Glucagon secretion and autonomic signaling during hypoglycemia in late pregnancy

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Canniff, Kathryn M., Marta S. Smith, D. Brooks Lacy, Phillip E. Williams, and Mary Courtney Moore. Glucagon secretion and autonomic signaling during hypoglycemia in late pregnancy. Am J Physiol Regul Integr Comp Physiol 291: R788–R795, 2006. First published March 23, 2006; doi:10.1152/ajpregu.00125.2006.—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 [nonpregnant 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 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 nonpregnant 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 and sympathoadrenal stimuli and altered sensing of circulating and/or intraislet insulin.

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 nonpregnant 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

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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 approximately caudal to the SPDV-portal juncture and 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 μCi/min) infusion of [3H]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 mU·kg⁻¹·min⁻¹ in nonpregnant and 2.2 mU·kg⁻¹·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 increment over 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, acetocetate, 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 × FA × p + [(A × FA) + (P × FP)]. Where H, A, and P indicate the hepatic vein, femoral artery, and portal vein substrate concentration, respectively, and FA and FP 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 = (Epi_{\text{A}} - Epi_{\text{SPDV}})/Epi_{\text{A}}. 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 (Rg) and disappearance (Ra) 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 R² 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 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).
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

<table>
<thead>
<tr>
<th>Parameter and Group</th>
<th>Basal Period</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
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<td>Insulin, pmol/l</td>
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<tr>
<td>NPe</td>
<td>32 ± 5</td>
<td>380 ± 72</td>
<td>356 ± 32</td>
<td>351 ± 45</td>
<td>292 ± 52</td>
<td>353 ± 45</td>
<td>343 ± 16</td>
<td>356 ± 43</td>
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<td>Pe</td>
<td>4 ± 2*</td>
<td>374 ± 52</td>
<td>447 ± 24</td>
<td>475 ± 18*</td>
<td>472 ± 24*</td>
<td>438 ± 19</td>
<td>460 ± 22*</td>
<td>493 ± 34*</td>
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<td>Glucagon, ng/l</td>
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<tr>
<td>NPe</td>
<td>42 ± 7</td>
<td>37 ± 8</td>
<td>29 ± 4</td>
<td>29 ± 6</td>
<td>31 ± 4</td>
<td>28 ± 4</td>
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<td>Pe</td>
<td>73 ± 24</td>
<td>47 ± 15</td>
<td>39 ± 13</td>
<td>35 ± 11</td>
<td>26 ± 7</td>
<td>25 ± 8</td>
<td>22 ± 9</td>
<td>27 ± 6</td>
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<td>Epinephrine, ng/l</td>
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<tr>
<td>NPe</td>
<td>60 ± 18</td>
<td>118 ± 40</td>
<td>149 ± 69</td>
<td>158 ± 36</td>
<td>81 ± 28</td>
<td>124 ± 49</td>
<td>120 ± 49</td>
<td>97 ± 42</td>
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<tr>
<td>Pe</td>
<td>171 ± 64</td>
<td>180 ± 65</td>
<td>181 ± 84</td>
<td>235 ± 53</td>
<td>152 ± 41</td>
<td>164 ± 64</td>
<td>224 ± 56</td>
<td>214 ± 33*</td>
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<td>Norepinephrine, ng/l</td>
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<tr>
<td>NPe</td>
<td>122 ± 17</td>
<td>144 ± 24</td>
<td>169 ± 36</td>
<td>188 ± 26</td>
<td>135 ± 11</td>
<td>184 ± 29</td>
<td>187 ± 29</td>
<td>160 ± 10</td>
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<td>Pe</td>
<td>212 ± 48</td>
<td>248 ± 77</td>
<td>237 ± 57</td>
<td>304 ± 92</td>
<td>252 ± 66</td>
<td>212 ± 55</td>
<td>302 ± 85</td>
<td>268 ± 66</td>
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<td>Cortisol, nmol/l</td>
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<tr>
<td>NPe</td>
<td>73 ± 11</td>
<td>103 ± 31</td>
<td>111 ± 32</td>
<td>108 ± 24</td>
<td>89 ± 15</td>
<td>79 ± 10</td>
<td>122 ± 19</td>
<td>111 ± 13</td>
</tr>
<tr>
<td>Pe</td>
<td>178 ± 22*</td>
<td>254 ± 67</td>
<td>211 ± 34*</td>
<td>171 ± 35</td>
<td>120 ± 29</td>
<td>143 ± 42</td>
<td>125 ± 17</td>
<td>132 ± 12</td>
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<tr>
<td>Arterial plasma glucose, mmol/l</td>
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<tr>
<td>NPe</td>
<td>6.1 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>5.8 ± 0.2</td>
<td>5.4 ± 0.4</td>
<td>5.4 ± 0.4</td>
<td>5.9 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>5.8 ± 0.5</td>
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<tr>
<td>Pe</td>
<td>4.2 ± 0.3*</td>
<td>4.9 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>5.4 ± 0.4</td>
<td>5.0 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>5.0 ± 0.3</td>
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<td>Net hepatic glucose balance, μmol·kg⁻¹·min⁻¹</td>
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<td>NPe</td>
<td>8.0 ± 2.0</td>
<td>0.4 ± 1.1</td>
<td>-0.7 ± 3.8</td>
<td>-3.6 ± 3.0</td>
<td>-1.9 ± 2.8</td>
<td>0.6 ± 2.9</td>
<td>-2.8 ± 2.0</td>
<td>-4.5 ± 0.4</td>
</tr>
<tr>
<td>Pe</td>
<td>12.1 ± 2.1</td>
<td>6.5 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>3.7 ± 2.0*</td>
<td>3.8 ± 0.9*</td>
<td>1.5 ± 1.3*</td>
<td>0.8 ± 1.6*</td>
<td>-1.7 ± 1.5</td>
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<tr>
<td>Glucose infusion rate, μmol·kg⁻¹·min⁻¹</td>
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<tr>
<td>NPe</td>
<td>0</td>
<td>22.0 ± 4.3</td>
<td>47.4 ± 13.5</td>
<td>59.7 ± 9.6</td>
<td>74.3 ± 7.6</td>
<td>82.7 ± 14.9</td>
<td>88.1 ± 24.0</td>
<td>93.2 ± 19.9</td>
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<tr>
<td>Pe</td>
<td>0</td>
<td>21.7 ± 3.5</td>
<td>26.3 ± 2.0</td>
<td>23.7 ± 6.2*</td>
<td>25.5 ± 4.6*</td>
<td>33.6 ± 4.0*</td>
<td>35.1 ± 2.4*</td>
<td>36.3 ± 3.7*</td>
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<td>Glucose Rₕ, μmol·kg⁻¹·min⁻¹</td>
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<tr>
<td>NPe</td>
<td>17.0 ± 2.0</td>
<td>34.7 ± 4.9</td>
<td>46.9 ± 5.9</td>
<td>70.5 ± 7.0</td>
<td>79.0 ± 6.0</td>
<td>79.0 ± 6.8</td>
<td>89.3 ± 10.0</td>
<td>93.2 ± 12.9</td>
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<tr>
<td>Pe</td>
<td>15.4 ± 1.7</td>
<td>21.6 ± 1.1</td>
<td>24.7 ± 1.7*</td>
<td>31.1 ± 3.7</td>
<td>34.5 ± 4.0*</td>
<td>36.9 ± 3.7*</td>
<td>38.8 ± 3.8*</td>
<td>40.7 ± 3.1*</td>
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</table>

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; Rₕ, 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).

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 ± 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 μg/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 ± 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 dogs 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 µmol·kg⁻¹·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 µmol·kg⁻¹·min⁻¹ during the last h), whereas the rate in the Ph group fell below basal (3.7 ± 0.5 µmol·kg⁻¹·min⁻¹; *P < 0.05).

### Table 2. Arterial plasma C-peptide concentrations in pregnant and nonpregnant dogs studied during hyperinsulinemic euglycemia and hypoglycemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
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<th>60</th>
<th>120</th>
<th>180</th>
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<tbody>
<tr>
<td>NPe</td>
<td>0.41±0.10</td>
<td>0.24±0.07</td>
<td>0.19±0.04</td>
<td>0.13±0.04</td>
<td>0.19±0.08</td>
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<td>Pe</td>
<td>0.23±0.04</td>
<td>0.25±0.05</td>
<td>0.23±0.03</td>
<td>0.23±0.06</td>
<td>0.25±0.08</td>
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<tr>
<td>NPh</td>
<td>0.49±0.05</td>
<td>0.35±0.05</td>
<td>0.21±0.06</td>
<td>0.21±0.06</td>
<td>0.22±0.05</td>
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<tr>
<td>Ph</td>
<td>0.32±0.02*</td>
<td>0.21±0.02*</td>
<td>0.21±0.03</td>
<td>0.18±0.03</td>
<td>0.21±0.03</td>
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</tbody>
</table>

*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.*
Basal endogenous glucose \( R_a \) was not different between the nonpregnant and pregnant groups (16.8 ± 1.4 vs. 16.4 ± 1.5 \( \mu mol \cdot kg^{-1} \cdot min^{-1} \) 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 ± 1.6 \( \mu mol \cdot kg^{-1} \cdot min^{-1} \) during the last h). In contrast, endogenous \( R_a \) in Ph was below basal in the last hour (7.1 ± 1.1 \( \mu mol \cdot kg^{-1} \cdot min^{-1} \), \( P < 0.05 \) between groups). Glucose \( R_a \) was significantly greater in Ph than in NPh by the end of study (25.9 ± 2.5 and 19.6 ± 2.9 \( \mu mol \cdot kg^{-1} \cdot min^{-1} \), 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 ± 1.1 \( \mu mol \cdot kg^{-1} \cdot min^{-1} \), respectively).

### Table 3. Best-subset regression analysis of glucagon secretion with selected metabolic variables in hypoglycemic dogs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pregnant</th>
<th>Nonpregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔGlucose</td>
<td>0.040</td>
<td>0.309</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>NS</td>
<td>0.043</td>
</tr>
<tr>
<td>PP</td>
<td>0.026</td>
<td>NS</td>
</tr>
<tr>
<td>ΔC-peptide</td>
<td>0.078</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.043</td>
<td>NS</td>
</tr>
<tr>
<td>NE spillover</td>
<td>0.021</td>
<td>0.065</td>
</tr>
<tr>
<td>Adjusted ( R^2 )</td>
<td>0.995</td>
<td>0.825</td>
</tr>
</tbody>
</table>

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.
as that of glucagon. Consistent with the blunted hormonal response, glycogenolysis was reduced in the Ph group, and consequently NHGO and endogenous glucose Ra declined from basal. In contrast, in the NPh group, after a transient fall, NHGO increased above basal and endogenous Ra returned to basal rates. As a result, there was a difference in glucose production of 8–9 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) 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 intrainstlet 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 intrainstlet 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 intrainstlet insulin on the glucagon response (e.g., 23, 36), the intrainstlet insulin concentration (including both endogenous and exogenous insulin) is unknown. In any event, the interpretation of the in vivo intrainstlet 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 intrainstlet 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. \( \alpha \)- and \( \beta \)-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.

GRANTS

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REFERENCES


