Insulin Sensitively Controls the Glucagon Response to Mild Hypoglycemia in the Dog

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In the present study, we examined how the arterial insulin level alters the α-cell response to a fall in plasma glucose in the conscious overnight fasted dog. 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 R 3401 (10 mg/kg), a glycogen phosphorylase inhibitor, was administered orally to decrease glucose output in each of four groups ($n = 5$). In group 1, saline was infused. In group 2, insulin was infused peripherally (3.6 pmol · kg^{-1} · min^{-1}), and the arterial plasma glucose level was clamped to the level seen in group 1. In group 3, saline was infused, and euglycemia was maintained. In group 4, insulin (3.6 pmol · kg^{-1} · min^{-1}) was given, and euglycemia was maintained by glucose infusion. In group 1, drug administration decreased the arterial plasma glucose level (mmol/l) from 5.8 ± 0.2 (basal) to 5.2 ± 0.3 and 4.4 ± 0.3 by 30 and 90 min, respectively ($P < 0.01$). Arterial plasma insulin levels (pmol/l) and the hepatic portal-arterial difference in plasma insulin (pmol/l) decreased ($P < 0.01$) from 78 ± 18 and 90 ± 24 to 24 ± 6 and 12 ± 6 over the first 30 min of the test period. The arterial glucagon levels (ng/l) and the hepatic portal-arterial difference in plasma glucagon (ng/l) rose from 43 ± 5 and 5 ± 2 to 51 ± 5 and 10 ± 5 by 30 min ($P < 0.05$) and to 79 ± 16 and 31 ± 15 ($P < 0.05$) by 90 min, respectively. In group 2, in response to insulin infusion, arterial insulin (pmol/l) was elevated from 48 ± 6 to 132 ± 6 to an average of 156 ± 6. The hepatic portal-arterial difference in plasma insulin was eliminated, indicating a complete inhibition of endogenous insulin release. The arterial glucagon level (ng/l) and the hepatic portal-arterial difference in plasma glucagon (ng/l) did not rise significantly (40 ± 5 and 7 ± 4 at basal, 44 ± 4 and 9 ± 4 at 90 min, and 44 ± 8 and 15 ± 7 at 180 min). In group 3, when euglycemia was maintained, the insulin and glucagon levels and the hepatic portal-arterial difference remained constant. In group 4, the arterial plasma glucose level remained basal (5.9 ± 1.1 mmol/l) throughout, whereas insulin infusion increased the arterial insulin level to an average of 138 ± 6 pmol/l. The hepatic portal-arterial difference in plasma insulin was again eliminated. Arterial glucagon level (ng/l) and the hepatic portal-arterial difference in plasma glucagon (ng/l) did not change significantly (43 ± 2 and 9 ± 2 at basal, 39 ± 3 and 9 ± 2 at 90 min, and 37 ± 3 and 7 ± 2 at 180 min). Thus, a difference of ~120 pmol/l in arterial insulin completely abolished the response of the α-cell to mild hypoglycemia. *Diabetes* 51:3033–3042, 2002

Prevention of hypoglycemia results from the dissipation of insulin action and the activation of various counterregulatory systems. During a hyperinsulinemic clamp, the plasma C-peptide concentration decreases, even when the plasma glucose level falls only slightly (5.1 to 4.9 mmol/l) (1,2). This result indicates that insulin secretion is sensitively affected by a decline in the plasma glucose concentration and/or a rise in insulin per se. The glycemic thresholds required for the initiation of the counterregulatory responses to insulin-induced hypoglycemia are well established (3). Plasma glucagon, epinephrine, growth hormone, cortisol, and norepinephrine begin to rise only when the plasma glucose level reaches 3.8, 3.8, 3.7, 3.1, and 3.6 mmol/l, respectively. The glycemic thresholds for the above hormones are reasonably similar both in the human (4–7) and the dog (8–11). It is clear that, in the presence of hyperinsulinemia, none of the hormonal components of the counterregulatory system are brought into play until the plasma glucose level drops to <3.9 mmol/l.

Phlorizin is known to lower plasma glucose by preventing the reabsorption of glucose from the glomerular ultrafiltrate. Gauthier et al. (12) used an intravenous infusion of phlorizin rather than insulin to decrease arterial plasma glucose from 6.11 to 5.22 mmol/l. This in turn caused glucagon to rise significantly from 50 ± 11 to 120 ± 25 ng/l. Unfortunately, their study contained no euglycemic control protocol, so it remains unclear whether the rise in glucagon resulted from a direct effect of phlorizin on the α-cells or a response to hypoglycemia itself. Nevertheless, their data raise the possibility that in the absence of hyperinsulinemia, glucagon release from the α-cell may occur with much smaller reduction in plasma glucose than currently appreciated.

Recently, we examined the counterregulatory changes that occur in response to insulin-independent hypoglycemia induced by oral treatment with BAY R 3401 (13). BAY R 3401 is a novel compound that can reduce blood glucose levels by selectively inhibiting glycogen phosphorylase and thereby reducing hepatic glucose production (14,15). The use of this drug, in combination with the glucose clamp technique, allowed us to create mild hypoglycemia (5.8 to 4.4 mmol/l) so that we could examine the relation-

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CNS, central nervous system; FFA, free fatty acid; NEFA, nonesterified fatty acid; NHGO, net hepatic glucose output.

ship between the decrement in the plasma glucose level per se and α- and β-cell function in the conscious dog in the absence of hyperinsulinemic conditions. We showed that insulin secretion decreased by 80 and 100% in response to decrements in glucose of 0.3 and 0.6 mmol/l, respectively. Simultaneously, we found that a fall of 0.6 mmol/l in plasma glucose (from 5.8 to 5.2 mmol/l) caused a 200% increase in glucagon secretion, giving support to the hypothesis that the latter increases to a greater extent in response to insulin-independent hypoglycemia than to insulin-dependent hypoglycemia (13).

Hyperinsulinemia has been shown to decrease plasma glucagon levels in the presence of euglycemia (8,16,17), and high circulating insulin levels are known to have a direct inhibitory effect on the α-cell (18,19). Thus, after the injection or infusion of exogenous insulin, it is possible that the effects of hypoglycemia per se on glucagon secretion are modified by hyperinsulinemia. In response to fasting, on the other hand, insulin falls as the blood glucose declines, and it is possible that the deficit in insulin allows a sensitive response of the α-cell. In the present study, therefore, we tested the hypothesis that small changes in arterial insulin brought about by infusion can suppress glucagon's response to a mild fall in plasma glucose in conscious overnight fasted dogs.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Experiments were performed on 20 overnight-fasted mongrel dogs (17.4–29.0 kg, mean 23.1 ± 1.1 kg) of either sex that had been fed a standard meat and chow diet once daily (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). 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 endotrachial anesthesia (15 mg/kg body wt pentothal sodium presurgery and 1.0% isoflurane as an inhalation anesthetic during surgery), and catheters for blood sampling were placed into a femoral artery, the portal, an hepatic, a jejunal, and a splenic vein as previously described (8–11,13,16). On the day of the experiment, the catheters were exteriorized under local anesthesia (2% lidocaine; Abbott, Chicago), 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 equilibration period (−140 to −40 min), a 40-min control period (−40 to 0 min), and a 180-min test period (0–180 min). Four experimental protocols were used. During the test period, a 0.5% methyl cellulose/saline solution (50 ml) with BAY R 3401 (10 mg/kg) was given by mouth at 0 min in each of the four groups. The plasma glucose level was then monitored every 5 min. In protocol 1, saline was infused, and the glucose level was allowed to drop. In protocol 2, insulin was infused peripherally at 3.6 pmol · kg^{-1} · min^{-1}, and glucose was infused peripherally to clamp the arterial plasma glucose level to that in protocol 1. In protocol 3, glucose was infused peripherally to maintain euglycemia. In protocol 4, insulin was infused peripherally at 3.6 pmol · kg^{-1} · min^{-1}, and glucose was infused so that the plasma glucose level was maintained at a euglycemic value.

Data from protocols 1 and 3 are contained as part of the database in another article that addresses a separate topic (13). The representation of the data facilitates demonstration of the effect of hyperinsulinemia on glucagon secretion in response to mild hypoglycemia, which is the focus of the present article. The period when protocols 1 and 3 were performed overlapped with the period when protocols 2 and 4 were carried out.

Analytical procedures. Plasma glucose concentrations were determined using the glucose oxidase method with a Beckman glucose analyzer (Fullerton, CA) (20). Blood concentrations of glucose, lactate, glycerol, ketones, and alanine were determined according to the method of Lloyd et al. (21), 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 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 $H \times (F_a + F_p) - [(A \times F_a) + (P \times F_p)]$, 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. Gluconeogenic flux 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) converted to glucose equivalents (22). The net glycogenolytic rate was obtained by subtracting the gluconeogenic flux 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 (23). Glucagon release was calculated using the formula $F_p \times [P_g - (0.85 \times A_g)]$, where A_g and P_g are the arterial and hepatic portal plasma glucagon concentrations, respectively, and F_p is hepatic portal plasma flow. A_g is multiplied by 0.85 because the gut fractional extraction of glucagon is 15% (24). Insulin release was calculated using the formula $F_p \times [P_I - (0.70 \times A_I)]$, where A_I and P_I are the arterial and hepatic portal plasma insulin concentrations, respectively, and F_p is hepatic portal plasma flow. A_I is multiplied by 0.7 because the gut fractional extraction of insulin is 30% (25).

Statistical analysis. Data are expressed as means ± SE. A one-way ANOVA for repeated measures was used to analyze changes over time. A two-way ANOVA for repeated measures was used to analyze the difference between time course. When significant changes were obtained over time, post hoc comparisons were made using a paired t test (26).

RESULTS

Hepatic blood flow. The hepatic arterial blood flow (5.4 ± 0.6, 6.1 ± 0.8, 4.7 ± 0.3, and 4.2 ± 0.5 ml · kg^{-1} · min^{-1} in the hypoinsulinemic-hypoglycemic, hyperinsulinemic-hypoglycemic, euinsulinemic-euglycemic, and hyperinsulinemic-euglycemic groups, respectively) and portal blood flow (20.6 ± 2.2, 25.4 ± 2.4, 19.2 ± 1.2, and 20.4 ± 2.7 ml · kg^{-1} · min^{-1} in the four groups, respectively) did not change over time or with treatment (data not shown).

Plasma glucose level and NHGO. BAY R 3401 administration decreased NHGO (μmol · kg^{-1} · min^{-1}) in the hypoinsulinemic-hypoglycemic group from 8.9 ± 1.7 to 5.0 ± 1.1 by 60 min ($P < 0.05$) (Fig. 1), after which it rose slightly. As a result, the arterial plasma glucose level (mmol/l) fell from 5.8 ± 0.2 to a minimum of 4.4 ± 0.3 ($P < 0.01$). In the hyperinsulinemic-hypoglycemic group, NHGO decreased from 10.9 ± 2.2 to zero within 2 h ($P < 0.01$). The plasma glucose level in the hyperinsulinemic-hypoglycemic group was clamped to the level observed in the hypoglycemic group, falling from 6.1 ± 0.1 (basal) to 4.6 ± 0.1 mmol/l. When the plasma glucose level was maintained at its basal value (5.9 ± 0.1 mmol/l) by exogenous glucose infusion in the presence of drug and basal insulin, the liver took up glucose at −3.3 ± 0.6 μmol · kg^{-1} · min^{-1} by the end of the experiment. When the plasma glucose level was maintained at a euglycemic value (6.1 ± 0.1 mmol/l) by glucose infusion in the presence of hyperinsulinemia and drugs, the liver took up glucose at −4.7 ± 1.7 μmol · kg^{-1} · min^{-1} by 60 min ($P < 0.01$), after which it remained unchanged. Thus, the presence of mild hyperinsulinemia blocked the hypoglycemia-induced stimulation of NHGO and hastened the switch to net hepatic glucose uptake in the presence of euglycemia.

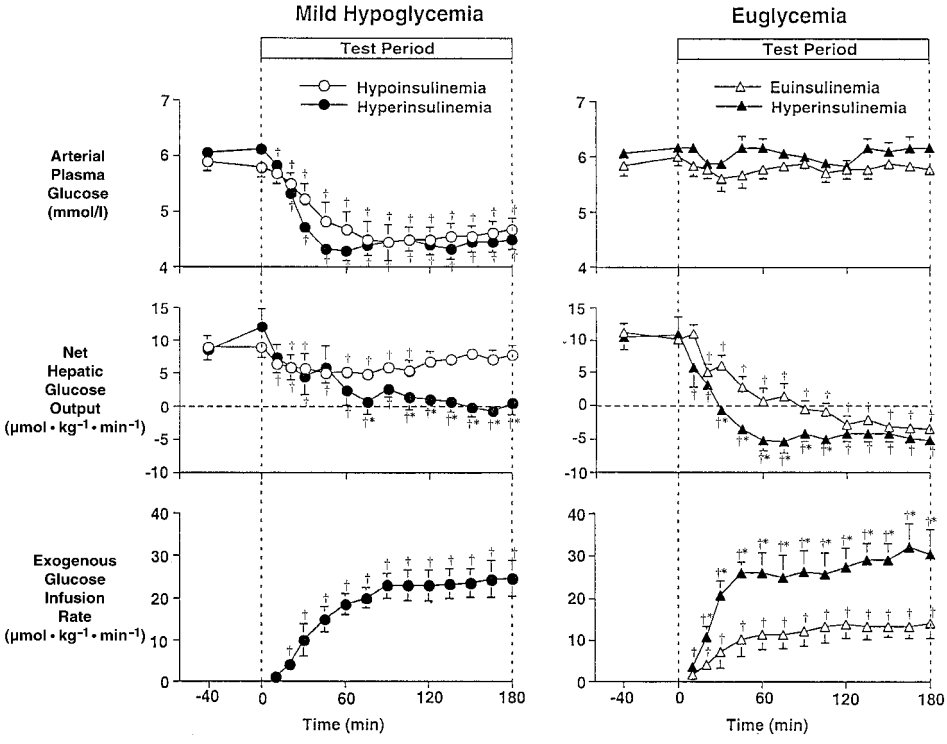

FIG. 1. Arterial plasma glucose levels, net hepatic glucose balances, and exogenous glucose infusion rates before and after oral administration of BAY R 3401 with and without hyperinsulinemia in the presence of mild hypoglycemia and euglycemia in 18-h–fasted conscious dogs. Animals received oral bolus injection of BAY R 3401 (10 mg/kg) at 0 min. Values are means ± SE. Each group consisted of five dogs. †Significantly different from control period in identical group ($P < 0.05$). *Significantly different from the corresponding value in the absence of hyperinsulinemia ($P < 0.05$).

Hormone levels. As shown in Fig. 2, and as was reported previously (13), arterial and hepatic portal plasma insulin levels (pmol/l) in the hypoinsulinemic-hypoglycemic group decreased from 78 ± 18 and 168 ± 36 to 24 ± 6 and 36 ± 12 during the first 30 min ($P < 0.01$) and to 18 ± 6 and 24 ± 6 by 90 min ($P < 0.01$), respectively. The hepatic portal-arterial difference in plasma insulin (pmol/l) decreased from 90 ± 24 to 12 ± 6 by 30 min ($P < 0.05$) and to 6 ± 3 by 90 min ($P < 0.05$). The insulin secretion rates (pmol · kg^{-1} · min^{-1}) decreased from 1.5 ± 0.4 to 0.3 ± 0.1 by 30 min ($P < 0.05$) and 0.2 ± 0.1 by 90 min ($P < 0.05$). The arterial and hepatic portal glucagon levels (ng/l) rose from 43 ± 5 and 48 ± 6 to 51 ± 5 and 61 ± 7 by 30 min ($P < 0.05$) and to 79 ± 16 and 109 ± 30 by 90 min ($P < 0.05$), after which they drifted down slightly (Fig. 3). The hepatic portal-arterial difference in plasma glucagon increased from 5 ± 2 (basal) to 10 ± 5 by 30 min ($P < 0.05$) and to 30 ± 15 by 90 min ($P < 0.05$), after which it remained at 20 ± 13. The glucagon secretion rate (pg · kg^{-1} · min^{-1}) increased from 112 ± 57 (basal) to 230 ± 113 by 30 min

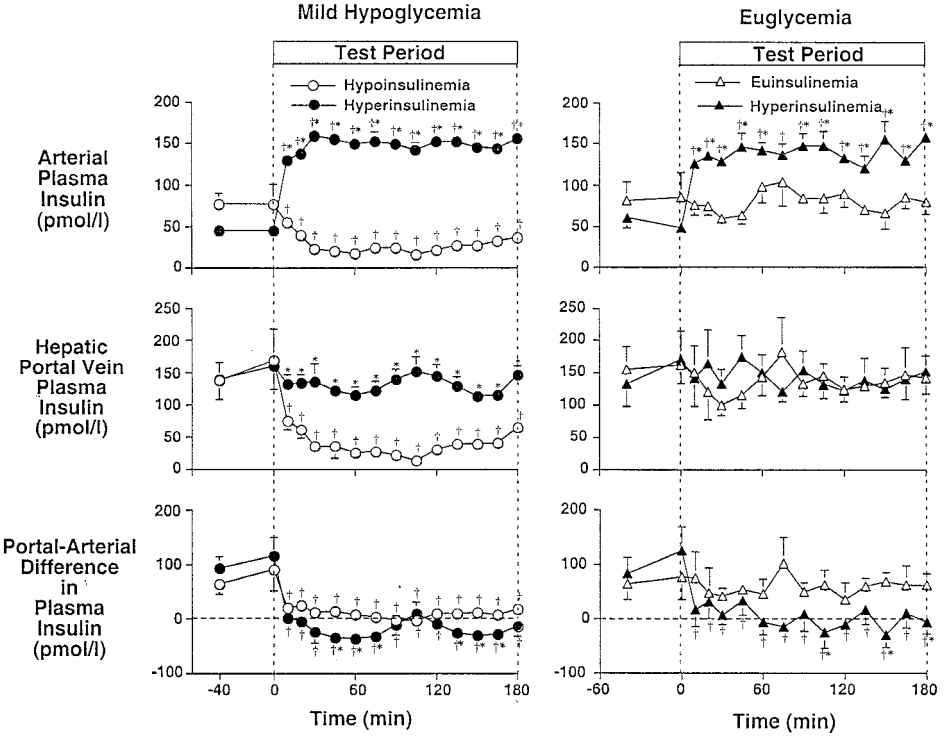

FIG. 2. Arterial and hepatic portal levels and the portal-arterial difference in plasma insulin before and after oral administration of BAY R 3401 with and without hyperinsulinemia in the presence of mild hypoglycemia and euglycemia in 18-h–fasted conscious dogs. Animals received oral bolus injection of BAY R 3401 (10 mg/kg) at 0 min. Values are means ± SE. Each group consisted of five dogs. †Significantly different from control period in identical group ($P < 0.05$). *Significantly different from the corresponding value in the absence of hyperinsulinemia ($P < 0.05$).

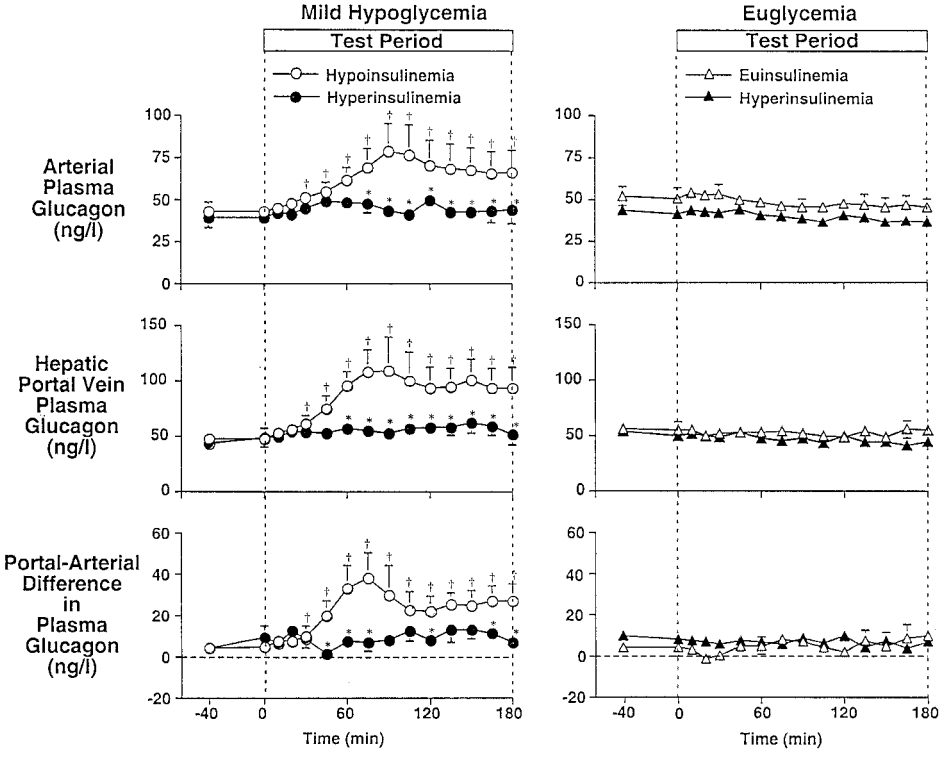

FIG. 3. Arterial and hepatic portal levels and the portal-arterial difference in plasma glucagon before and after oral administration of BAY R 3401 with and without hyperinsulinemia in the presence of mild hypoglycemia and euglycemia in 18-h–fasted conscious dogs. Animals received oral bolus injection of BAY R 3401 (10 mg/kg) at 0 min. Values are means ± SE. Each group consists of five dogs. †Significantly different from control period in identical group ($P < 0.05$). *Significantly different from the corresponding value in the absence of hyperinsulinemia ($P < 0.05$).

($P < 0.05$) and to 556 ± 273 by 90 min ($P < 0.05$), after which it decreased to 466 ± 232 by 180 min.

During hyperinsulinemic hypoglycemia, as shown in Fig. 2, the arterial plasma insulin level (pmol/l) increased from 48 ± 6 to 156 ± 6 during the first 30 min ($P < 0.01$), after which it remained constant (156 ± 24). The portal plasma insulin levels (pmol/l) remained at a basal value (138 ± 36 pmol/l). Therefore, the hepatic portal-arterial difference in plasma insulin (pmol/l) decreased from 90 ± 36 to −30 ± 18 by 30 min ($P < 0.01$) and remained nonsignificantly different from zero (−24 ± 18) thereafter, as did insulin secretion. The arterial and hepatic portal vein glucagon levels (ng/l) did not rise in response to the decline in plasma glucose (40 ± 5 and 46 ± 7 to 45 ± 3 and 54 ± 4 at 30 min and 44 ± 8 and 51 ± 9 at 180 min, respectively) (Fig. 3). The hepatic portal-arterial difference in plasma glucagon (ng/l) and glucagon secretion ($pg \cdot kg^{-1} \cdot min^{-1}$) also did not change significantly (6 ± 4 and 216 ± 126 [basal] to 9 ± 2 and 283 ± 71 by 30 min and 7 ± 3 and 252 ± 116 at 180 min, respectively).

When euglycemia was maintained in the presence of the drug, the arterial plasma insulin and glucagon levels did not change significantly; likewise, the portal hormone levels, their hepatic portal-arterial differences, and their secretion rates also remained unchanged (Figs. 2 and 3).

When insulin was raised in the presence of euglycemia, the arterial plasma insulin levels (pmol/l) rose from 54 ± 6 to 126 ± 6 by 30 min ($P < 0.01$) and averaged 138 ± 6 during the rest of the test period. However, hepatic portal plasma insulin levels did not rise from basal (150 ± 42 pmol/l). The hepatic portal-arterial difference in plasma insulin (pmol/l) decreased rapidly from 102 ± 36 to 18 ± 42 by 10 min ($P < 0.05$) and thereafter averaged −6 ± 24 (Fig. 2). Thus, insulin secretion was completely inhibited. The arterial and hepatic portal vein glucagon levels and the hepatic portal-arterial difference in plasma glucagon (ng/l) did not change (43 ± 2, 52 ± 2, and 9 ± 1 to 37 ± 3, 44 ± 3, and 7 ± 2 at 180 min during the test period, respectively). The glucagon secretion rate ($pg \cdot kg^{-1} \cdot min^{-1}$) also remained unchanged (basal of 150 ± 17 to 150 ± 52 at 180 min) during euglycemic hyperinsulinemia. These data indicate that the rise in arterial insulin blocked the hypoglycemia induced α-cell response and did not suppress basal glucagon secretion.

As shown in Table 1, the arterial norepinephrine, epinephrine, and cortisol levels did not change significantly in the absence of hyperinsulinemia regardless of whether euglycemia or hypoglycemia were present. In the presence of hyperinsulinemia, on the other hand, regardless of the blood glucose levels, arterial plasma norepinephrine, epinephrine, and cortisol levels tended to increase, but only cortisol changed significantly.

Arterial levels and net hepatic balances of plasma nonesterified fatty acids and arterial blood ketone, glycerol, lactate, and alanine. As shown in Table 2, when hypoinsulinemic hypoglycemia occurred, the arterial levels and net hepatic uptakes of nonesterified fatty acids (NEFAs), 3-hydroxybutyrate, and glycerol significantly increased. When hypoglycemia occurred in the presence of hyperinsulinemia and when euglycemia was maintained in the absence of elevated insulin, the arterial levels and net hepatic uptake of these metabolites did not change significantly. On the other hand, when plasma glucose levels were maintained in the presence of hyperinsulinemia and drugs, the arterial levels and net hepatic uptake of these metabolites fell significantly. In both cases, therefore, hyperinsulinemia reduced lipolysis and ketogenesis.

In the presence of hypoinsulinemic hypoglycemia, hyperinsulinemic hypoglycemia, euinsulinemic euglycemia, and hyperinsulinemic euglycemia, arterial lactate levels fell and net hepatic lactate balance shifted in favor of

TABLE 1
Arterial plasma levels of norepinephrine, epinephrine, and cortisol before and after oral administration of BAY R 3401 in the presence of non–insulin-induced hypoglycemia with and without mild hyperinsulinemia and in the presence of euglycemia with and without mild hyperinsulinemia in 18-h–fasted conscious dogs

Group	Control period	BAY R 3401 with or without peripheral glucose and/or insulin infusion (min)						
		10	20	30	60	90	120	180
Hypoglycemia								
Norepinephrine (pmol/l)	981 ± 177	993 ± 171	898 ± 171	1,069 ± 136	1,011 ± 100	969 ± 124	1,046 ± 130	1,099 ± 254
Epinephrine (pmol/l)	426 ± 60	513 ± 98	437 ± 98	475 ± 71	458 ± 87	458 ± 60	431 ± 82	453 ± 93
Cortisol (nmol/l)	41 ± 8	50 ± 11	55 ± 11	58 ± 17	50 ± 8	47 ± 8	63 ± 19	66 ± 33
Hypoglycemia + insulin								
Norepinephrine (pmol/l)	1,454 ± 242	1,188 ± 272	1,336 ± 272	1,543 ± 272	1,714 ± 313	1,720 ± 266	1,667 ± 219	1,856 ± 242
Epinephrine (pmol/l)	758 ± 147	519 ± 98	415 ± 98	770 ± 153	857 ± 153	830 ± 158	961 ± 104	1,037 ± 191
Cortisol (nmol/l)	50 ± 14	52 ± 11	50 ± 11	74 ± 19	88 ± 22	99 ± 19*	99 ± 19*	74 ± 14
Euglycemia								
Norepinephrine (pmol/l)	881 ± 207	946 ± 154	934 ± 154	786 ± 183	798 ± 154	893 ± 201	822 ± 195	851 ± 177
Epinephrine (pmol/l)	420 ± 180	409 ± 87	284 ± 87	295 ± 115	273 ± 33	272 ± 175	218 ± 104	442 ± 196
Cortisol (nmol/l)	58 ± 14	61 ± 14	58 ± 14	55 ± 22	36 ± 6	47 ± 8	55 ± 14	74 ± 36
Euglycemia + insulin								
Norepinephrine (pmol/l)	940 ± 100	916 ± 89	1011 ± 136	958 ± 106	1058 ± 166	916 ± 112	1023 ± 142	1383 ± 148
Epinephrine (pmol/l)	524 ± 136	551 ± 142	579 ± 158	579 ± 175	633 ± 186	464 ± 109	475 ± 164	628 ± 218
Cortisol (nmol/l)	58 ± 11	66 ± 8	63 ± 8	94 ± 14*	91 ± 11*	77 ± 3	88 ± 27	110 ± 19*

Data for each group are means ± SE from five dogs. *Significantly changed from the values during the control period in the same group.

increased uptake. The arterial blood alanine levels decreased in all four groups. Net hepatic alanine uptake increased during hypoinsulinemic hypoglycemia, but did not change during hyperinsulinemic hypoglycemia, euinsulinemic euglycemia, or hyperinsulinemic euglycemia.

Glycogenolysis and gluconeogenesis. BAY R 3401 caused net hepatic glycogenolysis to decrease from 7.39 ± 0.78 to 1.83 ± 1.83 μmol glucose equivalent · kg^{-1} · min^{-1} by 30 min in the hypoglycemic group, after which it was effectively zero (Table 3). As a result, both the plasma glucose level and the plasma insulin level fell. On the other hand, gluconeogenic flux started to rise 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 flux was almost equivalent to NHGO, indicating that gluconeogenesis (the conversion of gluconeogenically derived glucose-6-phosphate to plasma glucose) was responsible for almost all glucose production. When hyperinsulinemia was added to BAY R 3401 delivery, net glycogenolysis was suppressed more rapidly and to a greater extent, eventually switching to net glycogen synthesis (−6.9 ± 0.9 μmol glucose equivalent · kg^{-1} · min^{-1} at 180 min). The glucose level was clamped to the hypoglycemia seen in the former group. On the other hand, gluconeogenic flux rose from 4.9 ± 1.4 to 8.8 ± 1.9 μmol glucose equivalent · kg^{-1} · min^{-1} by the end of the study. Thus, although the presence of insulin caused more net glycogen synthesis, it failed to decrease gluconeogenic flux. Since NHGO was zero, however, gluconeogenesis (the conversion of gluconeogenic precursors into plasma glucose) was inhibited, and the gluconeogenic-derived carbon in the glucose-6-phosphate pool was directed to glycogen. In the presence of euinsulinemic euglycemia, BAY R 3401 caused net glycogenolysis to decline from 8.2 ± 1.5 to −7.3 ± 1.4 μmol glucose equivalent · kg^{-1} · min^{-1}. When hyperinsulinemia accompanied euglycemia and drugs, net glycogen flux switched rapidly from breakdown to synthesis, reaching 6.5 ± 1.7 μmol glucose equivalent · kg^{-1} · min^{-1} by the end of the study. Gluconeogenic flux did not change significantly in the presence of euglycemia, regardless of the presence or absence of insulin infusion. Once again, however, the gluconeogenic rate was zero in both groups, and the gluconeogenically derived carbon was stored in glycogen.

DISCUSSION

α-Cell response to hyperinsulinemia. Glucagon secretion was increased threefold in response to the non–insulin-induced fall in plasma glucose from 5.8 to 5.2 mmol/l. As such, the plasma glucose threshold was well above the well-recognized concentration of 3.8 mmol/l observed in the presence of insulin-induced hypoglycemia in humans (3–7) and dogs (8–11). In a previous study (10), we showed that 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 fall of 1.6 mmol/l in plasma glucose level failed to cause the plasma glucagon levels to rise, whereas, as we have also previously shown, in the presence of hypoinsulinemia, a fall of 1.4 mmol/l (from 5.8 to 4.4 mmol/l) caused a sixfold rise in glucagon secretion. These data therefore led to the hypothesis that was tested in the present study, namely, that glucagon secretion is sensitively inhibited by insulin such that the α-cell response to hypoglycemia is markedly reduced by the presence of insulin. Because it is known that glucagon secretion increases markedly in response to deep insulin-induced hypoglycemia, our data suggest that a deeper hypoglycemia results in an increase in neural input to the α-cell or some other change that then overcomes insulin's inhibitory action. Our data also suggest that if a deep hypoglycemia could be produced in the absence of elevated insulin, the rise in plasma glucagon would be much larger than that observed in the presence of equivalent insulin-induced hypoglycemia.

It has been shown that insulin has direct effects on the α-cell to suppress glucagon secretion (18,27–29). Anatomical and physiological studies suggest that blood in the

TABLE 2
Arterial levels and net hepatic balances of plasma NEFAs, 3-hydroxybutyrate, glycerol, lactate, and alanine before and after oral administration of BAY R 3401 in the presence of non–insulin-induced hypoglycemia with and without mild hyperinsulinemia and in the presence of the maintenance of euglycemia with and without mild hyperinsulinemia 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*
Hypoglycemia + insulin								
Plasma level (mmol/l)	0.82 ± 0.08	0.72 ± 0.09	0.53 ± 0.09	0.64 ± 0.10	0.73 ± 0.14	0.73 ± 0.14	0.79 ± 0.10	0.77 ± 0.164
NHB (μmol · kg^{-1} · min^{-1})	−3.18 ± 0.56	−3.27 ± 0.40	−1.95 ± 0.40	−2.39 ± 0.20	−3.20 ± 1.02	−3.47 ± 0.54	−2.90 ± 0.19	−2.06 ± 0.61
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
Euglycemia + insulin								
Plasma level (mmol/l)	0.65 ± 0.09	0.68 ± 0.09	0.56 ± 0.09	0.48 ± 0.08	0.34 ± 0.06*	0.31 ± 0.05*	0.33 ± 0.06*	0.29 ± 0.06*
NHB (μmol · kg^{-1} · min^{-1})	−1.65 ± 0.22	−1.92 ± 0.27	−1.44 ± 0.30	−0.86 ± 0.24	−0.71 ± 0.19*	−0.48 ± 0.18*	−0.73 ± 0.23*	−0.58 ± 0.17*
3-Hydroxybutyrate								
Hypoglycemia								
Blood level (μmol/l)	20 ± 3	19 ± 2	15 ± 2	16 ± 3	28 ± 4	49 ± 4*	65 ± 7*	111 ± 20*
NHB (μmol · kg^{-1} · min^{-1})	0.72 ± 0.14	0.64 ± 0.12	0.76 ± 0.12	0.66 ± 0.11	1.17 ± 0.17	1.93 ± 0.21*	2.52 ± 0.47*	3.91 ± 1.25*
Hypoglycemia + insulin								
Blood level (μmol/l)	29 ± 8	21 ± 3	19 ± 3	14 ± 3	32 ± 15	32 ± 15	25 ± 9	24 ± 8
NHB (μmol · kg^{-1} · min^{-1})	1.41 ± 0.45	0.75 ± 0.17	0.82 ± 0.17	0.87 ± 0.19	2.02 ± 0.89	1.69 ± 0.83	1.30 ± 0.62	1.86 ± 1.04
Euglycemia								
Blood level (μmol/l)	33 ± 11	24 ± 6	27 ± 6	32 ± 3	24 ± 3	14 ± 1*	17 ± 4	31 ± 15
NHB (μmol · kg^{-1} · min^{-1})	1.06 ± 0.38	0.60 ± 0.1	0.94 ± 0.1	1.01 ± 0.08	1.10 ± 0.06	0.61 ± 0.05	0.90 ± 0.15	1.00 ± 0.33
Euglycemia + insulin								
Blood level (μmol/l)	19 ± 3	21 ± 3	14 ± 3	13 ± 3	5 ± 1*	4 ± 1*	6 ± 1*	4 ± 2*
NHB (μmol · kg^{-1} · min^{-1})	0.60 ± 0.08	0.49 ± 0.04	0.54 ± 0.06	0.40 ± 0.03	0.27 ± 0.07*	0.24 ± 0.03*	0.23 ± 0.08*	0.15 ± 0.07*
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*
Hypoglycemia + insulin								
Blood level (μmol/l)	88 ± 10	68 ± 9	57 ± 9	78 ± 6	92 ± 18	88 ± 6	105 ± 10	108 ± 9
NHB (μmol · kg^{-1} · min^{-1})	−2.20 ± 0.28	−1.42 ± 0.21	−1.49 ± 0.21	−2.06 ± 0.34	−2.60 ± 0.65	−2.36 ± 0.28	−2.88 ± 0.46	−3.34 ± 0.61
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
Euglycemia + insulin								
Blood level (μmol/l)	68 ± 7	51 ± 7	49 ± 8	45 ± 6*	38 ± 7*	36 ± 7*	43 ± 7*	53 ± 9*
NHB (μmol · kg^{-1} · min^{-1})	−1.08 ± 0.20	−0.78 ± 0.20	−0.79 ± 0.19	−0.61 ± 0.10	−0.56 ± 0.04*	−0.52 ± 0.07*	−0.59 ± 0.13*	−0.78 ± 0.18*
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*
Hypoglycemia + insulin								
Blood level (μmol/l)	716 ± 147	630 ± 129	616 ± 129	542 ± 109	464 ± 112	506 ± 129	619 ± 136	548 ± 52
NHB (μmol · kg^{-1} · min^{-1})	−2.09 ± 1.71	−2.23 ± 1.19	−3.84 ± 1.19	−5.82 ± 2.76*	−5.45 ± 2.44*	−4.80 ± 1.32*	−6.56 ± 1.97*	−8.48 ± 2.22*
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
Euglycemia + insulin								
Blood level (μmol/l)	775 ± 120	700 ± 120	650 ± 80	560 ± 60	450 ± 40*	370 ± 20*	370 ± 30*	420 ± 40*
NHB (μmol · kg^{-1} · min^{-1})	7.73 ± 4.55	2.51 ± 5.10	0.29 ± 3.24	1.99 ± 2.09	0.32 ± 1.09*	−0.96 ± 0.92*	−0.91 ± 0.56*	−1.87 ± 0.57*
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*
Hypoglycemia + insulin								
Blood level (μmol/l)	340 ± 29	325 ± 33	312 ± 33	310 ± 40	254 ± 36*	217 ± 30*	198 ± 27*	172 ± 17*
NHB (μmol · kg^{-1} · min^{-1})	−2.75 ± 0.37	−2.98 ± 0.58	−2.79 ± 0.59	−3.38 ± 0.70	−3.23 ± 0.88	−3.05 ± 0.73	−2.87 ± 0.60	−2.90 ± 0.52
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
Euglycemia + insulin								
Blood level (μmol/l)	390 ± 45	370 ± 50	370 ± 50	330 ± 40	290 ± 30*	210 ± 20*	190 ± 20*	170 ± 20*
NHB (μmol · kg^{-1} · min^{-1})	−1.93 ± 0.39	−2.23 ± 0.39	−2.22 ± 0.41	−2.04 ± 0.25	−2.29 ± 0.43	−1.73 ± 0.26	−1.67 ± 0.20	−1.65 ± 0.24

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

TABLE 3
Hepatic gluconeogenic flux and net glycogenolysis before and after oral administration of BAY R 3401 in the presence of hypoglycemia or euglycemia with and without hyperinsulinemia 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 (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*
Hypoglycemia + insulin	4.90 ± 1.37	4.81 ± 1.28	5.46 ± 1.29	7.32 ± 2.25	7.26 ± 2.43	6.63 ± 1.53	7.59 ± 1.82	8.81 ± 1.94
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
Euglycemia + insulin	2.47 ± 0.46	2.62 ± 0.49	2.62 ± 0.51	2.35 ± 0.30	2.57 ± 0.45	2.47 ± 0.76	2.42 ± 0.45	2.98 ± 0.62
Net 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*
Hypoglycemia + insulin	7.49 ± 1.47	4.36 ± 1.09	2.26 ± 1.06*	−1.10 ± 1.51*	−3.54 ± 1.28*	−2.96 ± 0.82*	−4.76 ± 0.49*	−6.87 ± 0.93*
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*
Euglycemia + insulin	13.74 ± 4.21	5.83 ± 4.84	2.31 ± 2.00*	−0.29 ± 1.08*	−5.91 ± 2.61*	−4.86 ± 2.09*	−4.81 ± 1.78*	−6.48 ± 1.73*

Data for each group are means ± SE from five dogs. *Significantly changed from the values during the 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 NHGO, lactate production, and glucose oxidation.

islet flows from β-cells to α-cells in dogs (30) as well as in rats (31,32). Therefore, α-cells are exposed to high concentrations of intra-islet insulin in the presence of euglycemia. In the presence of mild hypoglycemia (Fig. 2), on the other hand, insulin secretion falls markedly, thereby lowering the insulin concentration to which α-cells are exposed. It has been shown that 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 (27,33,34). Acute insulin deficiency in humans (35,36), as well as alloxan- or streptozotocin-induced diabetes in animals, are associated with hyperglucagonemia (37). It is therefore likely that the decrease in intra-islet insulin contributed in some way to the increased glucagon secretion that we observed in response to mild hypoglycemia.

In the present study, modest arterial hyperinsulinemia almost completely suppressed the increment of glucagon secretion that occurred in response to hypoinsulinemic hypoglycemia (Fig. 3) even though the hypoglycemia was identical in the two groups. It is also clear that this was due to an inhibition of the α-cell response to hypoglycemia because when insulin was infused in the presence of euglycemia, it failed to decrease basal glucagons secretion. Because ~30% of insulin in the arterial blood is cleared by the intestine when blood passes through it (25), it is clear that endogenous insulin secretion was completely suppressed in this protocol. Accordingly, the α-cells were probably exposed to a concentration of insulin that was equivalent to the arterial insulin level. Therefore, the rise in plasma insulin levels caused by exogenous insulin infusion would have induced only a small increase in the intra-islet insulin concentration relative to that present in the hypoglycemic-hyperinsulinemic setting. Nevertheless, it appears to have been enough to inhibit glucagon secretion in response to mild hypoglycemia. Alternatively, the arterial or portal insulin levels themselves may be the key determinants of glucagon secretion. Thus, when each or both fall, a signal (neural or humoral) is generated that then controls the α-cells.

In the presence of euglycemia in the present study, mild hyperinsulinemia (~150 pmol/l) did not alter basal glucagon secretion. In contrast, we (10,16) and others (8) have shown previously that larger increases in the arterial plasma insulin level (to ~400–1,440 pmol/l) can decrease plasma glucagon levels by 30–40% in the presence of euglycemia in conscious dogs. It is possible that the rise in the plasma insulin concentration that we brought about in the present study was not large enough to cause a detectable inhibition of basal glucagon secretion under euglycemic conditions. Further, it is unclear whether the inhibition of glucagon secretion by hyperinsulinemia that we observed in the presence of euglycemia in our previous studies was due to a direct action of insulin on the α-cell (8,10,16). Several in vitro studies have shown that high concentrations of insulin (0.01–3.3 units/ml) do not suppress glucagon secretion in the presence of 5.5 mmol/l glucose in perifused islets isolated from normal rats (18,34,38).

Electrical stimulation of the pancreatic sympathetic nerves (39), local infusion of the classic sympathetic neurotransmitter norepinephrine (40), and a pancreatic sympathetic neuropeptide such as galanin (41) all increase glucagon secretion. Similarly, electrical stimulation of parasympathetic nerves (42,43), local infusion of the classic parasympathetic neurotransmitter acetylcholine (44), and a pancreatic parasympathetic neuropeptide such as vasoactive intestinal peptide (45,46) all stimulate glucagon release from the α-cell. Each of these autonomic inputs is capable of stimulating glucagon secretion. We have no evidence, however, that increased central nervous system (CNS) input can explain the rise in glucagon secretion seen during hypoinsulinemic hypoglycemia in the present study. In fact, we did not observe any change in the level of circulatory norepinephrine. Further, a fall of only 1.4 mmol/l in plasma glucose level would not have activated the autonomic nervous system. In addition, in another

recent study, we showed that non–insulin-induced hypoglycemia caused glucagon secretion even in dogs with denervated pancreata (47). Therefore, it is unlikely that the rise in arterial insulin level would have blocked α-cell function by inhibiting CNS input, because if anything, it would have had the opposite effect.

It is known that amino acids have stimulatory effects and that FFAs and ketones have inhibitory effects on glucagon secretion from the α-cell (34,48,49). As shown in Table 2, the blood alanine concentration decreased in the hypoinsulinemic-hypoglycemic group, and the increase in glucagon secretion could not be explained. Likewise, the NEFA and ketone levels rose again, precluding them from explaining the rise in the glucagon secretion seen in response to non–insulin-induced hypoglycemia. Furthermore, raising insulin failed to change the plasma alanine level and decreased FFA and ketones levels, meaning that alterations in the levels of these metabolites cannot explain the insulin-induced inhibition of glucagon secretion.

In summary, the increase in glucagon secretion seen in response to a small decline of blood glucose occurs only when the arterial, portal vein, and intra-islet concentration of insulin are very low. Even maintenance of an arterial concentration (and presumably intra-islet insulin concentrations) of ~150 pmol/l suppresses the increase in glucagon secretion seen in response to a small fall in blood glucose. An effect of insulin on the CNS or blood metabolite levels cannot explain its effect on glucagon release. It is therefore likely to have resulted from a direct effect of insulin on the α-cell or local neural reflexes.

β-Cell response to hyperinsulinemia. When the arterial plasma glucose level fell by 0.3 mmol/l as a result of administration of glycogen phosphorylase inhibition, insulin secretion decreased by 80%. This result confirms that insulin secretion is very sensitively suppressed in response to a declining plasma glucose level, in agreement with earlier data (2,3,6). When euglycemia was maintained, plasma insulin levels and insulin secretion did not change in response to the drug, indicating that it had no direct effect on the β-cell. When insulin was infused through a leg vein to create mild hyperinsulinemia in the presence of euglycemia, the resulting rise in arterial plasma insulin was not accompanied by a rise in the hepatic portal vein plasma insulin level. The hepatic portal vein–arterial difference in plasma insulin (pmol/l) decreased from 102 ± 36 to 18 ± 42 by 10 min and thereafter averaged −6 ± 24 (Fig. 2). Thus, endogenous insulin secretion was decreased markedly and rapidly by a selective rise in arterial insulin per se. In the dog, the insulin concentration in the pancreatic vein is four to five times higher than that in the portal vein (24). Because the mass of the islets represents only a small part of the total mass of the pancreatic tissue, the insulin concentration in the interstitial fluid surrounding the islets and in the venules draining the islets should be higher than that in the pancreatic vein. When endogenous insulin secretion is suppressed completely, the insulin concentration in the interstitial fluid surrounding the islets would be expected to be similar to the concentration of the hormone in arterial plasma. If so, the insulin concentration in islet interstitial fluid during the hyperinsulinemic-euglycemic period was probably lower than that during the control period of the same protocol. This finding raises the question of how insulin inhibited its own secretion.

It has been suggested that insulin can act directly on the brain. Davis et al. (9) showed that increased insulin levels can amplify the sympathetic nervous system response to hypoglycemia in the dog, normal human (5,50), and insulin-dependent diabetic human (51,52). Furthermore, studies in which microneurography was used have shown that insulin can increase sympathetic nerve activity, even under euglycemic conditions (53,54). Epinephrine and norepinephrine are known to inhibit insulin secretion through an α_2-adrenergic mechanism (55,56). 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 that activation of the sympathetic nerves by the islets of Langerhans induces the release of norepinephrine (57) and galanin (58), both of which inhibit insulin release (59). Stagner et al. (60) examined the effects of systemic infusion of insulin ($1\ mU \cdot kg^{-1} \cdot min^{-1}$) on endogenous insulin and C-peptide secretion from in situ perfused pancreata isolated vascularly but yet innervated in anesthetized dogs. They showed that the systemic infusion of insulin suppressed insulin and C-peptide secretion but that no changes in the pancreatic hormone secretion occurred after the sympathetic nerves were sectioned. It is not clear in the present study, however, whether the small rise in arterial insulin could have amplified the sympathetic discharge to the β-cells so as to decrease insulin secretion. If so, it was not reflected by a change in epinephrine and norepinephrine in plasma.

Amino acids, FFAs, and ketones have been shown to stimulate insulin secretion (48). Because significant decreases in their concentrations occurred in the presence of euglycemic hyperinsulinemia, it is possible that changes in the plasma concentrations of these substances contributed in some way to the decrease in insulin secretion that was observed in response to euglycemic hyperinsulinemia.

In summary, a physiological increase in the arterial insulin level strongly suppressed endogenous insulin secretion even in the presence of euglycemia. The effect of increased insulin levels on insulin secretion may occur via their action on the brain, secondary to their effects on metabolite levels in the blood, or because of a direct action of arterial insulin on β-cells.

Hepatic glucose metabolism. In our previous study (14), inhibition of phosphorylase by BAY R 3401, in the presence of a pancreatic clamp to keep plasma insulin and glucagon at the basal levels and glucose infusion to maintain euglycemia, had little effect on the net hepatic uptake of gluconeogenic precursors. The latter provides a good estimate of gluconeogenic flux rate (22), thus suggesting that inhibition of glycogen breakdown per se did not activate the gluconeogenic pathway. In the present study, when euglycemia was maintained (in the presence of basal insulin and glucagon), inhibition of phosphorylase by BAY R 3401 shifted net glycogen flux from breakdown to synthesis but, consistent with the data in our previous study (14), did not affect hepatic gluconeogenic flux. The selective rise in arterial insulin in the presence of euglycemia did not affect the hepatic gluconeogenic rate. However, the suppression of net glycogenolysis occurred

somewhat more rapidly when the insulin level was increased. When mild hypoglycemia was allowed to occur in the presence of the phosphorylase inhibitor, the hepatic gluconeogenic rate doubled, regardless of the presence or absence of insulin infusion and the associated changes in plasma insulin and glucagon. Therefore, these results suggest that the changes in hepatic sinusoidal insulin (from 135 to 29 pmol/l) and glucagon (from 48 to 98 ng/l) seen in response to mild hypoglycemia had little effect on the acute increase in net hepatic gluconeogenic flux seen in response to hypoglycemia, at least when glycogenolysis was impaired. This finding is in agreement with our previous suggestions (61,62) and indicates that hypoglycemia per se may be the major factor increasing gluconeogenic flux under our experimental conditions. Further, it is clear that almost all of the glucose being produced by the liver in response to non–insulin-induced hypoglycemia was gluconeogenic in origin.

In euglycemic-euinsulinemic animals, the inhibition of phosphorylase suppressed NHGO without changing gluconeogenic flux. Because net glycogen flux is determined by the balance between glycogen phosphorylase activity and glycogen synthase activity (63), the inhibition of phosphorylase must have shifted the net flux toward synthesis, and, as a result, the gluconeogenic precursors taken up by the liver were incorporated into glycogen. The shift of net hepatic glucose balance from output to uptake, and 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. As noted above, the presence of mild hyperinsulinemia in combination with euglycemia and phosphorylase inhibition accelerated the shift of net hepatic glucose and glycogen flux from output and breakdown to uptake and synthesis, respectively, but did not alter gluconeogenic flux (Fig. 1 and Table 3). Therefore, the effect of hyperinsulinemia in this context was to more rapidly activate glycogen synthesis.

In the hypoglycemic-hypoinsulinemic animals, inhibition of phosphorylase decreased NHGO 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, which activate glycogen synthase, fell (64), and the plasma concentration of glucagon, which inhibits glycogen synthase, rose (64). 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. Indeed, when mild hepatic euinsulinemia was maintained in the presence of hypoglycemia and basal hepatic sinusoidal glucagon levels, net glycogen flux shifted from breakdown to synthesis even in the presence of hypoglycemia. Therefore, glycogen synthase activity appears to have been sensitively regulated by small changes in plasma insulin and/or glucagon levels.

The present study shows that *1*) insulin secretion from β-cells in vivo decreases sensitively in response to mild hyperinsulinemia induced by exogenous insulin infusion, even in the presence of euglycemia; *2*) mild hyperinsulinemia completely inhibits the increase in glucagon secretion normally caused in response to mild non–insulin-induced hypoglycemia; *3*) the decrease in plasma insulin and increase in plasma glucagon levels that occur in response to mild hypoglycemia exert their regulatory effect on glycogen metabolism but not on gluconeogenic flux; *4*) mild hypoglycemia per se plays a major role in acutely increasing gluconeogenic flux in response to mild non–insulin-induced hypoglycemia; and *5*) coincident inhibition of glycogen deposition (by hormonal changes) and a stimulation of gluconeogenic flux (by hypoglycemia) results in gluconeogenesis, which prevents serious hypoglycemia in response to hepatic phosphorylase inhibition.

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REFERENCES

1. Heller SR, Cryer PE: Hypoinsulinemia is not arterial to glucose recovery from hypoglycemia in humans. *Am J Physiol* 261:E41–E48, 1991
2. Schwart NS, Clutter WE, Shah SD, Cryer PE: Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. *J Clin Invest* 79:777–781, 1987
3. Cryer PE: Hierarchy of physiological responses to hypoglycemia: relevance to clinical hypoglycemia in type 1 (insulin dependent) diabetes mellitus. *Horm Metab Res* 29:92–96, 1997
4. Cohen N, Rossetti L, Shimovich 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
5. 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
6. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Ciofetta M, Modarelli F, DiVincenzo A, Annibale B, Lepare M, Lalli C, DelSindaco 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
7. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991
8. 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
9. 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
10. 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
11. 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
12. 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
13. Flatten N, Igawa K, Shiota M, Emshwiller MG, Neal DW, Cherrington AD:

Alpha and β-cell responses to mild non-insulin induced hypoglycemia in the conscious dog. *Diabetes* 50:367–375, 2001
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. Bergans N, Stalmans W, Goldman S, Vanstapel F: Molecular mode of inhibition of glycogenolysis in rat liver by the dihydropyridine derivative, BAY R3401: inhibition and inactivation of glycogen phosphorylase by an activated metabolite. *Diabetes* 49:1419–1426, 2000
16. 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
17. 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
18. 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
19. Liljenquist JE, Horwitz DL, Jennings AS, ChiassonJ-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
20. 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
21. 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
22. Goldstein R, Palmer B, Lin R, Massillon D: The effects of chronic hypercortisolemia on gluconeogenesis assessed using two independent methods in vivo (Abstract). *Diabetes* 44 (Suppl. 1):55A, 1995
23. Jacot E, DeFronzo RA, Jequier E, Meader 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
24. Dunning BE, Scott MF, Neal DW, Cherrington AD: Direct quantification of norepinephrine spillover and hormone output from the pancreas of the conscious dog. *Am J Physiol* 272:E746–E755, 1997
25. Sindelar DK, Balcom JH, Chu CA, Neal DW, Cherrington AD: A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. *Diabetes* 45:1594–1604, 1996
26. Snedecor GW, Cochran WG: *Statistical Methods.* 6th ed. Ames, Iowa, State University Press, 1967
27. 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
28. Samols E, Weir GC, Bonner-Weir S: Intraislet insulin-glucagon-somatostatin relationships. In *Glucagon II*. Lebebre PJ, Ed. Berlin, Springer, 1983, p. 133–173
29. Samols E, Stagner JI: Intra-islet regulation. *Am J Med* 85:31–35, 1988
30. Stagner JI, Samols E, Bouner-Weir S: β → α → δ pancreatic islet cellular perfusion in dogs. *Diabetes* 37:1715–1721, 1988
31. Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 31:883–889, 1982
32. Stagner JI, Samols E: Retrograde perfusion as a method for testing the relative effects of glucose on the A cell. *J Clin Invest* 77:1034–1037, 1986
33. 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
34. 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
35. Gerich JE, Tsalikian E, Lorenzi M, Karam JH, Bier DM: Plasma glucagon and alanine response to acute insulin deficiency in man. *J Clin Endocrinol Metab* 40:526–529, 1975
36. Unger RH, Madison L, Muller W: Abnormal alpha cell function in diabetes: response to insulin. *Diabetes* 21:301–309, 1972
37. Braaten JT, Faloona GR, Unger RH: The effect of insulin on the alpha cell response to hyperglycemia in long-standing alloxian diabetes. *J Clin Invest* 53:1017–1021, 1974
38. Buchanan KD, Mawkinney WAA: Glucagon release from isolated pancreas in streptozotocin-treated rats. *Diabetes* 22:794–800, 1973
39. 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
40. Ahren B, Veith RC, Taborsky GJ Jr: Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog. I. Effects on basal release of insulin and glucagon. *Endocrinology* 121:323–331, 1987
41. Dunning BE, Ahren B, Veith RC, Bottcher G, Sundler F, Taborsky GJ Jr: Galanin: a novel pancreatic neuropeptide. *Am J Physiol* 251:E127–E133, 1986
42. Bloom SR, Edwards AV: Pancreatic endocrine responses to stimulation of the peripheral ends of the vagus nerves in conscious calves. *J Physiol* 315:31–41, 1981
43. Kaneko A, Miki E, Kosaka K: Effects of vagal simulation on glucagon and insulin secretion. *Endocrinology* 95:1005–1010, 1974
44. Kaneko A, Kajinuma H, Kosaka K, Nakao K: Stimulation of insulin secretion by parasympathomimetic agents. *Endocrinology* 83:651–658, 1968
45. 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
46. 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
47. Sherck SM, Shiota M, Saccomando J, Cardin S, Alien EJ, Hastings JR, Neal DW, Williams PE, Cherrington AD: Pancreatic response to mild non-insulin-induced hypoglycemia does not involve extrinsic neural input. *Diabetes* 50:2487–2496, 2001
48. Gerich JE, Charles MA, Grodsky GM: Regulation of pancreatic insulin and glucagon secretion. *Ann Rev Physiol* 38:353–389, 1976
49. 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
50. Davis SN, Goldstein RE, Jacobs J, Price L, Wolfe R, Cherrington AD: The effect of differing insulin levels on the hormonal and metabolic responses to equivalent hypoglycemia in normal man. *Diabetes* 42:263–272, 1993
51. Kerr D, Reza M, Smith N, Leatherdale B: Importance of insulin in subjective, cognitive, and hormonal responses to hypoglycemia in patients with IDDM. *Diabetes* 40:1057–1062, 1991
52. Davis M, Mellman M, Shamoon H: Physiologic hyperinsulinemia enhances counterregulatory hormone responses to hypoglycemia in IDDM. *J Clin Endocrinol Metab* 76:1383–1385, 1993
53. 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
54. Berne C, Fagius J, Pollare T, Hjemidahl P: The sympathetic response to euglycemic hyperinsulinemia. *Diabetologia* 35:873–879, 1992
55. Porte D Jr, Robertson RP: Control of insulin secretion by catecholamines, stress, and the sympathetic nervous system. *Fed Proc* 32:1792–1796, 1973
56. Strubbe JH, Steffens AB: Neural control of insulin secretion. *Horm Metab Res* 25:507–512, 1993
57. Christensen NJ, Iversen J: Release of large amounts of noradrenaline from the isolated perfused canine pancreas during glucose deprivation. *Diabetologia* 9:396–399, 1973
58. Dunning BE, Havel PJ, Veith RC, Taborsky GJ Jr: Pancreatic and extrapancreatic galanin release during sympathetic neural activation. *Am J Physiol* 258:E436–E444, 1990
59. Ahren B, Ostenson CG, Efendic S: Other islet peptides. In *The Endocrine Pancreas.* Samols E, Ed. New York, Raven Press, 1991, p. 153–173
60. Stagner J, Samols E, Polonsky K, Pugh W: Lack of direct inhibition of insulin secretion by exogenous insulin in the canine pancreas. *J Clin Invest* 78:1193–1198, 1986
61. 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.* Efendic S, Ostenson CG, Vranic M, Eds. New York, Plenum Press, 1993, p. 199–208
62. 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
63. 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
64. Hems DA, Whitton PD: Control of hepatic glycogenolysis. *Physiol Rev* 60:1–50, 1980