Insulin resistance and impaired baroreflex gain during pregnancy

Daisy L. Daubert,1* Mee-Young Chung,1,2* and Virginia L. Brooks1

1Oregon Health & Science University, Department of Physiology and Pharmacology, Portland, Oregon; and 2The Catholic University of Korea, Department of Anesthesiology and Pain Medicine, Seoul, Korea

Submitted 28 August 2006; accepted in final form 10 February 2007

Daubert DL, Chung M-Y, Brooks VL. Insulin resistance and impaired baroreflex gain during pregnancy. Am J Physiol Regul Integr Comp Physiol 292: 2188–2195, 2007. First published February 15, 2007; doi:10.1152/ajpregu.00614.2006.—Pregnancy decreases baroreflex gain, but the underlying mechanism is unclear. Insulin resistance, which has been associated with reduced transport of insulin into the brain, is a consistent feature of many conditions exhibiting impaired baroreflex gain, including pregnancy. Therefore, using conscious pregnant and nonpregnant rabbits, we tested the novel hypothesis that the pregnancy-induced impairment in baroreflex gain is due to insulin resistance and reduced brain insulin. Baroreflex gain was determined by quantifying changes in heart rate in response to stepwise steady-state changes in arterial pressure, secondary to infusion of nitroprusside and phenylephrine. We found that insulin sensitivity and in baroreflex gain exhibited similar time courses throughout pregnancy, reaching significantly lower levels at 3 wk of gestation and remaining reduced at 4 wk (term is 31 days). Treatment of rabbits with the insulin-sensitizing drug rosiglitazone during pregnancy almost completely normalized baroreflex gain. Finally, pregnancy significantly lowered cerebrospinal fluid insulin concentrations. These data identify insulin resistance as a mechanism underlying pregnancy-induced baroreflex impairment and suggest, for the first time in any condition, that decreased brain insulin concentrations may be the link between reductions in peripheral insulin sensitivity and baroreflex gain.

Insulin resistance and impaired baroreflex gain during pregnancy.

The maternal cardiovascular system undergoes considerable transformation during pregnancy (for reviews, see Refs. 39, 44). Many of these adaptations, such as the increases in blood volume and cardiac output, are clearly beneficial for the fetus. However, the progressive baroreflex impairment that accompanies pregnancy (52) poses significant risk for the mother. Decreased baroreflex gain may, by reducing the ability to maintain blood pressure (BP) in the face of hypotensive challenges, underlie the increased incidence of orthostatic hypotension in pregnant women (18), as well as the decreased tolerance of pregnant animals to hemorrhage (5). Importantly, a major cause of human maternal peripartum mortality is uncontrollable hemorrhage (7). Despite this clear clinical significance, the mechanism for the suppression of baroreflex gain remains unclear.

Multiple conditions other than pregnancy are associated with decreases in baroreflex gain, such as obesity (26), hypertension (25), congestive heart failure (8), type 2 diabetes mellitus (50), and aging (38). Interestingly, a hallmark of each of these pathophysiological states, as well as pregnancy, is a reduction in insulin sensitivity (10, 11, 20, 22, 55). This association suggests a mechanistic link between insulin resistance and suppressed baroreflex gain. Indirect evidence to support this hypothesis is that weight loss in obese humans improves baroreflex control of muscle sympathetic nerve activity as it increases insulin sensitivity (27). However, whether insulin resistance contributes to baroreflex impairment in pregnancy is unknown. Moreover, the mechanism by which reductions in peripheral insulin sensitivity could decrease baroreflex gain has not been identified in any condition.

Previous research indicates that the brain is the site at which pregnancy depresses baroreflex function (3, 37). Insulin receptors are present in numerous but discrete sites in the brain, many of which interact with brain stem baroreflex pathways (56, 61). Moreover, insulin gains access from the systemic circulation to the brain via transendothelial transport across the blood-brain barrier (1, 23, 62), and in the case of two insulin resistant states, obesity (20) and Alzheimer disease (36), insulin transport is reduced, resulting in decreased brain insulin concentrations (14, 33, 57). Because insulin acts in the brain to enhance baroreflex gain (40, 47), decreased brain insulin concentrations may underlie the impaired baroreflex gain. However, whether a decline in brain insulin levels is a critical determinant of the decreased baroreflex gain during pregnancy, or any condition, is unknown.

Therefore, we hypothesized that, during pregnancy, decreased insulin sensitivity and, subsequently reduced brain insulin concentrations, drive the impaired baroreflex gain. To test this hypothesis, we determined 1) whether the suppression of baroreflex gain during pregnancy correlates with the reduction in insulin sensitivity, 2) whether the time course for the decline in baroreflex gain matches the time course for development of insulin resistance throughout pregnancy, 3) whether normalization of insulin sensitivity during pregnancy by administration of the insulin sensitizing drug, rosiglitazone, restores baroreflex gain, and 4) whether pregnancy is associated with reduced cerebrospinal fluid insulin concentrations.

METHODS

Female New Zealand White rabbits (Western Oregon Rabbit, Philomath, OR) weighing 3.8 ± 0.1 kg (nonpregnant, n = 28) were used for these experiments. The rabbits were received when they were 14 wk old and were allowed a minimum of 5 days to acclimate to the new environment before any surgery was performed. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Surgical Preparation

Surgery was performed to implant nonoccluding abdominal aortic and vena cava catheters as previously described (28) for the measurement of mean arterial BP and heart rate (HR) and the infusion of drugs, respectively. Briefly, the animals were initially anesthetized with a cocktail containing ketamine (58.8 mg/kg), xylazine (5.9 mg/kg), and acepromazine (1.2 mg/kg) administered subcutaneously. The rabbits were then intubated and ventilated with 100% oxygen throughout surgery. A surgical plane of anesthesia was maintained by administration of ketamine intravenously as needed. A midline abdominal incision was made, and polyethylene catheters with silastic tips were implanted nonocclusively in the abdominal aorta (one) and vena cava (two). The catheters were tunneled subcutaneously from the abdominal cavity and exited at the nape of the neck. The catheters were flushed immediately after surgery and then three times weekly with sterile 0.9% saline and filled with heparin (1,000 U/ml) to maintain patency. Rabbits were allowed at least 2 wk to recover from this surgery before any experiments were performed. During this time, the rabbits were conditioned to the black Plexiglas box used for restraint during experiments.

To determine whether insulin levels in the cerebrospinal fluid are decreased during pregnancy, we performed surgery to implant canulas into the cisterna magna for the collection of cerebrospinal fluid using a technique modified from Vistelle et al. (60). Animals were sedated with 150 mg ketamine, further anesthetized with isoflurane (5% in oxygen), and intubated. Rabbits were then maintained on 2% isoflurane in oxygen throughout the surgery. With the rabbit's head immobilized in a stereotaxic frame, a midsagittal incision was made. The dorsal surface of the parietal, intraparietal, and occipital bones was cleared of muscle and periosteum. Using dental burrs, we made a hole in the occipital bone. A small incision was made in the dura mater, and polytetrafluoroethylene Teflon tubing (cannula, 0.022 in. inner diameter, 0.010 in. wall thickness; Zeus Industrial Products, Orangeburg, SC) was inserted a distance of 14 mm from the surface of the dura mater so that the distal end lay in the cisterna magna. A bluntly cut 23-gauge needle was glued into the end of the cannula, and a 2-cm-length, 1-cm-diameter open plastic cylinder (made from the distal end of a 3-ml syringe) was then placed around the cannula with the needle hub to help protect and secure the cannula. A screw was placed in the skull, and dental cement was used to fill the cylinder, close the hole in the skull, and secure the cannula to the skull. The muscle and skin were then sutured closed around the plastic cylinder. A stopcock plug screwed into the needle hub capped the cannula. To maintain patency, 500–1,000 μl of cerebrospinal fluid were collected daily from the canulas, which were then filled with the antibiotic enrofloxacin (7.6 mg/ml). No samples were used for assay until at least 1 wk after surgery and until at least 2 days after the cerebrospinal fluid was clear (not clouded with blood). Microscopic analysis of cerebrospinal fluid from three rabbits at this time demonstrated zero red blood cells per 10 μl of cerebrospinal fluid.

For the vascular catheter surgery, rabbits were given an intramuscular injection of enrofloxacin (22.7 mg) just before the surgery and an intravenous injection of this antibiotic for the 4 days after surgery (22.7 mg/day). For the cisterna magna cannula surgery, the rabbits were given subcutaneous injections of chloramphenicol (100 mg) just before the surgery and the following day to prevent infection. All animals also received buprenorphine hydrochloride (0.09 mg sc) 2–3 h after each surgery and again the following day to minimize pain.

Measurement of Baroreflex Function

On the experimental day, the rabbits were placed in the experimental box and allowed ~30 min to acclimate. Mean arterial BP and HR were measured continuously via the aortic catheter using a Statham pressure transducer, a Grass tachometer, and a Grass polygraph. To determine the baroreflex relationship between BP and HR, arterial pressure was first lowered by intravenous infusion of increasing doses of nitroprusside (1.5, 3, 6, 12, 24, 48, 63 μg·kg⁻¹·min⁻¹ for non-pregnant; 0.8, 1.5, 3, 6, 12, 24, 48, 63 μg·kg⁻¹·min⁻¹ for pregnant; 37.5 μg·kg⁻¹·min⁻¹ in 5% dextrose in water vehicle). After a 30–60-min rest period, BP was then raised by intravenous infusion of increasing doses of phenylephrine (0.5, 1, 2, 4 μg·kg⁻¹·min⁻¹; 12.5 μg·kg⁻¹·min⁻¹ in 5% dextrose in water vehicle). Doses of nitroprusside and phenylephrine were increased by increasing the flow rate. Each dose was infused until BP and HR stabilized. ~2–8 min; usually ~5–10 ml of fluid were infused for each nitroprusside or phenylephrine segment of the reflex curve.

Measurement of Insulin Sensitivity

On the experimental day, after a 12- to 18-h fast, the rabbits were placed in the experimental box and allowed ~30 min to settle. Insulin sensitivity was then determined by the hyperinsulinemic-euglycemic method, as described by DeFronzo et al. (16), usually the day before or the day after the measurement of baroreflex function. Heparin (1,000 U/ml) was infused into the arterial catheter at a rate of 1 ml/h to maintain patency. Five blood samples were collected from the arterial catheter over at least 30 min. Blood was either spun down and the plasma analyzed on a Beckman Glucose Analyzer II, or the blood was analyzed on a Freestyle Flash handheld blood glucose monitor. Insulin sensitivity was the same (within 1%) in nine animals whether the Beckman or the Freestyle was used. The highest and the lowest values for plasma or blood glucose were dropped, and the range of euglycemia was determined from the remaining three values. A priming dose of insulin in normal saline (21 μU/kg) was then infused into a venous catheter over 10 min followed by a constant infusion of insulin (1.2 μU·kg⁻¹·min⁻¹ with a flow rate of 21 μl/min). Dextrose (95 μg·kg⁻¹·min⁻¹ in water) was infused in the other venous catheter starting at a rate of 2 mg·kg⁻¹·min⁻¹ 4 min after the start of the priming insulin dose. After the initial insulin dose, the dextrose infusion was increased to 3 mg·kg⁻¹·min⁻¹. Plasma or blood glucose values were then measured every 10–15 min, and the dextrose infusion rate was altered until plasma and/or blood glucose values were back in the euglycemic range. Two to three samples were collected over the next 20–45 min to ensure that the euglycemia was at steady state. Steady-state euglycemia was usually reached within 3–4 h of the start of the insulin infusion. The dextrose infusion rate required to reestablish euglycemia was taken as an index of insulin sensitivity; lower dextrose infusion rates indicated decreased insulin sensitivity.

Measurements of Insulin Concentrations

After an overnight (12–18 h) fast, cerebrospinal fluid (1 ml/day) was collected from the cisterna magna cannula; heparinized blood (3–6 ml/day) was collected from an ear artery. The blood was then spun down, and the plasma was collected, frozen, and stored along with the cerebrospinal fluid at −20°C until assayed. Because of the low concentration of insulin in the cerebrospinal fluid (sometimes below 0.02 ng/ml), these samples were concentrated before assay. To do this, cerebrospinal fluid collected from the same rabbit over 4–6 (nonpregnant) or 2 (pregnant) consecutive days was pooled. This pooled cerebrospinal fluid was then concentrated with the use of Centricron Ultrapel YM-3 centrifugal filter devices (Millipore, Bedford, MA). After concentration of the samples was completed, the samples were again frozen at −20°C and stored until shipment to the Oregon National Primate Research Center, where the assay was performed.

Insulin levels were quantified with the Linco Research sensitive rat radioimmunoassay kit, which uses a guinea pig anti-rat insulin antibody and can specifically detect rat, pig, sheep, hamster, and mouse insulin with 100% recovery. Validation of the assay for rabbit included tests for parallelism; halving the volume of cerebrospinal fluid reduced the measured insulin level by 53 ± 3% (n = 15); halving the
volume of plasma reduced the insulin level by 46 ± 2% (n = 14). In addition, recovery of rat insulin spiked into samples was 85% in plasma and 98% in cerebrospinal fluid. Plasma and cerebrospinal fluid samples from each rabbit in the nonpregnant and pregnant state were assayed at the same time; intra- and interassay coefficients each averaged 8.4%.

Protocols

Protocol 1: are insulin sensitivity and baroreflex gain correlated? Insulin sensitivity and baroreflex function were measured in 21 nonpregnant and 14 near-term pregnant rabbits (days 29–30 of pregnancy), and the linear relationship between these variables was determined.

Protocol 2: does the time course for the decrease in baroreflex gain during pregnancy match the time course for the decrease in insulin sensitivity? In five rabbits, insulin sensitivity and baroreflex gain were measured before pregnancy and at days 15–16 (2 wk), 22–23 (3 wk), and 29–30 (4 wk) of gestation, to determine at which point baroreflex gain and insulin sensitivity significantly decrease. In one rabbit, baroreflex gain and insulin sensitivity were not measured at day 29–30 as the catheters had lost patency by this time.

Protocols 3 and 4: do insulin resistance and impaired baroreflex gain contribute to the development of gestational diabetes? Basal BP, mmHg 65 ± 1 57 ± 1

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Protocol 2</th>
<th>Protocol 3</th>
<th>Protocol 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal BP, mmHg</td>
<td>65 ± 1</td>
<td>57 ± 1</td>
<td>57 ± 1</td>
</tr>
<tr>
<td>Basal HR, beats/min</td>
<td>157 ± 2</td>
<td>183 ± 7**</td>
<td>183 ± 7*</td>
</tr>
<tr>
<td>Maximum HR, beats/min</td>
<td>303 ± 4</td>
<td>276 ± 6*</td>
<td>276 ± 6*</td>
</tr>
<tr>
<td>Minimum HR, beats/min</td>
<td>131 ± 3</td>
<td>158 ± 6*</td>
<td>158 ± 6*</td>
</tr>
<tr>
<td>Maximum gain, beats·min⁻¹·mmHg⁻¹</td>
<td>50 ± 1</td>
<td>15 ± 2*</td>
<td>15 ± 2*</td>
</tr>
<tr>
<td>Insulin sensitivity, mg dextrose·kg⁻¹·min⁻¹</td>
<td>9.9 ± 0.3</td>
<td>4.5 ± 0.3*</td>
<td>4.5 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; HR, heart rate; BP₉₀, mean arterial pressure at the midpoint between the minimum and maximum HR. *Significantly different from nonpregnant results (P < 0.05).

Protocol 3: does preventing the decrease in insulin sensitivity during pregnancy prevent the decrease in baroreflex gain? Insulin sensitivity and baroreflex gain were determined in five rabbits when they were nonpregnant, at days 29 and 30 during a control first pregnancy, and at days 29 and 30 of a second pregnancy during which the rabbits were continuously treated with the insulin-sensitizing drug rosiglitazone. Rosiglitazone (14 mg/day) was administered orally once daily in 5 ml of yogurt. Four rabbits were also given 5 ml of yogurt without rosiglitazone daily during their first pregnancy to determine whether yogurt alone had any effect on insulin sensitivity or baroreflex gain.

Five rabbits were studied to determine the effects of rosiglitazone in the nonpregnant state. In all of these rabbits, insulin sensitivity and baroreflex gain were first measured in the virgin control state. Insulin sensitivity and baroreflex gain were again tested after rosiglitazone treatment (29–30 days) in two rabbits that remained virgins. Because previous experiments indicate that baroreflex gain is similar in non-pregnant rabbits before and after pregnancy (2), in one rabbit, rosiglitazone treatment (30 days) was begun 9 days after delivery of her first litter, and, in two rabbits, rosiglitazone was given throughout their second pregnancy and was continued 2 wk after delivery. There were no differences in responses between rabbits, so these data were combined.

Protocol 4: do cerebrospinal fluid insulin concentrations decrease during pregnancy? In nine rabbits, cerebrospinal fluid was collected on multiple days before pregnancy and then again after 2 and 4 wk of pregnancy. In three rabbits, cerebrospinal fluid was also collected 1 wk after delivery. In six rabbits, blood samples were drawn on 1 day at each of these time intervals.

Data Analysis

The sigmoidal baroreflex relationships between mean arterial BP and HR generated in each experiment were fitted and compared with the Boltzmann sigmoidal equation: HR = A + B/(1 + e⁹(C – mean arterial pressure)/D), where A equals the minimum HR, B equals the HR range, C equals the mean arterial pressure at the midpoint between the minimum and maximum HR, and D is the slope coefficient. Maximum gain was calculated by dividing the HR range by four times the slope coefficient. Because of the exponential nature of baroreflex gain, the log of this parameter was used for linear regression analyses.

Basal BP and HR, curve-fitting parameters, insulin sensitivity, and cerebrospinal fluid and plasma insulin concentrations were compared between groups using the Student’s t-test or one-way ANOVA for repeated measures and the post hoc Bonferroni correction test. A least-squares linear regression was used to determine the correlation coefficient between baroreflex gain and insulin sensitivity in protocols 1 and 2. All data are reported as means ± SE.
Table 1. Basal and baroreflex curve-fitting parameters in rabbits before pregnancy and at 2, 3, and 4 wk of pregnancy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonpregnant</th>
<th>2 wk Pregnant</th>
<th>3 wk Pregnant</th>
<th>4 wk Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal BP, mmHg</td>
<td>65 ± 2</td>
<td>64 ± 3</td>
<td>63 ± 2</td>
<td>55 ± 2*</td>
</tr>
<tr>
<td>Basal HR, beats/min</td>
<td>157 ± 5</td>
<td>160 ± 5</td>
<td>162 ± 12</td>
<td>183 ± 14*</td>
</tr>
<tr>
<td>Maximum HR, beats/min</td>
<td>298 ± 4</td>
<td>284 ± 10</td>
<td>294 ± 16</td>
<td>282 ± 20</td>
</tr>
<tr>
<td>Minimum HR, beats/min</td>
<td>135 ± 5</td>
<td>135 ± 11</td>
<td>133 ± 13</td>
<td>158 ± 15</td>
</tr>
<tr>
<td>BPso_{50}, mmHg</td>
<td>64 ± 2</td>
<td>63 ± 3</td>
<td>62 ± 2</td>
<td>53 ± 2*</td>
</tr>
<tr>
<td>Maximum gain, beats/min−1 · mmHg−1</td>
<td>76 ± 12</td>
<td>58 ± 10</td>
<td>32 ± 7*</td>
<td>16 ± 4*</td>
</tr>
<tr>
<td>Insulin sensitivity, mg dextrose·kg−1·min−1</td>
<td>10.1 ± 0.6</td>
<td>9.2 ± 1.1</td>
<td>6.3 ± 0.5*</td>
<td>5.0 ± 0.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 rabbits. *Significantly different from nonpregnant results (P < 0.05).

Protocol 1: Baroreflex Gain and Insulin Sensitivity Are Related in Pregnant and Nonpregnant Rabbits

Our first approach to test the hypothesis that the decrease in baroreflex gain during pregnancy is mediated by the decrease in insulin sensitivity was to determine whether these two parameters are correlated in nonpregnant and late pregnant rabbits (29–30 days pregnant; gestation is 31 days). Pregnancy decreased baroreflex gain, the maximum slope of the linear segment of the sigmoidal baroreflex relationship (Fig. 1A, Table 1). Insulin sensitivity also fell (Table 1), and these two parameters were correlated (Fig. 1B; r² = 0.59). In addition, as previously described (2, 3, 6, 52), pregnancy decreased basal BP and the baroreflex maximum HR, increased the basal and baroreflex minimum HR, and left-shifted the baroreflex curve (shortened the minimum arterial pressure at the midpoint between the minimum and maximum HR; Fig. 1A, Table 1).

Protocol 2: Changes in Baroreflex Gain and Insulin Sensitivity During Pregnancy Exhibit Similar Time Courses

Baroreflex gain decreases gradually during pregnancy to reach its nadir just before delivery (52). If the fall in baroreflex gain is mediated by the decrease in insulin sensitivity, then insulin resistance should also develop near the end of gestation. In rabbits studied throughout pregnancy, baroreflex gain was decreased significantly at 3 and 4 wk of gestation (Fig. 2A, Table 2). Insulin sensitivity fell at the same time (Fig. 2A, Table 2), and these changes were correlated (Fig. 2B; r² = 0.38). Moreover, linear regression analysis of insulin sensitivity and baroreflex gain within individual animals yielded r² ranging from 0.43 to 0.86 (mean = 0.65 ± 0.08). In contrast, other baroreflex and basal cardiovascular parameters were not significantly altered until 4 wk of gestation, at which time changes were generally similar to those observed in protocol 1 (Table 2).

Protocol 3: Rosiglitazone Treatment Reverses the Effects of Pregnancy on Insulin Sensitivity and Baroreflex Gain

If the decrease in insulin sensitivity underlies the impaired baroreflex gain during pregnancy, then prevention of insulin resistance should normalize baroreflex gain. When pregnant rabbits were studied near term after treatment with the insulin-sensitizing drug rosiglitazone throughout pregnancy, insulin sensitivity was increased compared with results shown with normal pregnancy (Fig. 3, Table 3); however, rosiglitazone did not completely reverse the pregnancy-induced decrease in insulin sensitivity (Fig. 3, Table 3). Similarly, rosiglitazone increased baroreflex gain in pregnant rabbits compared with untreated pregnant animals, but gain still remained below the values of nonpregnant rabbits (Fig. 3, Table 3). Rosiglitazone exerted no other effects on basal or baroreflex-mediated cardiovascular function during pregnancy (Fig. 3, Table 3). In nonpregnant rosiglitazone-treated rabbits, baroreflex gain and insulin sensitivity were not altered, but baroreflex-induced maximum HR was decreased (Fig. 4, Table 4).

Daily administration of the rosiglitazone yogurt vehicle also had no effect on baroreflex function or insulin sensitivity. At 4-wk gestation, rabbits that received yogurt during pregnancy exhibited a decrease in baroreflex gain (from 77 ± 20 to 21 ±
7 beats·min⁻¹·mmHg⁻¹; P < 0.05) and insulin sensitivity (from 10.4 ± 0.8 to 5.1 ± 1.0 mg dextrose·kg⁻¹·min⁻¹; P < 0.05, n = 4), which is not different from rabbits receiving no vehicle (from 45 ± 9 to 13 ± 2 beats·min⁻¹·mmHg⁻¹ and from 9.7 ± 0.6 to 4.3 ± 0.3 mg dextrose·kg⁻¹·min⁻¹; P < 0.05, n = 10).

All values for untreated pregnant rabbits in this study were from a first pregnancy, and all values for rosiglitazone-treated pregnant rabbits were from a second pregnancy. Therefore, the possibility that the increases in baroreflex gain and insulin sensitivity were a confounding factor of multiple pregnancies should be considered. However, in a previous study (2) baroreflex gain was reduced (P < 0.05) near term to a similar level during a second pregnancy (12 ± 3 beats·min⁻¹·mmHg⁻¹; n = 5) as during a first pregnancy (13 ± 1 beats·min⁻¹·mmHg⁻¹; n = 3) compared with values obtained before pregnancy (24 ± 3 and 19 ± 3 beats·min⁻¹·mmHg⁻¹, respectively). Also, in one untreated rabbit in the present study, insulin sensitivity was reduced from 10.0 to 4.0 mg dextrose·kg⁻¹·min⁻¹ during a first pregnancy. During a second pregnancy, insulin sensitivity was decreased similarly to 3.6 mg dextrose·kg⁻¹·min⁻¹.

**Pregnancy Decreases Cerebrospinal Fluid Insulin Concentrations**

To test the hypothesis that decreases in brain insulin concentrations contribute to the decrease in baroreflex gain, we measured plasma and cerebrospinal fluid insulin concentrations before and during pregnancy. Plasma insulin levels were unaltered by pregnancy (Fig. 5), as previously reported in the rabbit (24). However, although cerebrospinal fluid insulin concentrations were similar in rabbits in the nonpregnant state and at midgestation, reductions of more than 50% were evident near term [from 25 ± 7 to 10 ± 2 pg/ml; P < 0.01 (Fig. 5)].
In three rabbits, cerebrospinal fluid insulin levels returned to normal after delivery (from 46 ± 13% control at term to 94 ± 18% control 1 wk after delivery; P < 0.01).

DISCUSSION

The purpose of this study was to test the novel hypothesis that the decrease in baroreflex gain during pregnancy is mediated by decreased insulin sensitivity. The new findings were that 1) baroreflex gain and insulin sensitivity are highly and positively correlated in nonpregnant and pregnant rabbits, 2) the declines in baroreflex gain and insulin sensitivity exhibit the same time courses, with each decreasing in the second half of gestation, 3) treatment of rabbits with the insulin-sensitizing drug rosiglitazone throughout pregnancy almost completely normalized the falls in both insulin sensitivity and baroreflex gain, and 4) late pregnancy is associated with reduced cerebrospinal fluid insulin concentrations. These data support the hypothesis that pregnancy suppresses baroreflex gain by decreasing insulin sensitivity and brain insulin concentrations.

It has long been known that pregnancy impairs baroreflex gain (4, 31, 41), and significant research has been directed at uncovering the mechanism. Our laboratory and others have ruled out a role for ANG II (6, 15, 46) and nitric oxide (15). Maslamanli and Heesch (41) have implicated a metabolite of progesterone, allopregnanolone, because infusion of this neurosteroid in virgin rats decreases baroreflex gain similarly to that shown during pregnancy. However, because the actions of this metabolite cannot be blocked without termination of pregnancy, it remains uncertain whether endogenous allopregnanolone is fully responsible for the pregnancy-induced decrease in baroreflex gain. Moreover, in pregnant rats, allopregnanolone levels drop immediately preceding delivery (12), a time when baroreflex gain has reached its lowest level. Thus other mediators must be involved.

The association between insulin resistance and impaired baroreflex gain in numerous conditions (10, 11, 20, 22, 26, 55) led us to hypothesize that during pregnancy these variables are mechanistically linked. Pregnancy-induced insulin resistance develops secondary to the actions of several hormones, including glucocorticoids, estrogen, progesterone, placental lactogen, and TNF-α (13, 35, 43). These hormones may decrease insulin sensitivity by increasing free fatty acid levels (21, 29, 58), which can inhibit insulin signaling by serine phosphorylation of the insulin receptor and insulin receptor substrate proteins (32, 53). In support of our hypothesis, we first found that decreases in both insulin sensitivity and baroreflex gain were correlated in pregnant and nonpregnant rabbits, similar to the reduction in insulin sensitivity and baroreflex gain that occurs in obese humans and is reversed by weight loss (27). We also showed, for the first time in any condition, that the time course for the decrease in baroreflex gain matches the decrease in insulin sensitivity. These two results are in accordance with the hypothesis that the decrease in insulin sensitivity drives the decrease in baroreflex gain. However, these correlations, while tight, do not prove cause and effect. Therefore, we next tested whether preventing the decrease in insulin sensitivity during pregnancy normalizes baroreflex gain.

To do this, rabbits were treated with the insulin-sensitizing drug rosiglitazone. Rosiglitazone is a member of the drug class thiazolidinediones, which are agonists of the peroxisome proliferator-activated receptor-γ (PPAR-γ). PPAR-γ is a ligand-activated transcription factor that acts predominantly as a regulator of lipid metabolism (59). Exactly how PPAR-γ activation increases insulin sensitivity is not completely understood and probably includes multiple mechanisms. Rosiglitazone may act in part by increasing insulin receptor number and activity (by decreasing inhibitory serine phosphorylation). Indirect effects include decreases in the levels of free fatty acid levels and TNF-α (59), which, as described above, decrease insulin signaling.

In the present study, rosiglitazone substantially attenuated the decrease in insulin sensitivity with pregnancy and, to a similar extent, the reduction in baroreflex gain. These data strongly implicate insulin resistance as the cause of the baroreflex impairment. In nonpregnant rabbits, rosiglitazone had no effect on baroreflex gain or insulin sensitivity but did decrease baroreflex-induced maximum HR. This effect of rosiglitazone, therefore, appears to be independent of its ability to improve insulin sensitivity.

Interestingly, rosiglitazone did not reverse the effects of pregnancy on other aspects of baroreflex function, such as the increase in minimum HR (4). Our group (3) has previously demonstrated that the decrease in baroreflex gain is mediated by reductions in the sympathetic component of HR control, whereas the increase in minimum HR is due to impaired parasympathetic control of HR. Therefore, insulin resistance may impair baroreflex control of the sympathetic nervous system with less effect on the parasympathetic nervous system.
If the decrease in baroreflex gain is due to alterations in insulin sensitivity, the next question is, how does this occur? Much of insulin in the brain is of pancreatic origin (1); however, insulin is a large polypeptide that cannot pass through the blood-brain-barrier unassisted (1). It has been proposed that insulin gains access to the brain via a receptor-mediated process in which insulin binds to its receptor on the capillary wall, and this complex is internalized by endocytosis (1, 23, 62). The insulin-insulin receptor complex is then expelled by exocytosis on the brain side of the endothelial cell (1, 23, 62). Such a transport process has been demonstrated in cultured aortic endothelial cells, in which insulin transport is blocked by insulin receptor antibodies (34). Because the endothelial cells of the blood-brain barrier also contain insulin receptors and can transport insulin intact from the blood to the brain (1, 23, 62), we hypothesized that insulin resistance decreases transport of insulin from plasma to brain. Moreover, because insulin increases baroreflex gain when chronically administered into the lateral ventricles (47), reduced brain insulin concentrations could, therefore, underlie the decreased baroreflex gain. In support of this hypothesis, we found that cerebrospinal fluid insulin concentrations were unchanged at midgestation, when baroreflex gain is normal, but decreased at term, when gain reaches its nadir. Therefore, we conclude that the pregnancy-induced decrease in insulin sensitivity, by decreasing brain insulin concentrations, contributes to the decrease in baroreflex gain. We speculate that the decreased brain insulin concentrations are secondary to impaired transport, since deficits in transport have been described in other insulin-resistant states (23, 62). In addition to reductions in insulin transport into the brain and brain insulin concentrations, insulin resistance may also suppress neuronal insulin signaling (17), thus decreasing the effectiveness of the insulin that does reach the brain. Therefore, pregnancy may also impair baroreflex gain by decreasing brain insulin receptor responsiveness; future experiments are required to test this hypothesis.

It is not clear why decreases in insulin sensitivity are associated with reductions in insulin transport into the brain or even exactly how insulin is transported into the brain. It is also not known where insulin might be acting in the brain to increase baroreflex gain. Insulin receptors are concentrated in several brain regions important in control of the autonomic nervous system, including the nucleus of the solitary tract (NTS), the paraventricular nucleus of the hypothalamus (PVN), and the ventrolateral medulla (30, 61). Insulin hyperpolarizes neurons, thereby decreasing their activity (19, 48, 49). Because inactivation of the PVN enhances baroreflex gain (9), insulin may act in PVN to increase gain. Alternatively, insulin may suppress PVN activity indirectly, by binding to receptors in the arcuate nucleus, since this hypothalamic site contains high levels of insulin receptors and projects to the PVN (45, 51). Application of insulin onto the dorsal medulla results in enhancement of baroreflex gain (40), suggesting that insulin might act in the NTS to exert this effect. However, microinjection of insulin into the NTS decreases the activity of barosensitive neurons (54) and decreases baroreflex gain (42), suggesting a possible role for other nuclei in the brain stem (rostral and/or caudal ventrolateral medulla).

In summary, the results of this study support the hypothesis that, during pregnancy, insulin resistance is associated with decreased brain insulin concentrations, which may drive the decrease in baroreflex gain. It is tempting to speculate that this association may also underlie the decreased gain observed in a number of other conditions in which insulin sensitivity is reduced, such as type 2 diabetes mellitus and obesity.

ACKNOWLEDGMENTS

We thank Corrina L. Freeman for technical assistance and Terrence Chu in Dr. David Hess’ laboratory at the Oregon National Primate Research Center for measurements of insulin concentrations.

GRANTS

This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-35872 and HL-70962 (to V. L. Brooks) and by American Heart Association, Pacific Mountain Affiliate Fellowship 0315254Z (to D. L. Daubert).

REFERENCES

PREGNANCY, INSULIN RESISTANCE, AND IMPAIRED BAROREFLEX GAIN


