pathogenesis of NIDDM*. Ostenson CG, Efendic S, Vranic M, Eds. New York, Plenum Press, 1993, p.199–208
66. Cherrington AD, Stevenson RW, Steiner KE, Davis MA, Myers SR, Adkins BA, Abumrad NN, Williams PE: Insulin, glucagon, and glucose as regulators of hepatic glucose uptake and production in vivo. *Diabetes Metab Rev* 3:307–332, 1987