Published in final edited form as:

Am J Physiol Regul Integr Comp Physiol. 2006 September ; 291(3): R788–R795.

Glucagon secretion and autonomic signaling during hypoglycemia in late pregnancy

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Abstract

We examined net pancreatic norepinephrine (NE) spillover, pancreatic polypeptide (PP) release, and the decrement in C-peptide to identify factors involved in the blunted counterregulatory glucagon response in pregnancy. Conscious pregnant [pregnant hypoglycemic (Ph); 3rd trimester; $n = 8$] and nonpregnant [nonpregnant hypoglycemic (NPh); $n = 6$] dogs were studied during insulin-induced (~12-fold basal insulin concentrations) hypoglycemia (plasma glucose 3.1 mM). Additional dogs were studied during hyperinsulinemic euglycemia [non-pregnant euglycemic (NPe), $n = 4$; pregnant euglycemic (Pe), $n = 5$; plasma glucose 6 mM]. Arterial glucagon concentrations declined similarly in NPe and Pe. Areas under the curve (AUCs) of the changes in glucagon and epinephrine were seven- and threefold greater in NPh than Ph ($P < 0.05$ between groups for both). Glucagon secretion fell below basal in NPe, Pe, and Ph but rose significantly in NPh. C-peptide declined 0.25 ± 0.06 , 0.12 ± 0.11 , 0.28 ± 0.05 , and 0.13 ± 0.02 ng/ml in NPe, Pe, NPh, and Ph, respectively ($P < 0.05$, NPh vs. Ph). AUCs of NE spillover were 516 ± 274 , 265 ± 303 , 506 ± 94 , and -63 ± 79 ng, respectively ($P < 0.05$, NPh vs. Ph). The AUC of PP release was approximately threefold greater in NPh than Ph ($P < 0.05$) but not different between euglycemic groups. The current evidence strongly suggests that the blunting of glucagon secretion during insulin-induced hypoglycemia in pregnancy is related to generalized impairment of a number of different signals, including parasympathetic and sympathoadrenal stimuli and altered sensing of circulating and/or intraislet insulin.

Keywords

pancreatic polypeptide; norepinephrine; epinephrine; hypoglycemic counterregulation; C-peptide

The standard of care for pregnant women with diabetes [whether type 1, type 2, or gestational (GDM)] involves maintenance of near normoglycemia to reduce the risk of adverse perinatal and obstetrical outcomes. Intensive insulin therapy is often required to achieve the therapeutic goals. Unfortunately, insulin-treated pregnant women suffer from more frequent and severe episodes of hypoglycemia than their nonpregnant counterparts on comparable therapy (19, 43), for reasons that are incompletely understood. Intensive insulin therapy in general has been associated with higher rates of hypoglycemia (41), and multiple episodes of hypoglycemia may result in a decrease in the counterregulatory response over time (15). However, intensive insulin therapy is unlikely to be the sole cause of the frequent hypoglycemic episodes and the impaired

counterregulatory response in pregnancy. In a study of women with GDM undergoing continuous glucose monitoring, hypoglycemia was observed even among women receiving no pharmacological therapy (5). Moreover, studies in women, rats, and dogs have shown that the counterregulatory response to hypoglycemia is diminished in pregnancy, and this may be because of pregnancy itself, rather the result of chronic insulin therapy (13,38,39).

Glucagon release is of special interest in regard to the impairment of the hypoglycemic counterregulatory response. In normal individuals, the initial response to a fall in blood glucose includes a suppression of insulin secretion and an increase in the release of glucagon and epinephrine (Epi; see Ref. 15). People receiving exogenous insulin are unable to reduce their circulating insulin concentrations and therefore rely more heavily on counterregulatory hormones to respond to hypoglycemia. Glucagon's ability to bring about a rapid increase in hepatic glucose production makes it the key counterregulatory hormone under normal circumstances (15).

Autonomic inputs (pancreatic sympathetic and parasympathetic neural stimuli and sympathoadrenal stimulation via Epi) are involved in the release of glucagon in response to hypoglycemia (26,27). In our previous study of hypoglycemia during late pregnancy in the dog, we observed that the Epi response was not different from that in nonpregnant controls, but the arterial norepinephrine (NE) concentrations were reduced significantly (13). Most of the circulating NE in hypoglycemic humans is reported to arise from adrenal secretion (18), but the dissociation between the Epi and NE data indicated that sympathetic stimulation might be reduced in pregnancy. Thus the question arose as to whether pancreatic sympathetic signaling is suppressed during pregnancy and this, in turn, reduces the glucagon response. Moreover, the decrement in intraislet insulin (with C-peptide serving as a marker of the change) is reported to be a key factor in determining glucagon output in hypoglycemia (33). To determine the importance of autonomic signaling and the decrement in intraislet insulin, we examined the response to hypoglycemia in pregnant and non-pregnant dogs in which catheters were chronically placed in the superior pancreaticoduodenal vein (SPDV) to allow assessment of pancreatic NE spillover, an index of pancreatic sympathetic activity (21). We simultaneously assessed pancreatic polypeptide (PP) release as an indicator of parasympathetic stimulation of the pancreas (21,40) and evaluated changes in plasma C-peptide as an index of endogenous insulin secretion. Late pregnancy was chosen as the time for study because GDM is primarily encountered in the second and third trimesters and because recent data indicate that hypoglycemia, particularly in the postprandial and nocturnal periods, occurs relatively frequently during the third trimester in women with diabetes, especially GDM (10,46,47). Altered counterregulatory responses are likely to contribute to the prevalence of hypoglycemia in late pregnancy.

RESEARCH DESIGN AND METHODS

Animals and Surgical Procedures

Experiments were performed on overnight-fasted (18 h), conscious female mongrel dogs, either nonpregnant (with basal progesterone and estradiol concentrations) or in the 8th wk of pregnancy (normal canine gestation ~9 wk). The animals were divided into the following four groups: pregnant euglycemic (Pe, $n = 5$), nonpregnant euglycemic (NPe, $n = 4$), pregnant hypoglycemic (Ph, $n = 8$), and nonpregnant hypoglycemic (NPh, $n = 6$). Diet and housing have been described previously (14). This protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee.

The animals underwent surgical insertion of sampling catheters in the femoral artery, left common hepatic vein, and hepatic portal vein ~17 days before experimentation. The SPDV was ligated adjacent to the duodenum at the caudal extreme of the duodenal lobe of the

pancreas, and a silicone rubber cannula (0.04 in. ID and 0.085 in. OD; Baxter Scientific, Deerfield, IL) was introduced and positioned so that the tip rested ~3 cm from the juncture of the SPDV and the portal vein. Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the portal vein and hepatic artery as previously described (4,14). A flow probe was also placed on the SPDV immediately caudal to the SPDV-portal juncture and ~1.5 cm rostral from the tip of the cannula (21). The external ends of the catheters and flow probes were placed in subcutaneous pockets. On the day of the experiment, the catheter ends were removed from the pockets under local anesthesia, and the catheters were emptied of their contents and flushed with saline. The animal was placed in a Pavlov harness, and intravenous access was obtained through the cephalic veins. Criteria for inclusion and experimental preparation were as previously described (10).

Experimental Design

Each experiment consisted of a 120-min period of dye and tracer equilibration (–150 to –30 min), a 30-min basal sampling period (–30 to 0 min), and a 180-min experimental period (0 to 180 min). A primed (38 μCi), continuous (0.35 $\mu\text{Ci}/\text{min}$) infusion of [$3\text{-}^3\text{H}$]glucose (New England Nuclear, Boston, MA) was delivered from –150 min until the conclusion of the experiment. Indocyanine green (ICG) dye was also infused throughout the experiment (0.08 mg/min; Sigma, St. Louis, MO). At 0 min, peripheral infusion of pork insulin was begun at $1.8 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in nonpregnant and $2.2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in pregnant animals, to account for the increased insulin clearance during pregnancy (9). Glucose was infused peripherally, and 0.2-ml aliquots of blood were sampled every 5 min to measure the plasma glucose. The animals in the euglycemic protocols were given enough exogenous glucose to clamp their plasma glucose at approximately basal concentrations (~6 mmol/l), whereas the animals in the hypoglycemic protocols were maintained at plasma glucose levels ~3 mmol/l. Blood samples were taken from the hepatic, portal, SPDV, and femoral artery catheters every 7.5–30 min as previously described (13). The SPDV catheters failed to function in one dog in the NPh group, two in the Ph group, and one in the NPe group; all data not dependent on the SPDV catheter are included for those animals. At the conclusion of the experimental period, the dogs were killed with pentobarbital sodium, and catheter tip placement was verified.

Analytical Procedures

Parameters measured include hematocrit, plasma glucose, insulin, glucagon, cortisol, Epi, NE, PP, C-peptide, nonesterified fatty acids, estradiol, and progesterone. Blood concentrations of lactate, glycerol, β -hydroxybutyrate, acetoacetate, alanine, glutamine, glutamate, serine, threonine, and glycine were also measured (data not shown). All assays were carried out as described previously (4,14).

Calculations

Total hepatic blood flow was assessed by hepatic extraction of ICG and ultrasonic flow probes (12). The ultrasonic probes functioned adequately in all dogs; therefore, all calculations rely on data from the probes. Calculations performed with the ICG data do not differ significantly, however. Net hepatic substrate balance was calculated as: $H \times F_{A+P} - [(A \times F_A) + (P \times F_P)]$ where H, A, and P indicate the hepatic vein, femoral artery, and portal vein substrate concentration, respectively, and F_A and F_P are the arterial and portal vein flows (blood or plasma, as appropriate).

Pancreatic glucagon output was calculated as:

$$\text{Output} = ([\text{glucagon}]_{\text{SPDV}} - [\text{glucagon}]_{\text{FA}}) \times (1 - \text{hematocrit}) \times (\text{blood flow})_{\text{SPDV}}$$

where [glucagon] is hormone concentration and FA is the femoral artery. Spillover of NE was calculated similarly but modified to account for NE extraction from arterial blood by the pancreas (1,21) in the following manner:

$$\text{Spillover} = ([\text{NE}]_{\text{SPDV}} - \text{arterial contribution to } [\text{NE}]_{\text{SPDV}}) \times (1 - \text{hematocrit}) \times (\text{blood flow})_{\text{SPDV}}$$

where arterial contribution to $[\text{NE}]_{\text{SPDV}} = [\text{NE}]_{\text{A}} \times (1 - \text{fractional extraction of NE})$ and fractional extraction of NE = fractional extraction of Epi = $(\text{Epi}_{\text{A}} - \text{Epi}_{\text{SPDV}}) / \text{Epi}_{\text{FA}}$. These calculations provide information about net NE spillover, not total release, because they do not correct for reuptake of endogenous NE. Nevertheless, net spillover serves as a useful index of pancreatic sympathetic stimulation (21).

The rates of gluconeogenic flux from circulating precursors and net hepatic glycogenolysis were estimated using the arteriovenous difference technique described previously (14). Glucose rates of appearance (R_{a}) and disappearance (R_{d}) were calculated using a two-compartment model (32) with dog parameters (20).

The area under the curve (AUC) was determined by means of the trapezoidal calculation. Statistical comparisons were made with ANOVA with post hoc analysis by Tukey's test, using SigmaStat (SYSTAT, Point Richmond, CA). Repeated-measures ANOVA was applied to time course data. Best subset regressions were calculated with SigmaStat, using adjusted R^2 as the criterion for fit.

RESULTS

Hormone Data

Arterial concentrations—Serum progesterone concentrations obtained on the day of study were 0.6 ± 0.1 and 8.8 ± 1.0 ng/ml in the nonpregnant and pregnant dogs, respectively ($P < 0.05$).

Basal insulin concentrations were greater in the nonpregnant dogs than in their pregnant counterparts (Table 1 and Fig. 1), as previously reported (13,14). Insulin concentrations during the hyperinsulinemic period were significantly higher in Pe than in NPe (Table 1; $P < 0.05$). However, there were no significant differences in either the circulating plasma concentrations of insulin (481 ± 45 and 437 ± 38 pmol/l) or the increment over basal concentrations (414 ± 45 vs. 412 ± 36 pmol/l) in NPh and Ph, respectively (Fig. 1).

Basal arterial glucagon concentrations were similar in all four groups (Table 1 and Fig. 2). Arterial plasma glucagon concentrations declined significantly in both euglycemic groups during insulin infusion, and the concentrations were not different between groups at any time (Table 1). Arterial plasma glucagon concentrations rose briskly in NPh as glucose concentrations fell (maximum increase 28 ng/l), with the response waning after the first 90 min (Fig. 2). In contrast, the glucagon concentrations in Ph did not rise significantly above basal. Overall, the AUC of the change in arterial glucagon from basal was approximately sevenfold larger in NPh than in Ph ($2,776 \pm 426$ vs. 382 ± 489 ng; $P < 0.05$).

Arterial cortisol concentrations were higher ($P < 0.05$) in Pe than NPe in the basal period (Table 1), but the concentrations rose similarly (50–65%) during hyperinsulinemic euglycemia and were not significantly different between groups after 30 min. The cortisol concentrations in the hypoglycemic groups did not differ in the basal period; both hypoglycemic groups showed an increase in cortisol during the first half of the experimental period and a waning of this response during the final 90 min, but the response was blunted significantly in Ph vs. NPh ($P < 0.05$ for repeated-measures ANOVA; Fig. 2).

Arterial Epi was not significantly different among the four groups during the basal period. The euglycemic groups responded to hyperinsulinemia with a modest 40–100% increase in Epi during the experimental period, with experimental period concentrations in Pe being higher than those in NPe (Table 1). Both hypoglycemic groups experienced a much more significant rise in Epi than the euglycemic groups, but the increase was approximately threefold greater in NPh than in Ph (AUC of change 151 ± 38 vs. 46 ± 10 μg ; $P < 0.05$; Fig. 2). The euglycemic groups demonstrated a rise in arterial NE during hyperinsulinemia, but the change did not differ between the groups (Table 1). In contrast, the rise in NE was threefold greater in NPh than in Ph (AUC of change from basal 32.4 ± 5.8 vs. 9.5 ± 3.9 $\mu\text{g}/3$ h, $P < 0.05$).

Pancreatic secretion and signaling—The basal C-peptide concentrations in Pe tended to be lower than in NPe ($P = 0.12$), and those in Ph were significantly lower than in NPh (Table 2). C-peptide concentrations declined significantly from basal during the experimental period in all groups except Pe, and the concentrations did not differ among the four groups during the last 2 h of insulin infusion (Table 2).

Basal arterial levels of PP, net PP release, and net pancreatic NE spillover did not differ significantly in the pregnant and nonpregnant groups during euglycemia, and glucagon secretion declined similarly in both groups (Fig. 3). PP concentrations in the hypoglycemic groups increased two- to fourfold, and the change from basal in PP secretion was significantly greater in NPh than in Ph (AUC of the response $2,087 \pm 876$ vs. 590 ± 483 ng in NPh vs. Ph, $P < 0.05$). Net pancreatic NE spillover rose significantly ($P < 0.05$) during the experimental period in the NPh group. The Ph group exhibited an initial decline in NE spillover but returned to basal rates within 90 min; the rates never increased above basal (Fig. 3; $P < 0.05$ for Ph vs. NPh). During hyperinsulinemia, net pancreatic glucagon secretion fell below basal in Ph but rose significantly in NPh (Fig. 3; $P < 0.05$).

Best subset analysis was carried out in NPh and Ph to determine which factors assessed were most closely linked to glucagon secretion (Table 3). In NPh, the factors found to contribute in the model of best fit included only the change from basal in the plasma glucose concentration, AUC of the Epi response, and net pancreatic NE spillover. In Ph, not only did the change in glucose and pancreatic NE spillover enter the model, but also PP release, the decrement in C-peptide, and the arterial plasma insulin concentration during hyperinsulinemia.

Glucose Metabolism

The basal plasma glucose concentrations were significantly lower in the pregnant than in the nonpregnant groups, but glucose concentrations in nonpregnant and pregnant did not differ after 165 min (Table 1 and Fig. 1). The hypoglycemic groups experienced a 45–50% reduction in arterial plasma glucose (Fig. 1). Net hepatic glucose output (NHGO) was evident in all groups during the basal period, but the rate was significantly greater in the pregnant vs. nonpregnant groups (12.4 ± 1.1 vs. 8.9 ± 1.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for the combined Pe/Ph vs. NPe/NPh groups, $P < 0.05$). During insulin infusion, the euglycemic groups shifted to a low rate of net hepatic glucose uptake, with the shift to net hepatic glucose uptake being more rapid in the nonpregnant dogs ($P < 0.05$). The NPh group increased its NHGO during hypoglycemia (12.5 ± 1.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during the last h), whereas the rate in the Ph group fell below basal (3.7 ± 0.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$ between groups increasing the need for glucose infusion (Fig. 4).

Basal endogenous glucose R_a was not different between the nonpregnant and pregnant groups (16.8 ± 1.4 vs. 16.4 ± 1.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in NPe/NPh vs. Pe/Ph, respectively). The rates in the euglycemic groups decreased to values no different from zero during hyperinsulinemia (data not shown). Basal R_d was not different between the groups, but R_d increased significantly more in NPe than in Pe (Table 1). After an initial fall, endogenous R_a in NPh returned to basal

rates by 90 min and remained not different from basal for the remainder of the study ($15.1 \pm 1.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during the last h). In contrast, endogenous R_a in Ph was below basal in the last hour ($7.1 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$ between groups). Glucose R_d was significantly greater in Ph than in NPh by the end of study (25.9 ± 2.5 and $19.6 \pm 2.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P < 0.05$).

Net hepatic glycogenolysis during the basal period proceeded at similar rates in the nonpregnant and pregnant groups (8.0 ± 2.2 and $8.1 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively). With the onset of hyperinsulinemia, glycogenolysis was suppressed rapidly in both NPe and Pe. The rate of net hepatic gluconeogenic flux did not change significantly from basal in either euglycemic group. In NPh, glycogenolysis was markedly stimulated, with the rate peaking at $11.6 \pm 5.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at 60 min and then declining to $3.2 \pm 1.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ by the end of study. In contrast, in Ph the rate had fallen to $2.7 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ by 60 min, and there was no net glycogenolysis by 120 min ($P < 0.05$ vs. NPh). In NPh and Ph, gluconeogenic flux in the basal period was 3.7 ± 0.7 and $4.3 \pm 0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively ($P = 0.7$). By the end of study, it had doubled in NPh but increased only 50% in Ph ($P < 0.05$).

DISCUSSION

The peak glucagon concentration during the first 90 min of hypoglycemia in the nonpregnant dogs was approximately twofold ($P < 0.05$) that of the pregnant animals. In fact, the glucagon concentrations did not rise significantly above basal in the Ph group. The excursions of Epi, NE, and cortisol were also significantly reduced in the Ph group but not as completely as that of glucagon. Consistent with the blunted hormonal response, glycogenolysis was reduced in the Ph group, and consequently NHGO and endogenous glucose R_a declined from basal. In contrast, in the NPh group, after a transient fall, NHGO increased above basal and endogenous R_a returned to basal rates. As a result, there was a difference in glucose production of $8\text{--}9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ between the pregnant and nonpregnant dogs during hypoglycemia.

Redundant mechanisms appear to control glucagon secretion in the normal state. The alpha cell is able to respond sensitively to the glycemic level in hypoglycemia, but this ability is ablated in the presence of even very mild hyperinsulinemia (29). The decrement in intraislet insulin that occurs during hypoglycemia is reported to be related to the magnitude of the glucagon response (3,23,33,36). However, the exact extent to which a decrement in intraislet insulin accounts for the glucagon response to hypoglycemia remains unclear, as previously noted (23). Additionally, because insulin-induced hypoglycemia has been used in some of the studies of the effect of intraislet insulin on the glucagon response (e.g., 23, 36), the intraislet insulin concentration (including both endogenous and exogenous insulin) is unknown. In any event, the interpretation of the in vivo intraislet insulin experiments has relied primarily on the correlation between the glucagon response and the change in C-peptide; therefore, it is impossible to determine cause and effect from the data. Because of the low basal C-peptide concentrations in the pregnant vs. nonpregnant dogs (and the resulting very small decrement in C-peptide in the pregnant dogs), we cannot rule out the possibility that the impairment in glucagon release was at least partially because of a lack of change in endogenous insulin secretion. With the exception of the earliest portion of the hyperinsulinemic clamp period, however, the C-peptide concentrations did not differ between pregnant and nonpregnant dogs during hypoglycemia (Table 2). Thus there is no evidence that any difference in the absolute concentration of intraislet insulin during hypoglycemia was responsible for the marked difference in glucagon release between the NPh and Ph groups. The low basal insulin concentrations in late-pregnant dogs may seem paradoxical, given that the degree of insulin resistance in this model [30–60% reduction in glucose disposal during a hyperinsulinemic euglycemic clamp (current data and Ref. 12)] is virtually identical to that of healthy women in

the third trimester of pregnancy (8). The low basal glucose and insulin concentrations in the pregnant dog are likely related to the fact that the fetoplacental unit in the dog is approximately two times as great (as a proportion of total maternal body weight) as in the human, since glucose uptake in the fetoplacental unit is insulin independent. Also, the greater fetal burden might hasten the “accelerated starvation” of pregnancy (34). Nevertheless, it is now clear from continuous glucose monitoring that low glucose concentrations are common even among healthy late-pregnant women [with one study of nondiabetic obese and lean women finding average nocturnal glucose concentrations of 59 and 72 mg/dl, respectively (45)].

In addition to direct effects of glucose and/or insulin on the alpha cell, autonomic input via the parasympathetic innervation of the pancreas and the sympathoadrenal system (Epi and NE release from the adrenals and sympathetic stimuli to the pancreas) has been shown to stimulate glucagon release. α - and β -Adrenergic blockade with and without concomitant atropine administration established that the glucagon response during insulin-induced hypoglycemia is mediated by both muscarinic and adrenergic mechanisms (25,28). In a series of experiments in the human, dog, rat, mouse, and rhesus monkey, Havel et al. (24–28) demonstrated that at least one pancreatic autonomic input is essential for an intact glucagon response during insulin-induced hypoglycemia. The importance of autonomic control of the counterregulatory response has also been demonstrated in the conscious dog without the use of pharmacological blockade. When the brain was maintained euglycemic (with carotid and vertebral arterial glucose infusion) during insulin-induced hypoglycemia, the glucagon response in the dogs was reduced 79% compared with the response when the brain was allowed to participate in the hypoglycemia (7).

In the present experiments, the pregnant dogs exhibited no significant reduction in indicators of pancreatic autonomic activation in the basal state or in response to hyperinsulinemic euglycemia. Epi, NE, and cortisol concentrations rose to a similar extent in pregnant and nonpregnant dogs during the euglycemic clamp. The rise in Epi during hyperinsulinemic euglycemia was consistent with some (2,30,42), but not the majority (e.g., see Refs. 16,17, and 35) of, previous reports. In contrast to the euglycemic studies, the reduction in pancreatic PP release, arterial catecholamine concentrations, and net pancreatic NE spillover in Ph vs. NPh indicates that all three autonomic inputs were suppressed during hyperinsulinemic hypoglycemia in pregnancy. Table 3 suggests that Epi and sympathetic signaling to the pancreas are the predominant autonomic stimuli for glucagon release in hypoglycemia in the nonpregnant state. However, with the blunting of these stimuli during pregnancy, other factors such as parasympathetic signaling and perhaps the decrement in insulin secretion assume increased importance. Even with reliance on these additional stimuli, however, it is clear that the glucagon response to hypoglycemia is suppressed in pregnancy.

The cortisol response to hypoglycemia, although significantly blunted during pregnancy, was less affected than the catecholamines and glucagon in the current investigation. These data are generally consistent with the observations that ACTH stimulation of cortisol release is intact in normal pregnant women (31) and dogs (37), and the increment in cortisol in response to stress (moderate exercise) is actually enhanced in pregnant women (6). We have previously found that the cortisol responses of pregnant and nonpregnant dogs are indistinguishable during more severe hypoglycemia (2.3 mM; see Ref. 13), and thus there may be a reduction in the glycemic threshold for cortisol release during pregnancy rather than an inability to mount a normal cortisol response. During the basal period, the plasma cortisol concentrations were significantly higher in the Pe group than in the other three groups. The elevation of cortisol in the Pe group was most likely related to the low basal glucose concentrations in that group (4.2 ± 0.3 compared with 5.8 ± 0.3 mM in the Ph group). The Pe and Ph dogs were fasted an identical number of hours, were healthy as determined by our specified criteria and by daily veterinary supervision, and were randomly assigned to euglycemia or hypoglycemia. Thus the

discrepancy in basal glycemia had no apparent basis in treatment effects. However, at the end of the studies, the mean number of fetuses in the Pe and Ph groups was found to be 8.6 ± 0.4 vs. 6.1 ± 0.8 , respectively ($P < 0.05$), suggesting that the fetal burden played an important role in determining basal maternal glycemia. We have no data regarding cortisol concentrations in the pregnant dogs other than on the day of experimentation, but we have not found basal cortisol concentrations to be elevated during pregnancy in our previous investigations (12,13). Both total and free cortisol are two- to fourfold higher in pregnant women compared with nonpregnant controls (31). Consistent elevations of cortisol have not been found during pregnancy in dogs (11,37), with the exception of a peak in secretion 8–24 h before parturition (11). The animals in this investigation were timed pregnant and were studied at least 1 wk before their due date to avoid the hormonal perturbations associated with parturition. Cortisol's actions reduce glucose uptake in the insulin-sensitive tissues (15) and thus could have made a contribution to the insulin resistance evident in the Pe group. Glucocorticoids are also reported to have a sympathoinhibitory role; 1 wk of treatment with moderate doses of a glucocorticoid reduced plasma concentrations of NE in humans and rats (22,44) and muscle sympathetic neural activity in humans (22). Neither the concentrations of NE nor pancreatic NE spillover were reduced in the Pe vs. NPe dogs, suggesting that the elevation of cortisol in the Pe group had little effect on these parameters. Additionally, because there were no differences in the basal cortisol concentrations in NPh and Ph, we have no evidence indicating that elevation of cortisol was responsible for the reduction of net pancreatic NE spillover or NE concentrations in Ph.

In conclusion, the counterregulatory response to insulin-induced hypoglycemia has long been known to be suppressed during pregnancy. This investigation is the first in which it has been possible in pregnant animals to examine comprehensively and simultaneously numerous stimuli involved in regulation of glucagon secretion. In the nonpregnant animals, where an array of redundant mechanisms was intact, best-subset regression suggested Epi played a key role in stimulating glucagon secretion. In moderate hypoglycemia in pregnancy, however, the Epi response was markedly suppressed, and the redundant sympathetic and parasympathetic stimuli, as well as the decrement in C-peptide concentrations, were also blunted. Whether the alpha cell's (and the brain's) capacity to detect changes in glucose or insulin during hypoglycemia is impaired during pregnancy remains unclear. Nevertheless, the data point to a generalized blunting of hypoglycemic sensing in the pregnant state. Future work is needed to examine the effects of each autonomic input and the decrement in endogenous insulin secretion on glucagon secretion in the pregnant dog.

Acknowledgements

GRANTS

These studies were supported by National Institutes of Health Grant DK-58134 and by Diabetes Research and Training Center Grant SP-60-AM-20593.

References

1. Ahren B, Dunning BE, Havel PJ, Veith RC, Taborsky GJ Jr. Extraction of epinephrine and norepinephrine by the dog pancreas in vivo. *Metab Clin Exp* 1988;37:68–73. [PubMed: 3336287]
2. Arauz-Pacheco C, Lender D, Snell PG, Huet B, Ramirez LC, Breen L, Mora P, Raskin P. Relationship between insulin sensitivity, hyperinsulinemia, and insulin-mediated sympathetic activation in normotensive and hypertensive subjects. *Am J Hypertens* 1996;9:1172–1178. [PubMed: 8972887]
3. Banarar S, McGregor VP, Cryer PE. Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. *Diabetes* 2002;51:958–965. [PubMed: 11916913]
4. Batista MR, Smith MS, Snead WL, Connolly CC, Lacy DB, Moore MC. Chronic estradiol and progesterone treatment in the conscious dog: effects on insulin sensitivity and the response to

- hypoglycemia. *Am J Physiol Regul Integr Comp Physiol* 2005;289:R1064–R1073. [PubMed: 15961530]
5. Ben-Haroush A, Yogev Y, Chen R, Rosenn B, Hod M, Langer O. The postprandial glucose profile in the diabetic pregnancy. *Am J Obstet Gynecol* 2004;191:576–581. [PubMed: 15343240]
 6. Bessinger RC, McMurray RG, Hackney AC. Substrate utilization and hormonal responses to moderate intensity exercise during pregnancy and after delivery. *Am J Obstet Gynecol* 2002;186:757–764. [PubMed: 11967503]
 7. 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* 1989;38:7–16. [PubMed: 2642436]
 8. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr* 2000;71:1256S–1261S. [PubMed: 10799399]
 9. Catalano PM, Drago NM, Amini SB. Longitudinal changes in pancreatic beta-cell function and metabolic clearance rate of insulin in pregnant women with normal and abnormal glucose tolerance. *Diabetes Care* 1998;21:403–408. [PubMed: 9540023]
 10. Chen R, Yogev Y, Ben-Haroush A, Jovanovic L, Hod M, Phillip M. Continuous glucose monitoring for the evaluation and improved control of gestational diabetes mellitus. *J Matern Fetal Neonatal Med* 2003;14:256–260. [PubMed: 14738172]
 11. Concannon PW, Butler WR, Hansel W, Knight PJ, Hamilton JM. Parturition and lactation in the bitch: serum progesterone, cortisol and prolactin. *Biol Reprod* 1978;19:1113–1118. [PubMed: 743546]
 12. Connolly CC, Aglione LN, Smith MS, Lacy DB, Moore MC. Insulin action during late pregnancy in the conscious dog. *Am J Physiol Endocrinol Metab* 2004;286:E909–E915. [PubMed: 14749203]
 13. Connolly CC, Aglione LN, Smith MS, Lacy DB, Moore MC. Pregnancy impairs the counterregulatory response to insulin-induced hypoglycemia in the dog. *Am J Physiol Endocrinol Metab* 2004;287:E480–E488. [PubMed: 15126242]
 14. Connolly CC, Holste LC, Aglione LN, Neal DW, Lacy DB, Smith MS, Diamond MP, Cherrington AD, Chiasson JL. Alterations in basal glucose metabolism during late pregnancy in the conscious dog. *Am J Physiol Endocrinol Metab* 2000;279:E1166–E1177. [PubMed: 11052973]
 15. Cryer PE, Davis SN, Shamon H. Hypoglycemia in diabetes. *Diabetes Care* 2003;26:1902–1912. [PubMed: 12766131]
 16. 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 Endocrinol Metab* 1992;263:E688–E695.
 17. Davis SN, Shavers C, Costa F. Differential gender responses to hypoglycemia are due to alterations in CNS drive and not glycemic thresholds. *Am J Physiol Endocrinol Metab* 2000;279:E1054–E1063. [PubMed: 11052960]
 18. DeRosa MA, Cryer PE. Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation. *Am J Physiol Endocrinol Metab* 2004;287:E32–E41. [PubMed: 14970007]
 19. Diamond MP, Reece EA, Caprio S, Jones TW, Amiel S, DeGennaro N, Laudano A, Addabbo M, Sherwin RS, Tamborlane WV. Impairment of counterregulatory hormone responses to hypoglycemia in pregnant women with insulin-dependent diabetes mellitus. *Am J Obstet Gynecol* 1992;166:70–77. [PubMed: 1733221]
 20. Dobbins RL, Davis SN, Neal DW, Cobelli C, Jaspan J, Cherrington AD. Compartmental modeling of glucagon kinetics in the conscious dog. *Metab Clin Exp* 1995;44:452–459. [PubMed: 7723667]
 21. 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 Endocrinol Metab* 1997;272:E746–E755.
 22. Golczynska A, Lenders JW, Goldstein DS. Glucocorticoid-induced sympathoinhibition in humans. *Clin Pharmacol Ther* 1995;58:90–98. [PubMed: 7628186]
 23. Gosmanov NR, Szoke E, Israelian Z, Smith T, Cryer PE, Gerich JE, Meyer C. Role of the decrement in intraislet insulin for the glucagon response to hypoglycemia in humans. *Diabetes Care* 2005;28:1124–1131. [PubMed: 15855577]

24. Havel PJ, Ahren B. Activation of autonomic nerves and the adrenal medulla contributes to increased glucagon secretion during moderate insulin-induced hypoglycemia in women. *Diabetes* 1997;46:801–807. [PubMed: 9133547]
25. Havel PJ, Akpan JO, Curry DL, Stern JS, Gingerich RL, Ahren B. Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. *Am J Physiol Regul Integr Comp Physiol* 1993;265:R246–R254.
26. Havel PJ, Mundinger TO, Taborsky GJ Jr. Pancreatic sympathetic nerves contribute to increased glucagon secretion during severe hypoglycemia in dogs. *Am J Physiol Endocrinol Metab* 1996;270:E20–E26.
27. Havel PJ, Parry SJ, Stern JS, Akpan JO, Gingerich RL, Taborsky GJ Jr, Curry DL. Redundant parasympathetic and sympathoadrenal mediation of increased glucagon secretion during insulin-induced hypoglycemia in conscious rats. *Metab Clin Exp* 1994;43:860–866. [PubMed: 8028509]
28. Havel PJ, Valverde C. Autonomic mediation of glucagon secretion during insulin-induced hypoglycemia in rhesus monkeys. *Diabetes* 1996;45:960–966. [PubMed: 8666149]
29. Igawa K, Mugavero M, Shiota M, Neal DW, Cherrington AD. Insulin sensitively controls the glucagon response to mild hypoglycemia in the dog. *Diabetes* 2002;51:3033–3042. [PubMed: 12351444]
30. Laine H, Nuutila P, Luotolahti M, Meyer C, Elomaa T, Koskinen P, Ronnema T, Knuuti J. Insulin-induced increment of coronary flow reserve is not abolished by dexamethasone in healthy young men. *J Clin Endocrinol Metab* 2000;85:1868–1873. [PubMed: 10843167]
31. Lindsay JR, Nieman LK. The hypothalamic-pituitary-adrenal axis in pregnancy: challenges in disease detection and treatment. *Endocr Rev* 2005;26:775–799. [PubMed: 15827110]
32. Mari A. On the calculation of glucose rate of disappearance in nonsteady state. *Am J Physiol Endocrinol Metab* 1994;266:E825–E826.
33. McCrimmon RJ, Evans ML, Jacob RJ, Fan X, Zhu Y, Shulman GI, Sherwin RS. AICAR and phlorizin reverse the hypoglycemia-specific defect in glucagon secretion in the diabetic BB rat. *Am J Physiol Endocrinol Metab* 2002;283:E1076–E1083. [PubMed: 12376337]
34. Metzger BE, Ravnkar V, Vileisis RA, Freinkel N. Accelerated starvation” and the skipped breakfast in late normal pregnancy. *Lancet* 1982;1:588–592. [PubMed: 6121184]
35. Paramore DS, Fanelli CG, Shah SD, Cryer PE. Forearm norepinephrine spillover during standing, hyperinsulinemia, and hypoglycemia. *Am J Physiol Endocrinol Metab* 1998;275:E872–E881.
36. Raju B, Cryer PE. Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. *Diabetes* 2005;54:757–764. [PubMed: 15734853]
37. Reimers TJ, Mummery LK, McCann JP, Cowan RG, Concannon PW. Effects of reproductive state on concentrations of thyroxine, 3,5,3'-triiodothyronine and cortisol in serum of dogs. *Biol Reprod* 1984;31:148–154. [PubMed: 6087944]
38. Rosenn BM, Miodovnik M, Khoury JC, Siddiqi TA. Counterregulatory hormonal responses to hypoglycemia during pregnancy. *Obstet Gynecol* 1996;87:568–574. [PubMed: 8602310]
39. Rossi G, Lapaczewski P, Diamond MP, Jacob RJ, Shulman GI, Sherwin RS. Inhibitory effect of pregnancy on counterregulatory hormone responses to hypoglycemia in awake rat. *Diabetes* 1993;42:1440–1445. [PubMed: 8375583]
40. Schwartz TW. Pancreatic polypeptide: a unique model for vagal control of endocrine systems. *J Auton Nerv Syst* 1983;9:99–111. [PubMed: 6663018]
41. Simonson DC, Tamborlane WV, DeFronzo RA, Sherwin RS. Intensive insulin therapy reduces counterregulatory hormone responses to hypoglycemia in patients with type I diabetes. *Ann Intern Med* 1985;103:184–190. [PubMed: 3893253]
42. Tack CJ, Lenders JW, Willemsen JJ, van Druten JA, Thien T, Lutterman JA, Smits P. Insulin stimulates epinephrine release under euglycemic conditions in humans. *Metabolism* 1998;47:243–249. [PubMed: 9500557]
43. ter Braak EW, Evers IM, Willem Erkelens D, Visser GH. Maternal hypoglycemia during pregnancy in type 1 diabetes: maternal and fetal consequences. *Diabetes Metab Res Rev* 2002;18:96–105. [PubMed: 11994900]

44. Wolfovitz E, Pacak K, Abassi Z, Kopin IJ, Goldstein DS. Effects of hypercortisolemia or hyperinsulinemia on neurochemical indices of catecholamine release and synthesis in conscious rats. *J Auton Nerv Syst* 1995;54:104–112. [PubMed: 7499722]
45. Yogeve Y, Ben-Haroush A, Chen R, Rosenn B, Hod M, Langer O. Diurnal glycemic profile in obese and normal weight nondiabetic pregnant women. *Am J Obstet Gynecol* 2004;191:949–953. [PubMed: 15467570]
46. Yogeve Y, Ben-Haroush A, Chen R, Rosenn B, Hod M, Langer O. Undiagnosed asymptomatic hypoglycemia: diet, insulin, and glyburide for gestational diabetic pregnancy. *Obstet Gynecol* 2004;104:88–93. [PubMed: 15229005]
47. Yogeve Y, Chen R, Ben-Haroush A, Phillip M, Jovanovic L, Hod M. Continuous glucose monitoring for the evaluation of gravid women with type 1 diabetes mellitus. *Obstet Gynecol* 2003;101:633–638. [PubMed: 12681863]

Fig. 1.

Arterial plasma insulin and glucose concentrations and net hepatic glucose balance in nonpregnant and pregnant dogs studied in the basal state and during a hyperinsulinemic hypoglycemic clamp. NPh, nonpregnant hypoglycemic; Ph, pregnant hypoglycemic. $*P < 0.05$ between groups (significant main effect with ANOVA; asterisks indicate time points identified with post hoc tests).

Fig. 2.
Arterial plasma concentrations of glucagon, epinephrine, norepinephrine, and cortisol.
ANOVA indicated that all parameters were significantly different between groups. * $P < 0.05$
for post hoc analysis between groups.

Fig. 3.

Arterial plasma pancreatic polypeptide (PP), change from basal in net PP secretion, change from basal in net pancreatic norepinephrine (NE) spillover, and net glucagon secretion in hyperinsulinemic euglycemic dogs (*left*) and hypoglycemic dogs (*right*). Basal rates of PP secretion were 0.2 ± 0.2 , 5.5 ± 2.0 , 0.1 ± 0.1 , and 1.1 ± 0.6 ng/min in nonpregnant euglycemic (NPe), pregnant euglycemic (Pe), NPh, and Ph, respectively [not significant (NS) between NPe and Pe and between NPh and Ph]. Basal rates of NE spillover were 1.9 ± 1.0 , 8.9 ± 2.6 , 3.5 ± 1.4 , and 5.5 ± 1.3 ng/min in NPe, Pe, NPh, and Ph, respectively ($P < 0.05$ between NPe and Pe, NS between NPh and Ph). * $P < 0.05$ vs. the corresponding pregnant group (significant main effect with ANOVA, asterisks indicate time points identified with post hoc tests).

Fig. 4.

Glucose infusion rates, endogenous glucose rate of appearance (R_a), and glucose rate of disappearance (R_d) in the hypoglycemic groups. * $P < 0.05$ between groups (significant main effect with ANOVA; asterisks indicate time points identified with post hoc tests).

Table 1

Plasma hormone concentrations (arterial unless noted otherwise), glucose concentrations, and net hepatic glucose output in NPe and Pe dogs during the basal period and during a hyperinsulinemic clamp

Parameter and Group	Basal Period	Hyperinsulinemic Period, min						
		15	30	60	90	120	150	180
Insulin, pmol/l								
NPe	32±5	380±72	356±32	351±45	292±52	353±45	343±16	356±43
Pe	4±2*	374±52	447±24	475±18*	472±24*	438±19	460±22*	493±34*
Glucagon, ng/l								
NPe	42±7	37±8	29±4	29±6	31±4	28±4	21±5	28±6
Pe	73±24	47±15	39±13	35±11	26±7	25±8	22±9	27±6
Epinephrine, ng/l								
NPe	60±18	118±40	149±69	158±36	81±28	124±49	120±49	97±42
Pe	171±64	180±65	181±84	235±53	152±41	164±64	224±56	214±33*
Norepinephrine, ng/l								
NPe	122±17	144±24	169±36	188±26	135±11	184±29	187±29	160±10
Pe	212±48	248±77	237±57	304±92	252±66	212±55	302±85	268±66
Cortisol, nmol/l								
NPe	73±11	103±31	111±32	108±24	89±15	79±10	122±19	111±13
Pe	178±22*	254±67	211±34*	171±35	120±29	143±42	125±17	132±12
Arterial plasma glucose, mmol/l								
NPe	6.1±0.3	5.5±0.3	5.8±0.2	5.4±0.4	5.4±0.4	5.9±0.4	5.3±0.4	5.8±0.5
Pe	4.2±0.3*	4.9±0.4	5.3±0.4	5.4±0.4	5.0±0.2	5.1±0.2	4.9±0.2	5.0±0.3
Net hepatic glucose balance, μmol·kg ⁻¹ ·min ⁻¹								
NPe	8.0±2.0	0.4±4.1	-0.7±3.8	-3.6±3.0	-1.9±2.8	0.6±2.9	-2.8±2.0	-4.5±0.4
Pe	12.1±2.1	6.5±0.5	5.6±0.5	3.7±2.0*	3.8±0.9*	1.5±1.3*	0.8±1.6*	-1.7±1.5
Glucose infusion rate, μmol·kg ⁻¹ ·min ⁻¹								
NPe	0	22.0±4.3	47.4±13.5	59.7±9.6	74.3±7.6	82.7±14.9	88.1±24.0	93.2±19.9
Pe	0	21.7±3.5	26.3±2.0	23.7±6.2*	25.5±4.6*	33.6±4.0*	35.1±4.6*	36.3±3.7*
Glucose R _d , μmol·kg ⁻¹ ·min ⁻¹								
NPe	17.0±2.0	34.7±4.9	46.9±5.9	70.5±7.0*	79.6±6.0*	79.0±6.8	89.3±10.0	93.2±12.9
Pe	15.4±1.7	21.6±1.1	24.7±1.7*	31.1±3.7*	34.5±4.0*	36.9±3.7*	38.8±3.8*	40.7±3.1*

Date are means ± SE. Basal period values are means of samples taken between -30 and 0 min. Negative values indicate net hepatic uptake. NPe, nonpregnant euglycemic; Pe, pregnant euglycemic; R_d , rate of disappearance.

* $P < 0.05$ between groups (in all cases where there is an asterisk, there is a main effect by ANOVA; the asterisk marks only the time points indicated by post hoc analysis).

Arterial plasma C-peptide concentrations in pregnant and nonpregnant dogs studied during hyperinsulinemic euglycemia and hypoglycemia

Table 2

Group	Basal Period	Experimental Period, min		
		30	60	120
NPe	0.41±0.10	0.24±0.07	0.19±0.04	0.13±0.04
Pe	0.23±0.04	0.25±0.05	0.23±0.03	0.23±0.06
NPh	0.49±0.05	0.35±0.05	0.21±0.06	0.21±0.06
Ph	0.32±0.02	0.21±0.02	0.21±0.03	0.18±0.03

Data are means ± SE. Units are ng/ml. NPh, nonpregnant hypoglycemic; Ph, pregnant hypoglycemic. Basal period values are means of samples taken between -30 and 0 min.

* $P < 0.05$ between hypoglycemic groups.

Table 3

Best-subset regression analysis of glucagon secretion with selected metabolic variables in hypoglycemic dogs

Variables	Pregnant	Nonpregnant
Δ Glucose	0.040	0.309
Epinephrine	NS	0.043
PP	0.026	NS
Δ C-peptide	0.078	NS
Insulin	0.043	NS
NE spillover	0.021	0.065
Adjusted R^2	0.995	0.825

Data are P values, except for the row giving adjusted R^2 . Variables examined were the change in arterial plasma glucose (mean of the last h, when steady-state conditions existed, minus basal values), area under the curve (AUC) of the arterial plasma epinephrine response, AUC of pancreatic polypeptide (PP) release, change in arterial plasma C-peptide (calculated as described for glucose), the mean arterial plasma insulin concentrations during the insulin infusion, and AUC of net pancreatic norepinephrine (NE) spillover. AUCs are calculated as the change from basal values or rates. NS, using adjusted R^2 as the best criterion, parameter did not enter into the regression.