A Fall in Portal Vein Insulin Does Not Cause the Alpha-Cell Response to Mild, Non–Insulin-Induced Hypoglycemia in Conscious Dogs

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The aim of the present study was to determine whether a decrease in the portal vein insulin level during non–insulin-induced hypoglycemia is sensed and is responsible for the normal increase in glucagon release from the alpha cell. To address this aim, a glycogen phosphorylase inhibitor was used to create mild, non–insulin-induced hypoglycemia in 2 groups of 18-hour fasted conscious dogs. Arterial insulin was clamped at a basal level in both groups, but in one group (PE) the portal vein insulin level was permitted to fall by approximately 65% while in the other group (POR) it was clamped at a basal level. In both groups glucose was infused at a variable rate to clamp the plasma glucose level at approximately 70 mg/dL. Plasma glucagon (pg/mL) rose to indistinguishable maxima in both groups (56 ± 3 in PE and 67 ± 9 in POR). Likewise, glucagon secretion (pg/kg/min) increased similarly (189 ± 32 to 455 ± 203 in PE and 192 ± 50 to 686 ± 237 in POR). Thus, the increase in glucagon release was not inhibited when the portal vein insulin level was prevented from decreasing (POR group). Clearly, a fall in the portal vein insulin level is not required for a normal alpha-cell response to mild, non–insulin-induced hypoglycemia.

IN PREVIOUS STUDIES, administration of a glycogen phosphorylase inhibitor (BAY R3401)[1-4] caused mild hypoglycemia, a fall in insulin release, and a rise in glucagon secretion in conscious dogs.[5] To determine whether it was the decrease in insulin or the fall in glucose that caused the rise in glucagon, in a second study we created mild hypoglycemia using the glycogen phosphorylase inhibitor, only in this case one group of dogs received a small peripheral insulin infusion and another received saline.[6] Insulin infusion caused a 3-fold increase in the arterial insulin level and prevented the drop in the portal vein insulin level, whereas saline infusion was associated with a decrease in insulin in both vessels. Insulin infusion completely abolished the rise in plasma glucagon.

There are 3 possible explanations for the inhibition of glucagon secretion caused by insulin infusion in the above study. The first possibility is that the rise in arterial insulin could have directly inhibited alpha-cell secretion by preventing the intraislet insulin level from falling below a critical threshold. The second possibility is that the rise in arterial insulin could have been sensed at some unknown site and thereby inhibited glucagon secretion indirectly through a neural or humoral mechanism. The final possibility is that the maintenance of the portal vein insulin level at a basal value could have inhibited alpha-cell function through a neural or humoral mechanism. If the latter were to be the case, it would indicate that it is the fall in portal vein insulin that triggers the alpha-cell response to mild non–insulin-induced hypoglycemia.

There is some indirect evidence to support the latter theory. First, the identification of glucose sensors in the portal vein[7] raises the possibility that insulin could be sensed there, since all glucose sensors discovered to date contain glucokinase and GLUT2,[8-11] and since glucokinase is usually insulin-sensitive.[12] In fact, delivering glucose directly into the portal vein stimulated insulin secretion to a greater extent than peripheral glucose administration even when the arterial glucose levels were identical.[13] Additional evidence that pancreatic secretion may be affected by portal vein glucose levels is that mice lacking GLUT2 in nonpancreatic tissue (including the portal vein) exhibit an impaired control of glucagon secretion in response to low or high glucose levels.[14] Finally, it was shown that insulin infusion into the portal vein of the rat stimulated intestinal glucose uptake, implying the portal vein insulin level can affect an organ upstream of its blood flow.[10] Clearly, evidence exists to support the concept that signaling loops exist between glucose sensors in the portal vein and other organs.

The aim of the present study therefore was to test the hypothesis that the fall in the portal vein insulin level that occurs in response to mild non–insulin-induced hypoglycemia decreases glucose uptake by the portal glucose sensors and thereby causes an increase in alpha-cell secretion. To test this hypothesis, arterial insulin was clamped at a basal level in 2 groups of dogs exposed to mild hypoglycemia, but in one group the portal vein insulin level was permitted to fall while in the other group it was clamped at a basal level.

MATERIALS AND METHODS

Animals and Surgical Procedures

Studies were performed on 12 overnight-fasted conscious mongrel dogs of either sex (24.7 ± 0.6 kg). Animals were fed once daily a diet of meat (Kal Kan, Vernon, CA) and chow (Purina Lab Canine Diet No. 5006; Purina Mills, St Louis, MO) comprised of 46% carbohydrate, 34% protein, 14% fat, and 6% fiber based on dry weight. The animals 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.

Approximately 16 days prior to the study, a laparotomy was per-

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formed under general anesthesia (15 mg/kg body weight sodium thiopental presurgery; 1.0% isoflurane as an inhalation anesthetic during surgery). In all dogs, ultrasonic flow probes (Transonic Systems, Ithaca, NY) were positioned around the portal vein and a hepatic artery as previously described.[15] Silastic catheters (Dow Corning, Midline, MI) were inserted into a femoral artery, the portal vein, and the left common hepatic vein for blood sampling and into the splenic and jejunal veins for intraportal hormone delivery as previously described.[16] All of the dogs also had a catheter implanted in the stomach to facilitate drug delivery and a catheter implanted into the inferior vena cava for peripheral insulin infusion. The catheters were filled with heparinized saline (200 U/mL; Abbott Laboratories, North Chicago, IL) and the free ends were knotted. The free ends of the catheters and the flow probe leads were placed in subcutaneous pockets until the study day. The animals were studied only if the following criteria were met: (1) leukocyte count less than 18,000/μL, (2) hematocrit greater than 35%, (3) good appetite, and (4) normal stools.

On the morning of the study, the Transonic leads and the catheters were exteriorized under local anesthesia (2% lidocaine; Abbott Laboratories). Each dog was placed in a Pavlov harness, the contents of the catheters were aspirated, and they were flushed with saline and subsequently used for blood sampling. Angiocaths (20 gauge; Becton Dickinson, Sandy, UT) were inserted into the right and left cephalic veins for infusion of glucose (20% Dextrose; Baxter Healthcare, Deerfield, IL) and indocyanine green dye (Sigma), respectively.

Experimental Design

Each experiment consisted of a 90-minute equilibration period (−120 to −30 minutes) followed by a 30-minute control period (−30 to 0 minutes). During these periods, and during the 3-hour experimental period (0 to 180 minutes) that followed, indocyanine green dye (0.07 mg/min) was infused. At the onset of the experimental period, all dogs were given the glycogen phosphorylase inhibitor, Bay R3401 (Bayer, Pittsburgh, PA; 8 mg/kg in a 0.5% methyl cellulose/water solution [50 mL]), intragastrically via the stomach catheter to decrease the plasma glucose level. Additionally, dogs were given insulin (Eli Lilly, Indianapolis, IN), either peripherally (PE; 0.25 mU/kg/min via the inferior vena cava so as to maintain only basal arterial insulin levels) or intraportally (POR; 0.5 mU/kg/min via splenic and jejunal catheters, so as to maintain both basal arterial and portal vein insulin levels). Glucose was infused peripherally to clamp the plasma glucose level at 70 mg/dL. Note that previous administration of BAY R3401 and maintenance of euglycemia in conscious dogs did not result in any changes in hormone levels, showing that the drug does not directly influence alpha- or beta-cell secretion.[3] Additionally, there was no change in glucose utilization, suggesting that the effects of the drug on muscle phosphorylase were minimal.[3] Our previous work provides a more detailed description of the effects and action of Bay R3401.[17]

Analytical Procedures

The immediate processing of the samples and the measurement of whole blood glucose and metabolites (lactate, alanine, glycerol, beta-hydroxybutyrate [BOHB]) were described previously.[15,18] In addition, plasma levels of glucose, indocyanine green, catecholamines, and nonesterified fatty acids (NEFA) were measured as previously described, as were plasma immunoreactive levels of insulin, glucagon, cortisol, and pancreatic polypeptide.[15,18] Note that immunoreactive glucagon (IRG) values include approximately 15 to 25 pg/mL of cross-reacting, nonglucagon material, and that the level of the cross-reactant is equivalent and unchanging in all blood vessels, thus providing a constant background in the artery and the portal vein. Immunoreactive canine C-peptide (in plasma to which 500 KIU/mL Trasylol had been added; FBA Pharmaceuticals, New York, NY) was determined via double-antibody radioimmunoassay (Linco Research, St Charles, MO) with an interassay coefficient of variation of 5%.

Calculations

Glucagon secretion was calculated in each group by multiplying portal vein plasma flow by the [portal vein − arterial] plasma IRG difference. Note that the arterial glucagon concentrations were adjusted by multiplying arterial values by 0.92. This correction factor was used because the gut has been shown to destroy approximately 8% of the plasma IRG that reaches it.[19]

Insulin secretion was also determined in each group by multiplying portal vein plasma flow by the [portal vein − arterial] plasma immunoreactive insulin (IRI) difference. Note that the arterial insulin concentrations were adjusted by multiplying arterial values by 0.7 to account for the destruction (~30%) of plasma IRI by the gut.[20] This calculation was used for determining insulin secretion in the peripheral infusion group, but another adjustment was also necessary to determine insulin secretion in the group receiving portal insulin infusion. In POR, the known insulin infusion rate (500 μU/kg/min) was subtracted from the calculated insulin secretion rate.

The net hepatic balances of blood glucose, lactate, alanine, glycerol, BOHB, and plasma NEFA were calculated using both Transonic-determined and indocyanine green dye (ICG)-determined flow. The data shown are those calculated using Transonic-determined flow as this flow does not require an assumption about the distribution of arterial versus portal flow. Note that the same conclusions were drawn when ICG-determined flow was used to calculate the data. Equations used are as follows: Net Hepatic Substrate Balance = H · HF − [A · (AF) + P · (PF)], where A, P, and H are arterial, portal vein, and hepatic vein substrate concentrations (blood or plasma); AF and PF are the arterial and portal vein flow (blood or plasma) measured by the Transonic flow probes; and HF (total hepatic flow; blood or plasma) = AF + PF. Positive numbers for net hepatic substrate balance indicate net production while negative numbers indicate net uptake. Net hepatic uptake is presented as a positive value.

Area under the curve (AUC) for glucagon in each group was calculated for the entire experimental period (3 hours) using the trapezoidal rule for each dog. The AUC of the basal period (time-adjusted) was subtracted from the AUC of the corresponding experimental period of each dog to calculate the ΔAUC, thus accounting for any baseline differences between the groups.

Statistical Analysis

Data are expressed as mean ± SE. Statistical comparisons were made by 1- and 2-way analysis of variance (ANOVA) with repeated-measures design run on SYSTAT (Cary, NC). Post hoc analysis was performed using the univariate *F* test when ANOVA yielded significant results between or within the groups to determine more specifically when the differences existed (SYSTAT). Statistical significance was accepted at $P < .05$.

RESULTS

Glucose and Hormone Levels

The basal plasma glucose level (Fig 1) was similar in both groups. Upon administration of the glycogen phosphorylase inhibitor and infusion of insulin the plasma glucose level reached minima of 69 ± 2 and 70 ± 2 mg/dL in PE and POR, respectively ($P < .05$ for both groups; no significant difference [NS] between groups). The glucose infusion required to keep the plasma glucose level at approximately 70 mg/dL was not significantly different between the 2 groups (Table 1). Net hepatic glucose balance (NHGB; mg/kg/min; Fig 1) fell to

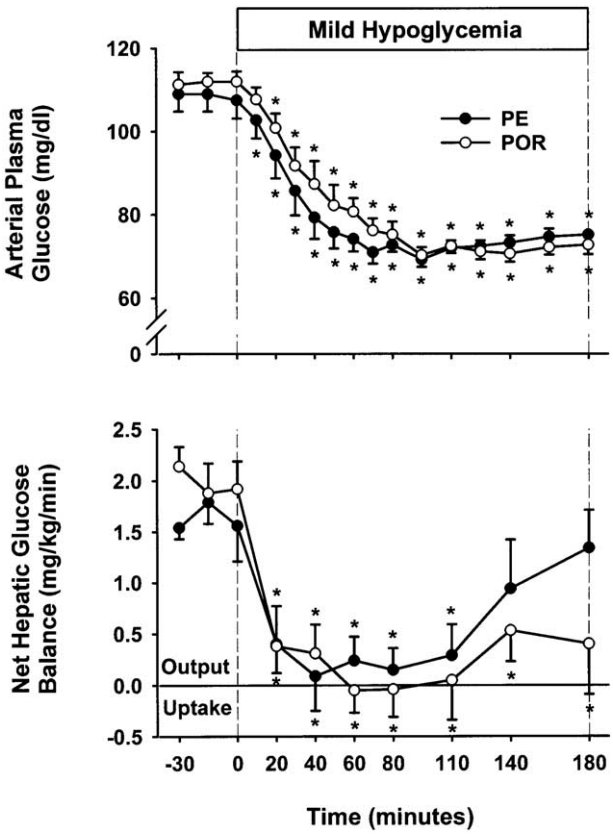

Fig 1. Arterial plasma glucose levels and net hepatic glucose balance in the control period (−30 to 0 min) and after administration of the glycogen phosphorylase inhibitor (8 mg/kg intragastrically at 0 min) and either peripheral (PE) or portal (POR) insulin (0.25 mU/kg/min or 0.5 mU/kg/min, respectively, from 0 to 180 min) in 18-hour fasted conscious dogs. Data are expressed as mean ± SEM. *$P < .05$ *v* basal period, n = 6 for PE and POR.

similar minima in both groups (PE: from 1.7 ± 0.2 to 0.1 ± 0.3; POR: from 2.0 ± 0.2 to 0.0 ± 0.2). It remained almost completely suppressed in POR but rose to 1.3 ± 0.4 in PE. The average hepatic blood flow (mL/kg/min) was similar between the 2 groups during the basal period (PE: 35 ± 3; POR: 35 ± 3) and throughout the experimental period (PE: 35 ± 3; POR: 35 ± 3).

Arterial plasma insulin (Fig 2; μU/mL) remained at basal levels in both groups. In contrast, in PE the portal insulin level dropped ($P < 0.05$), while in POR it rose minimally (NS). As a result the portal insulin levels were significantly different in the 2 groups ($P < .05$). Endogenous insulin secretion was calculated and fell significantly in both groups by the end of the study to near zero (PE: 333 ± 97 to 15 ± 3; POR: 289 ± 76 to −20 ± 77 μU/kg/min). Plasma C-peptide levels (Fig 2), an index of endogenous insulin secretion, fell by approximately 60% ($P < .05$) in both groups, with no significant difference between the groups.

Arterial plasma glucagon (Fig 3; pg/mL) rose in both groups (44 ± 2 to 56 ± 3; 39 ± 1 to 67 ± 9 for PE and POR, respectively; $P < .05$ for both groups), with no significant difference between groups. The rise in portal plasma glucagon mirrored the rise in arterial plasma glucagon ($P < .05$). The portal-arterial glucagon gradient (pg/mL) tended to increase in PE (7 ± 1 to 24 ± 7; $P = .09$) and increased significantly in POR (8 ± 3 to 37 ± 7; $P < .05$), and there was no significant difference between the 2 groups. The overall change from baseline AUC (pg over 3 h) in arterial plasma glucagon was 1,569 ± 584 in PE and 2,678 ± 767 in POR. The change in AUC for portal plasma glucagon was 3,446 ± 1,085 in PE and 5,263 ± 407 in POR, and for the portal-arterial plasma glucagon difference was 1,880 ± 967 in PE and 2,577 ± 828 in POR. Glucagon secretion increased significantly ($P < .05$) in both groups (from 189 ± 32 to a maximal value of 455 ± 203 and from 192 ± 50 to a maximal value of 686 ± 237 pg/kg/min in PE and POR, respectively). None of the above changes differed between the groups.

Norepinephrine and cortisol levels did not change significantly over time in either group. Likewise, pancreatic polypeptide levels did not rise appreciably. Epinephrine levels, on the other hand, increased modestly in POR (~100%; $P < .05$; Table 2) and tended to rise in PE (~40%; NS). There were no significant differences between groups for the norepinephrine, pancreatic polypeptide, or epinephrine levels. There was a significant difference ($P < .05$) between the cortisol levels in the 2 groups (40 to 80 minutes), but it was the result of differing baseline values.

Metabolite Levels and Balance

Arterial blood lactate and net hepatic lactate balance fell significantly in both groups in response to mild hypoglycemia ($P < .05$; Table 3). Similarly, arterial blood alanine levels fell significantly in both groups ($P < .05$; Table 3), while net hepatic alanine balance did not change significantly. There were no significant differences between PE and POR with regard to lactate or alanine at any time.

Table 1. Glucose Infusion Rate During the Control and Experimental Periods of 18-Hour Fasted Conscious Dogs Given a Glycogen Phosphorylase Inhibitor (8 mg/kg intragastrically at 0 minutes) and Either Peripheral or Portal Insulin Infusion (0.25 mU/kg/min or 0.5 mU/kg/min, respectively, from 0 to 180 minutes)

	Glucose Infusion Rate (mg/kg/min)												
	Control Period (min)	Experimental Period (min)											
Group	−30 to 0	0 to 30	40	50	60	70	80	95	110	125	140	160	180
PE	0	0	0.4 ± 0.3	0.6 ± 0.4	1.1 ± 0.5	1.2 ± 0.5	1.5 ± 0.7	1.1 ± 0.4	1.5 ± 0.6	1.2 ± 0.5	0.5 ± 0.2	0.6 ± 0.4	0.6 ± 0.4
POR	0	0	0	0.4 ± 0.5	0.7 ± 0.5	0.4 ± 0.4	1.0 ± 0.8	0.6 ± 0.4	1.6 ± 0.4	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.4	1.1 ± 0.5

NOTE. Data are mean ± SEM for each group. For both groups, n = 6. There was no significant difference between groups.

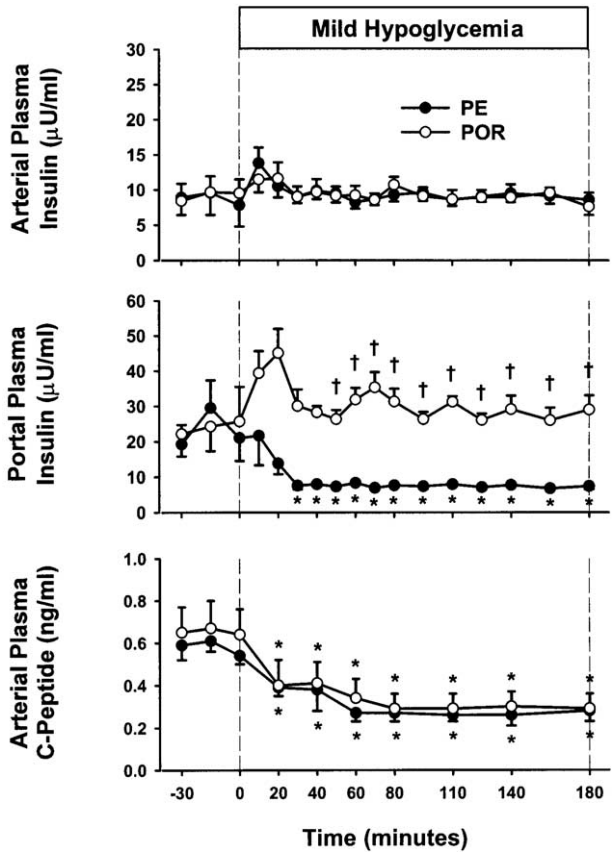

Fig 2. Arterial and portal plasma insulin and arterial plasma C-peptide levels in the control period (−30 to 0 min) and after administration of the glycogen phosphorylase inhibitor (8 mg/kg intragastrically at 0 min) and either peripheral (PE) or portal (POR) insulin (0.25 mU/kg/min or 0.5 mU/kg/min, respectively, from 0 to 180 min) in 18-hour fasted conscious dogs. Data are expressed as mean ± SEM. *$P < .05$ *v* basal period, n = 6 for PE and POR. †$P < .05$ for POR *v* PE.

Arterial blood glycerol levels rose in response to mild hypoglycemia in both groups, as did net hepatic glycerol uptake ($P < .05$; Table 3). Arterial plasma NEFA levels and arterial blood BOHB levels paralleled the changes in arterial blood glycerol, and increased in response to mild hypoglycemia ($P < .05$; Table 3). Net hepatic NEFA uptake increased in both groups, as did BOHB production. There were no significant differences between PE and POR with regard to glycerol, NEFA, or BOHB at any time.

DISCUSSION

A possible explanation for our previous finding that insulin blunted the glucagon response to mild non–insulin-induced hypoglycemia[6] was that the decrease in portal vein insulin, which would normally trigger glucagon release, was prevented by insulin infusion. For this to be the case it would mean that the portal insulin level can influence glucagon release. As previously mentioned, the portal vein contains glucose sensors,[7] which are likely to be insulin-sensitive.[8-12] Portal vein glucose elevation was shown to influence insulin[13] and glucagon[21] release, and portal vein insulin infusion was shown to affect glucose uptake of an organ upstream of its blood flow.[10] However, the present study did not find an affect of the portal insulin level on the alpha-cell response to mild non-insulin-induced hypoglycemia. In fact, these results are consistent with those from another group, which showed that although portal vein glucose delivery affected the release of the counter-regulatory hormones epinephrine and norepinephrine,[7,22-25] it did not significantly alter glucagon release during moderate insulin-induced hypoglycemia.[22-24] Additionally, results from a recent study showed that administration of oral glucose to humans, prior to inducing systemic hypoglycemia so as to diminish portal vein hypoglycemia, blunted the symptoms and epinephrine response but not the alpha-cell response to hypoglycemia.[26] Note, however, that this study[26] used supraphysiological insulin concentrations, which resulted in a complete lack of alpha-cell response to hypoglycemia, thereby making it impossible to detect a blunted response.

The present study showed that mild hypoglycemia stimulated a significant rise in glucagon secretion (3.5-fold) even

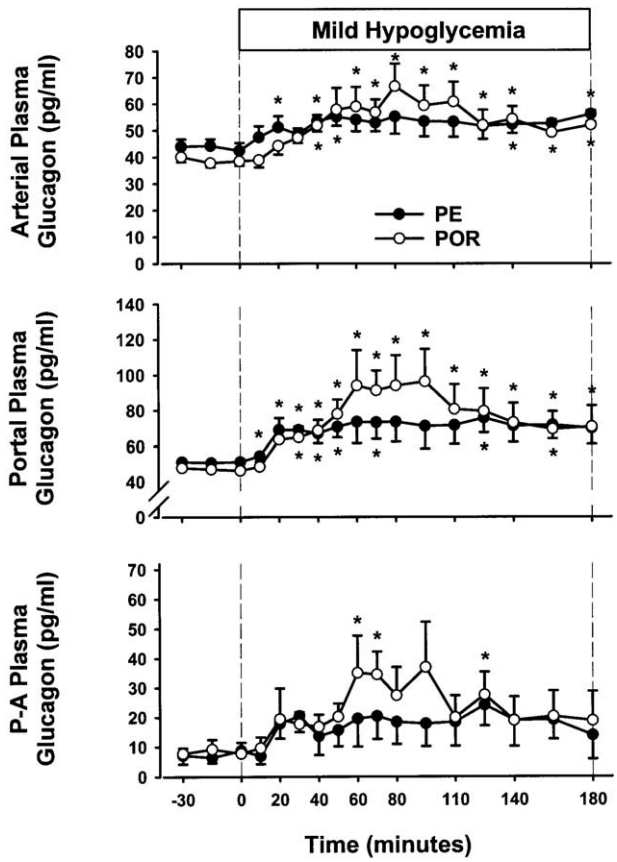

Fig 3. Arterial and portal plasma glucagon levels and the portal-arterial (P-A) glucagon gradient in the control period (−30 to 0 min) and after administration of the glycogen phosphorylase inhibitor (8 mg/kg intragastrically at 0 min) and either peripheral (PE) or portal (POR) insulin (0.25 mU/kg/min or 0.5 mU/kg/min, respectively, from 0 to 180 min) in 18-hour fasted conscious dogs. Data are expressed as mean ± SEM. *$P < .05$ *v* basal period, n = 6 for PE and POR. $P = .094$ for the P-A glucagon gradient of PE.

Table 2. Hormone Data During the Control and Experimental Periods of 18-Hour Fasted Conscious Dogs Given a Glycogen Phosphorylase Inhibitor (8 mg/kg intragastrically at 0 minutes) and Either Peripheral or Portal Insulin Infusion (0.25 mU/kg/min or 0.5 mU/kg/min, respectively, from 0 to 180 minutes)

	Control Period (min)	Experimental Period (min)						
	−30 to 0	20	40	60	80	110	140	180
Arterial plasma epinephrine (pg/mL)								
PE	205 ± 56	167 ± 56	199 ± 79	220 ± 73	226 ± 71	238 ± 59	232 ± 64	262 ± 82
POR	222 ± 28	252 ± 46	290 ± 47	259 ± 38	309 ± 85	371 ± 74*	427 ± 114	451 ± 92
Portal plasma epinephrine (pg/mL)								
PE	97 ± 32	85 ± 29	115 ± 40	103 ± 40	93 ± 39	106 ± 22	108 ± 29	145 ± 48
POR	102 ± 8	132 ± 19	131 ± 17*	131 ± 36	146 ± 44	164 ± 34	202 ± 61	182 ± 23*
Arterial plasma norepinephrine (pg/mL)								
PE	176 ± 34	172 ± 36	198 ± 55	206 ± 38	222 ± 45	214 ± 36	203 ± 38	232 ± 41
POR	187 ± 22	205 ± 44	184 ± 17	202 ± 19	278 ± 98	207 ± 33	211 ± 34	203 ± 35
Portal plasma norepinephrine (pg/mL)								
PE	200 ± 33	222 ± 48	243 ± 53	224 ± 43	278 ± 64	219 ± 30	233 ± 47	280 ± 52
POR	221 ± 16	232 ± 18	222 ± 14	237 ± 29	232 ± 27	231 ± 28	239 ± 26	234 ± 25
Arterial plasma cortisol (μg/dL)								
PE	2.0 ± 0.3	2.8 ± 0.5	2.9 ± 0.4	2.7 ± 0.5	2.9 ± 0.7	3.5 ± 0.5	3.2 ± 0.6	2.8 ± 0.6
POR	3.2 ± 0.6	3.7 ± 0.5	4.5 ± 0.6†	5.1 ± 0.8†	4.8 ± 0.7†	5.3 ± 1.4	4.2 ± 0.6	4.3 ± 1.0
Arterial plasma pancreatic polypeptide (pg/mL)								
PE	156 ± 23	232 ± 43	236 ± 23	233 ± 40	209 ± 41	160 ± 17	169 ± 38	178 ± 66
POR	142 ± 30	121 ± 34	125 ± 28	156 ± 42	209 ± 38*	209 ± 63	201 ± 48	175 ± 48

NOTE. Data are mean ± SEM for each group. For both groups, n = 6.

*$P < .05$ *v* control period.

†$P < .05$ for POR *v* PE.

when insulin remained constant in the arterial circulation and the portal vein (POR group). There are 3 possible explanations for this rise in glucagon secretion: (1) the reduction in the intra-islet insulin concentration (that must have occurred when endogenous insulin secretion was replaced with intraportal insulin infusion), (2) hypoglycemia per se, or (3) a combination of the two.

Evidence that insulin can directly inhibit the alpha cell (ie, evidence for the importance of the intra-islet insulin concentration) comes from the observation that insulin inhibits glucagon secretion in vitro.[27] In addition, high circulating insulin levels that would raise the intra-islet insulin concentration have been shown to directly inhibit glucagon secretion in humans[28] and in the perfused rat pancreas.[29] Insulin deficiency in vivo results in hyperglucagonemia in humans[30,31] and other animals.[32] Likewise, administration of anti-insulin serum to the perfused rat pancreas[33,34] or insulin antibodies to the perfused human pancreas[35] stimulated glucagon secretion. Finally, a recent study found that the glucagon response to hypoglycemia in the diabetic BB rat is not defective if hyperinsulinemia is prevented, thus showing that insulin can profoundly affect alpha-cell secretion.[36]

The likelihood that insulin directly affects glucagon release is related to the anatomical structure of the pancreatic islet. In the rat, blood flows from the core of the islet, which is composed of beta cells, to the periphery of the islet where the alpha cells are found.[37,38] However, in the human alpha and delta cells are interspersed throughout the islet and there is no structured beta-cell core.[39] The structure of the canine islet is not firmly established; however, one study showed, using light microscopy, that canine islets also display marked heterogeneity in the proportions and arrangements of the endocrine cells.[40] To confirm this observation, we examined the architecture of the dog islet in more detail by using epifluorescent microscopy on multiple samples of pancreatic tissue. Our results indicate that the structure of dog islets resembles that of human (ie, variable islets and interspersed cell types) rather than rodent islets (Brissova et al, Vanderbilt University, unpublished communication). Thus while directed flow might not play a role in the control of the alpha cell, diffusion from adjacent beta cells might.

When a number of studies are looked at collectively, it appears that the arterial insulin level itself, as opposed to the intra-islet insulin level, can control glucagon secretion. For example, in a previous study[41] when insulin was administered peripherally in relatively low amounts to conscious dogs and euglycemia was maintained, arterial insulin rose but C-peptide levels fell, indicating that endogenous insulin secretion was inhibited. Despite the rise in arterial insulin it was obvious that the intra-islet insulin levels must have decreased as a result of the fall in insulin secretion. Nevertheless, these changes resulted in a significant decrease in plasma glucagon levels.[41] Clearly the alpha cell responded more sensitively to the rise in arterial insulin than to the fall in intra-islet insulin during euglycemia. In addition, in our previous study[6] even though the glucose level fell and the intra-islet insulin level fell, both of which would be expected to result in an increase in glucagon release, the 3-fold rise in the arterial insulin level completely inhibited the alpha-cell response. Furthermore, it should be noted that much of the evidence for the intra-islet insulin

Table 3. Metabolic Data: During the Control and Experimental Periods of 18-Hour Fasted Conscious Dogs Given a Glycogen Phosphorylase Inhibitor (8 mg/kg intragastrically at 0 minutes) and Either Peripheral or Portal Insulin Infusion (0.25 mU/kg/min or 0.5 mU/kg/min, respectively, from 0 to 180 minutes)

	Control Period (min)	Experimental Period (min)						
	−30 to 0	20	40	60	80	110	140	180
Arterial blood lactate (μmol/L)								
PE	1125 ± 155	861 ± 144*	647 ± 112*	549 ± 119*	542 ± 143*	510 ± 143*	483 ± 125*	413 ± 80*
POR	957 ± 121	736 ± 82*	710 ± 81*	617 ± 57*	521 ± 28*	523 ± 42*	515 ± 33*	522 ± 43*
Arterial blood alanine (μmol/L)								
PE	428 ± 31	417 ± 27	362 ± 24*	330 ± 26*	276 ± 24*	236 ± 23*	187 ± 21*	159 ± 15*
POR	365 ± 31	341 ± 38*	317 ± 35*	293 ± 29*	235 ± 24*	196 ± 18*	177 ± 18*	179 ± 18*
Arterial blood glycerol (μmol/L)								
PE	57 ± 5	48 ± 6	69 ± 12	75 ± 13	83 ± 17	98 ± 17*	98 ± 14*	102 ± 22*
POR	77 ± 13	69 ± 9	83 ± 12	90 ± 19	93 ± 22	111 ± 23*	116 ± 18*	114 ± 25
Arterial Plasma NEFA (μmol/L)								
PE	453 ± 37	412 ± 32	560 ± 76	701 ± 119	799 ± 175*	951 ± 251*	1044 ± 235*	1018 ± 248*
POR	671 ± 123	579 ± 85	699 ± 147	866 ± 187	946 ± 257	1073 ± 226*	1139 ± 181*	1109 ± 232*
Arterial blood BOHB (μmol/L)								
PE	17 ± 2	14 ± 2	13 ± 2*	16 ± 3	22 ± 8	34 ± 17	45 ± 22	55 ± 26
POR	19 ± 3	16 ± 3	17 ± 5	26 ± 7	39 ± 15	39 ± 17	49 ± 24	56 ± 25
NHLB (μmol/kg/min)								
PE	14.3 ± 2.7	5.5 ± 2.7*	0.2 ± 2.4*	−1.1 ± 2.2*	−3.4 ± 1.1*	−4.4 ± 1.1*	−6.5 ± 0.6*	−6.1 ± 1.0*
POR	7.8 ± 4.0	2.0 ± 2.6	0.8 ± 2.6	−3.3 ± 0.9*	−4.1 ± 0.7*	−4.6 ± 1.4*	−5.4 ± 0.8*	−7.2 ± 0.9*
NHAU (μmol/kg/min)								
PE	3.6 ± 0.6	4.4 ± 0.5	4.4 ± 0.5	4.4 ± 0.5	3.9 ± 0.4	4.3 ± 0.5	3.2 ± 0.4	3.4 ± 0.4
POR	2.9 ± 0.4	3.3 ± 0.5	3.2 ± 0.4	3.8 ± 0.3	3.4 ± 0.2	3.0 ± 0.5	3.0 ± 0.3	3.2 ± 0.2
NHGlycU (μmol/kg/min)								
PE	1.2 ± 0.1	1.1 ± 0.1	1.8 ± 0.3	2.1 ± 0.4*	2.2 ± 0.5*	2.7 ± 0.5*	2.9 ± 0.5*	2.7 ± 0.6*
POR	1.8 ± 0.4	1.8 ± 0.4	2.1 ± 0.5	2.2 ± 0.6	2.5 ± 0.9	2.9 ± 0.7*	2.9 ± 0.5*	2.7 ± 0.6*
NH NEFA U (μmol/kg/min)								
PE	2.1 ± 0.3	1.8 ± 0.1	2.6 ± 0.4	3.3 ± 0.6	3.9 ± 0.8*	4.5 ± 1.1*	4.7 ± 1.4*	4.2 ± 1.2*
POR	2.9 ± 0.5	2.7 ± 0.5	2.5 ± 0.4	3.5 ± 0.6	3.0 ± 1.1	4.4 ± 0.9*	4.3 ± 0.9*	4.3 ± 1.2
NH BOHB P (μmol/kg/min)								
PE	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	1.0 ± 0.3	1.2 ± 0.4	1.4 ± 0.6	2.0 ± 0.9	2.3 ± 1.1
POR	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.3	1.4 ± 0.5	1.5 ± 0.5	1.8 ± 0.5	1.7 ± 0.5

NOTE. Data are mean ± SEM for each group. For both groups, n = 6. For net hepatic balance, negative and positive numbers mean net hepatic uptake and output, respectively.

Abbreviations: NHLB, net hepatic balance of lactate; NHAU, net hepatic uptake of alanine; NHGlycU, net hepatic uptake of glycerol; NH NEFA U, net hepatic uptake of NEFA; NH BOHBP, net hepatic production of BOHB.

*$P < .05$ *v* control period. ANOVA did show a significant increase from basal in arterial blood BOHB and NH BOHB P in both PE and POR, even though post hoc analysis did not find any single point that was significantly different from the control period. Furthermore, although the 40-minute point for the arterial BOHB levels in PE was significantly different, the overall main effect is the observed rise by the end of the experimental period.

concentration affecting glucagon release came from studies involving a perfused pancreas system,[29,33-35] making it possible that the results were due to changes in the insulin level in the pancreatic artery rather than around the islets per se.

In a recent study, Banarer et al tried to separate the effects of arterial and intra-islet insulin on glucagon secretion during hypoglycemia.[42] The investigators compared the glucagon response of healthy subjects to insulin-induced hypoglycemia alone with their response to insulin-induced hypoglycemia brought about in the presence of an infusion of the beta-cell secretagogue tolbutamide. Because the glucagon response was prevented during tolbutamide infusion, the authors concluded that intra-islet insulin inhibits the alpha-cell response to hypoglycemia. It was possible, however, that the higher arterial insulin levels evident in the tolbutamide group were responsible for blunting the alpha-cell response, as opposed to a rise in the intra-islet insulin concentration. For this reason, the investigators restudied some subjects with a higher insulin infusion rate, to see if the higher arterial insulin levels, in the absence of tolbutamide, could blunt the alpha-cell response.[42] They found that the higher arterial insulin levels did not blunt the glucagon response to hypoglycemia, implying that intra-islet insulin was key. However, interpretation of this study is difficult because when additional insulin was infused there was a tendency towards increased activation of the central nervous system, which could very well explain the enhanced glucagon response despite the inhibitory effect of the high arterial insulin.

As previously mentioned, it seems likely that hypoglycemia per se stimulates glucagon release (for a recent review see Taborsky et al[43]). The threshold, half-maximal, and maximal inhibition of glucagon by glucose occur at plasma glucose levels of 45, 90, and 180 mg/dL, respectively, according to in

vitro data.[44] Perfusion of the rat pancreas with low-glucose media increased glucagon secretion even though insulin levels were already maximally inhibited.[45] Although isolated rat alpha cells were not stimulated by low glucose, glucose was able to inhibit amino acid-induced glucagon release via a direct, insulin-independent action, implying that glucose can in fact exert direct effects on the alpha cell.[46] Finally, the present results suggest hypoglycemia per se may stimulate the alpha cell. For example, the increases in the arterial and portal glucagon levels seen when insulin was kept from falling in both vessels were similar in magnitude to those that occurred when insulin was allowed to decrease in response to hypoglycemia in our earlier studies.[5,6] However, glucose fell to a lower level in the present study than in the previous studies[5,6] (~70 mg/dL *v* ~80 mg/dL, respectively). Thus, a larger glucagon response may have been observed if arterial insulin had been permitted to fall in the current studies. Put another way, perhaps the 3-fold rise in the arterial insulin level brought about in the previous study[6] would not be sufficient to completely inhibit the alpha-cell response to the slightly deeper hypoglycemia evident in the present study.

It is widely accepted that elevations in plasma catecholamines can stimulate glucagon release. In the present study, epinephrine rose minimally (~200 pg/mL) in response to the hypoglycemia in the POR group, thus raising the possibility that a small increase in sympathetic drive may have influenced the increased glucagon secretion in the POR group. In a previous study, the glycogen phosphorylase inhibitor was given to conscious dogs that had previously undergone surgical pancreatic denervation.[18] There was greater than 97% destruction of sympathetic input to the pancreas and there was no increase in systemic catecholamines during mild hypoglycemia, yet the glucagon response to the mild hypoglycemia was robust. Based on this evidence, we do not believe that a minimal activation of the central nervous system was the stimulus for glucagon release in the present study.

Lactate and alanine levels in blood fell similarly in PE and POR, while net lactate uptake by the liver increased and net alanine uptake was maintained, revealing increased gluconeogenic flux during the inhibition of glycogenolysis. In contrast, arterial glycerol and NEFA rose equivalently in both groups, implying increased lipolysis in the presence of basal arterial insulin levels. These increments were expected because glucose inhibits lipolysis,[47,48] perhaps via an indirect mechanism involving the central nervous system,[48] and glucose fell after drug administration. The increase in lipolysis was the result of the hypoglycemia and not a side effect of the drug, because it was previously shown that when euglycemia was maintained in the presence of the drug lipolysis did not change.[5]

The changes in the blood concentrations of the metabolic substrates are unlikely to have caused the observed changes in glucagon secretion.[5,18] Glucagon secretion is stimulated by amino acids,[44,46] and alanine fell during the present study. Given the increase in fractional extraction of amino acids by glucagon, it is likely other amino acids would have fallen and thus not contributed to the rise in glucagon secretion. In contrast, glucagon secretion is inhibited by NEFA and ketones, which rose during the study.[44,46] Thus, these substrate changes should, if anything, have opposed the increase in glucagon that occurred in response to hypoglycemia.

In conclusion, a fall in portal vein insulin did not provide a signal for the increase in glucagon secretion seen in response to non–insulin-induced hypoglycemia. This finding suggests that the sensitive response of the alpha cell to non–insulin-induced hypoglycemia was attributable to a lowering of the interstitial fluid insulin level proximate to the alpha cell, to hypoglycemia per se, or to a combination of the two. However, it is clear that additional factors, such as the arterial insulin level and the central nervous system, influence alpha-cell secretion during other hypoglycemic situations.

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