Pregnancy impairs baroreflex control of heart rate in rats: role of insulin sensitivity

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Pregnancy impairs baroreflex control of heart rate in rats: role of insulin sensitivity. Am J Physiol Regul Integr Comp Physiol 298: R419–R426, 2010. First published November 25, 2009; doi:10.1152/ajpregu.00441.2009.—Recent studies in rabbits suggest that insulin resistance and reduced brain insulin contribute to impaired baroreflex control of heart rate (HR) during pregnancy; however, the mechanisms are unknown. The rat model is ideal to investigate these mechanisms because much is known about rat brain baroreflex neurocircuitry and insulin receptor locations. However, it is unclear in rats whether pregnancy impairs the HR baroreflex or whether insulin resistance is involved. Therefore, this study tested the hypothesis that in rats pregnancy decreases HR baroreflex sensitivity (BRS) and that this decrease is related to concurrent decreases in insulin sensitivity (IS). BRS was quantified before, during, and after pregnancy using complementary methods: 1) spontaneous BRS (sBRS) derived from sequence method analysis of telemetric, continuous arterial pressure recordings; and 2) maximal BRS of complete sigmoidal baroreflex relationships. IS was measured (hyperinsulinemic euglycemic clamp) to determine whether BRS and IS change in parallel. sBRS was reduced at midgestation [pregnancy day 10 (P10)], returned to nonpregnant (NP) levels on P18, and fell again at late gestation (P20) (sBRS in mmHg: NP, 1.66 ± 0.04; P10, 1.17 ± 0.11; P18, 1.55 ± 0.12; P20, 1.31 ± 0.05; n = 5; P < 0.05). Similar triphasic patterns were observed for both maximal BRS [in beats·min−1·mmHg−1: NP, 4.45 ± 0.52 (n = 10); P11–12, 2.76 ± 0.11 (n = 7); P17–18, 3.79 ± 0.14 (n = 5); P19–20, 2.32 ± 0.40 (n = 8); P < 0.0001] and previous and current measurements of IS (in mg glucose·kg−1·min−1: NP, 32 ± 2; P19–20, 15 ± 1; P < 0.0005). Furthermore, during pregnancy, the standard deviation (SD) of MAP increased, and the SD of HR decreased, indirectly suggesting baroreflex impairment. sBRS increased transiently during parturition, and sBRS, maximal BRS, and IS normalized 3–4 days postpartum. In conclusion, pregnancy decreases HR BRS in rats. The parallel temporal changes in BRS and IS suggest a mechanistic link.

hyperinsulinemic-euglycemic clamp; mean arterial pressure; telemetry; spontaneous baroreflex sensitivity; insulin resistance

PREGNANCY INDUCES PROFOUND alterations in the cardiovascular system, including a blunting of the baroreceptor reflex (9, 20). As a result, pregnant individuals exhibit an increased susceptibility to orthostatic hypotension and a reduced ability to maintain blood pressure during hemorrhage (11, 17). Two mechanisms have been proposed: during pregnancy 1) increases in the progesterone metabolite, allopregnanolone, blunt reflex responses by decreasing the maximal levels of renal sympathetic nerve activity achieved during hypotensive challenges (20); and 2) insulin resistance impairs the gain or sensitivity of baroreflex control of heart rate (HR), at least in rabbits (15).

Studies investigating these mechanisms indicate that pregnancy decreases baroreflex function via alterations in the brain (20, 27). The rostral ventrolateral medulla has been identified as a key site of action of allopregnanolone (13, 28). The mechanisms by which insulin resistance impairs brain regulation of the baroreflex are less clear, although one hypothesis is that reduced brain insulin is responsible (15). More specifically, decreased brain insulin is found in many insulin-resistant conditions, including pregnancy (15, 26). Because insulin enhances baroreflex sensitivity (BRS) via a central action (40, 44), falling brain insulin levels would induce a parallel decrease in BRS. However, the sites and mechanisms by which insulin acts in the brain to increase BRS, and whether these mechanisms are attenuated during pregnancy, are unknown. The rat is the ideal model to investigate these questions because of the numerous parallels between rat and human pregnancy (38) and because much is known in the rat concerning brain baroreflex neurocircuitry and the location of brain insulin receptors (47, 55). However, it is unclear whether and when pregnancy decreases HR BRS in rats. Moreover, the role of insulin resistance in pregnancy-induced BRS changes has not been previously investigated in the rat.

Therefore, the primary goal of this study was to test the hypothesis that HR BRS is reduced in the rat during pregnancy and to begin to test whether the reduction is related to the concurrent fall in insulin sensitivity. If BRS and insulin sensitivity are mechanistically linked, then reductions in BRS and insulin sensitivity would be expected to exhibit the same time course, as was observed in rabbits (15). Interestingly, in contrast to rabbit pregnancy in which insulin sensitivity drops precipitously at end gestation, as pregnancy progresses in the rat, insulin sensitivity falls at midgestation, then increases to near nonpregnant (NP) levels, and finally decreases again just before delivery (10, 29–31, 35, 45). However, whether BRS exhibits a similar pattern of change as insulin sensitivity during rat pregnancy is unknown. In addition, both insulin sensitivity and BRS would be expected to normalize in parallel during the postpartum period; however, the rates of normalization of BRS and insulin sensitivity following delivery are unknown. Therefore, we determined HR BRS in conscious rats on several selected days during pregnancy and during the early postpartum period by 1) estimating spontaneous BRS (sBRS) using sequence method analysis of telemetric, continuous arterial blood pressure recordings; and 2) estimating maximal HR BRS by using infusions of vasoactive drugs to induce changes in mean arterial pressure (MAP) that evoke steady-state baroreflex-mediated HR responses. In addition, we determined insu-
lin sensitivity by using the hyperinsulinemic euglycemic clamp in rats in the NP state, at end gestation, and during the postpartum period.

The secondary goal of this study was to determine whether BRS changes during delivery, which is also unknown. Oxytocin, which has been shown to enhance BRS (21, 23, 46), increases during delivery. Therefore, we tested the hypothesis that BRS increases during delivery by measuring sBRS just prior to, during, and just after delivery in rats instrumented with telemetry catheters.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats (n = 58; Charles River Laboratories), 12 wk old, were acclimated to the laboratory for at least 7 days prior to instrumentation. Animals were housed with a 12:12-h light-dark cycle. Food (LabDiet 5001) and water were provided ad libitum. All procedures were conducted in accordance with the National Institutes of Health Guide for the Health and Use of Laboratory Animals and were approved by the Oregon Health & Science University Animal Care and Use Committee.

Animal Survival Surgery

All surgeries were performed using aseptic technique. Rats received a single intramuscular injection of 30,000 units penicillin G (Hanford’s United States Veterinary Products) 10–15 min prior to incision and codeine (1 mg/100 ml) in their drinking water for 3 days postprocedure.

Telemetry catheter insertion. Anesthesia was induced with 5% isoflurane in 100% oxygen and was maintained with 1.5–2% isoflurane. Through a longitudinal inguinal incision, the pressure catheter (PAC 40, 10-cm catheter length; Data Sciences International, St. Paul, MN) was inserted via the femoral artery retrograde into the distal abdominal aorta, and the transmitter was inserted into a subcutaneous pocket in the flank. Rats recovered a minimum of 7 days prior to data collection.

Catheter insertion for full baroreflex curve measurement and insulin sensitivity measurements. After induction of anesthesia, a polyethylene arterial catheter (PE10 or PE50) was inserted through a small inguinal incision into the femoral artery and advanced into the distal abdominal aorta. In addition, two venous catheters (PE10) were inserted into the femoral vein and advanced into the distal inferior vena cava. The catheters were tunneled subcutaneously and were exteriorized between the scapulae. Catheter patency was maintained by flushing with sterile heparin saline (100 U/ml) at least 3 times per week. Rats recovered 3–5 days before experiments were conducted.

Experimental Protocols

Changes in sBRS before, during and after pregnancy within rats. Following instrumentation and recovery, five consecutive days of baseline data in NP rats were collected to capture the entire estrus cycle during which sBRS has been shown to vary (18). The female rat was then placed in a male rat’s home cage. Vaginal epithelium cytology was examined daily and the presence of sperm indicated pregnancy day 0; the female rat was then placed back in her home cage. Pregnancy progressed to delivery. Pups were removed within 24 h of delivery. In five rats, data collection continued during pregnancy days 10 and 12–14 and also during the last 3 days before delivery [day 21 or day 22; these 3 days are standardized in the figures as days 18–20, even though for the two rats that delivered on day 22, days 19–21 were assessed], and postpartum days 2–7. In a sixth rat, data were collected at end gestation and during the postpartum period.

sBRS was determined post hoc using the sequence method. In brief, arterial pressure was measured continuously at 1,000 Hz via the telemetry catheter and transmitter. The sequence method software (Hemolab, Iowa City, IA) identifies spontaneous baroreflex-mediated ramps in which both systolic pressure and pulse interval either simultaneously increase or decrease (3, 52). For each ramp or sequence identified, the software application uses linear regression (minimum r threshold was set at 0.8) to calculate the slope of each ramp, which is the sBRS (mmHg/ms).

A delay in the pulse interval associated with a changing systolic pressure is needed to take into account the time for a pressure change to trigger a change in HR. Based on an early study of the application of the sBRS technique in rats (42), many investigators have averaged the sBRS values obtained from delays of 3, 4, and 5 beats. However, whether different estimates of sBRS are obtained for each of these beat delays is not clear. Moreover, because pregnancy increases HR, a longer beat delay may be required for optimal results. To address these issues, we determined whether sBRS, or the number of identified ramps or sequences, varies among beat delays or between the pregnant and nonpregnant state. In five rats, these two variables were quantified for a 4-h interval (average of four 1-h assessments) in the middle of the light phase and of the dark phase of 1 day before pregnancy (on diestrus, determined from the cytology of vaginal smears) and again on day 20 of gestation. A larger number of sequences (P < 0.05) was identified with a 2- or 3-beat delay during the dark phase; importantly, however, there was no difference between reproductive states (See Supplemental Table 1; supplemental data for this article are available online at the Am J Physiol Regul Integr Comp Physiol website.). In addition, the number of detected sequences was significantly reduced when the rats were pregnant (P < 0.05; Supplemental Table 1). On the other hand, as shown in Supplemental Table 2, no significant differences were found between sBRS values determined at each beat delay. Based on these data, and in keeping with other studies in rats, we chose a three-beat delay for these studies.

Because sBRS, MAP, and HR exhibit a diurnal rhythm (18, 42, 54), changes in sBRS during pregnancy were derived on selected days from continuous measurements of arterial pressure 24 h/day. For each hour, artifacts and, at most, one sequence outlier [which was defined as a value at least three standard deviations (SD) from the mean] were removed. Hourly mean values of sBRS, MAP, HR, and the SD of MAP and HR were then calculated (Dataquest ART 4.0, Data Sciences International, St. Paul, MN). From these hourly data, for each day for each rat, a single 12-h light-phase and a single 12-h dark-phase mean value were determined. In addition, for all data, means were calculated for the 5-day NP baseline period, and means of the SD of MAP and HR were determined for midestrus (P12–14), end gestation (P18–20), and also postpartum periods (PP2–4 and PP5–7).

Changes in BRS using the pharmacologic method for full baroreflex curves. Separate groups of different rats, instrumented with PE catheters, were studied in the NP state (n = 10), pregnancy days 11 or 12 (n = 7), pregnancy days 17 or 18 (n = 5), pregnancy days 19 or 20 (n = 8), and postpartum days 3 or 4 (n = 5). The NP group was not studied on a specific day of the estrus cycle since preliminary data indicated that the surgery for instrumentation induced constant diestrus for a period that overlapped the day on which experiments were performed. Otherwise, these rats were handled in the same manner as the telemetry rats; in particular, pregnancy day 0 was established via cytology of vaginal smears, and the pups were removed within 24 h following delivery.

On the morning of the experimental day, maximal BRS was measured after attaching catheters to the recording equipment and infusion pumps. The rats rested, unrestrained, in their home cage and were allowed 1–2 h of equilibration before the experimental protocol was initiated.

MAP and HR were measured continuously via the arterial catheter using a Statham pressure transducer, a Grass tachometer, Grass polygraph, and a MP100 data acquisition and analysis system (sampling at 1,000 Hz; Biopac, Santa Barbara, CA). To determine barore-
The disruption of diurnal variations in BRS observed during pregnancy was sustained during the early postpartum period (Fig. 1). Nevertheless, sBRS achieved NP values within 3 (lights off) to 5 (lights on) days postpartum. MAP also rapidly increased, to reach prepregnant values by the end of gestation (days 19–20) (Fig. 1). In contrast, HR during the light phase was increased at midgestation and remained high until delivery.

MAP SD during the light phase was not altered during gestation (Fig. 2). However, during the dark active phase, MAP SD was increased at midgestation compared with the NP state and increased further (P < 0.05) just before delivery. Pregnancy suppressed HR SD during both the light and dark phases (Fig. 2).

**Statistical Analysis**

Two-way ANOVA for repeated measures [factors were time of day (light, dark) and days during or after gestation], followed by the post hoc Neuman-Keuls test, were used to assess significant within- and between-group differences in data collected by telemetry. Differences between groups in sigmoidal parameters from fits of complete baroreflex curves were determined with one-way ANOVA. Changes in sBRS, MAP, and HR during delivery were determined with one-way ANOVA for repeated measures. Finally, three-way ANOVA for repeated measures (factors were reproductive state, light-dark, and beat delay) was used to assess the influence of the beat delay on sBRS and the number of detected sequences. ANOVA results are provided in the figure or table legends. Data are reported as means ± SE. P < 0.05 was considered statistically significant.

**RESULTS**

**Pregnancy Decreased HR BRS in the Rat**

sBRS measurements. Consistent with previous reports (18, 42, 54), sBRS demonstrated significant diurnal differences in rats in the NP state (1.66 ± 0.04 ms/mmHg, lights on; 1.52 ± 0.07 ms/mmHg, lights off; P < 0.05). Interestingly, these diurnal variations in sBRS were not observed during pregnancy, except for gestational day 18 (P < 0.05; Fig. 1).

Compared with the NP state, during the dark phase, sBRS was suppressed on all gestational days assessed, including midgestation (days 10–12) and just prior to delivery (day 20) (Fig. 1). However, during lights on, sBRS decreased (days 10–14), increased (day 18), and then decreased (days 19–20) relative to NP values (Fig. 1).

Diurnal variations in both MAP and HR were maintained throughout gestation (P < 0.05, Fig. 1). Compared with the NP state, MAP was decreased at midgestation (P < 0.05, Fig. 1), and fell further just before delivery (days 18–20 lower than midgestation, P < 0.05). HR during the dark phase did not increase above prepregnant values until gestational days 19–20. In contrast, HR during the light phase was increased at midgestation and remained high until delivery.

MAP SD during the light phase was not altered during gestation (Fig. 2). However, during the dark active phase, MAP SD was increased at midgestation compared with the NP state and increased further (P < 0.05) just before delivery. Pregnancy suppressed HR SD during both the light and dark phases (Fig. 2).

**Maximal BRS measurements (light phase only).** Compared with the NP state, maximal BRS decreased on gestational days 11–12, normalized on gestational days 17–18, and then decreased at end gestation (days 19–20) (Fig. 3). Maximal baroreflex HR and HR range both tended to decrease at end gestation, but these changes did not achieve statistical significance (P = 0.08 and 0.057, respectively; Table 1). No other sigmoidal parameters were significantly altered during gestation, except for width, which was decreased on pregnancy day 20 (Table 1).

**Insulin Sensitivity and BRS Rapidly Normalized Postpartum**

sBRS measurements. The disruption of diurnal variations in BRS observed during pregnancy was sustained during the early postpartum period (Fig. 1). Nevertheless, sBRS achieved NP values within 3 (lights off) to 5 (lights on) days postpartum. MAP also rapidly increased, to reach prepregnant values by the
second postpartum day. HR returned to NP values by postpartum days 2 (lights off) to 3 (lights on); however, HR continued to fall and became significantly lower than prepregnant levels 5–7 days after delivery. Finally, MAP SD normalized within 4 days after delivery, while it took more than 7 days postpartum for HR SD to increase to NP values (Fig. 2).

**Maximal BRS measurements.** Similarly to the measurements of sBRS, maximal BRS, estimated from the generation of complete baroreflex curves, had resumed prepregnant levels 3–4 days postpartum (Fig. 3; Table 1).

**Insulin sensitivity.** As previously reported (10, 29, 30, 35, 37, 45), fasting plasma glucose levels were reduced in late pregnant rats (NP, 115 ± 5 mg/dl; P19 –20, 65 ± 4 mg/dl; P < 0.05). Glucose levels were increased in postpartum rats (95 ± 4 mg/dl; P < 0.05), but still remained low compared with NP rats (P < 0.05). At the conclusion of the clamp protocol, the levels of human insulin achieved by intravenous infusion were not different (P > 0.50) between groups (NP, 23.9 ± 5.8 μU/ml, n = 4; P20, 20.5 ± 3.8 μU/ml, n = 3; PP3–4, 25.6 ± 3.6 μU/ml, n = 6).

As expected, insulin sensitivity was significantly reduced at end gestation, compared with the NP state (Fig. 4; P < 0.05). On the other hand, by postpartum days 3–4, insulin sensitivity increased to become similar to values obtained during the NP state. Therefore, BRS and insulin sensitivity both achieved NP levels by 3–4 days postpartum.

**sBRS Increased During Delivery**

During the first 2 h of labor and delivery, sBRS increased (Table 2), and this increase was sustained until the last hour of delivery (to 1.62 ± 0.06 ms/mmHg; P < 0.05). sBRS then promptly returned to predelivery values after delivery of the last pup (Table 2). No changes in MAP or HR were observed during delivery (Table 2).

**DISCUSSION**

The primary purpose of the present studies was to test the hypothesis that pregnancy impairs baroreflex control of HR in rats and that this impairment is associated with pregnancy-
induced decreases in insulin sensitivity. In support of this hypothesis, we found that 1) HR BRS decreased transiently in rats at mid- and end gestation, in parallel with transient decreases in insulin sensitivity, and 2) both insulin sensitivity and BRS rapidly normalized postpartum. In addition, the use of telemetry allowed documentation that MAP is reduced and HR is increased at midgestation and, for the first time, that sBRS transiently increases during labor and delivery.

Changes in MAP and HR During Pregnancy and the Immediate Postpartum Period

It is well established that pregnancy increases HR and decreases MAP, due to a profound fall in systemic vascular resistance that is greater than the concurrent increase in cardiac output. In the present study, telemetric measurements of MAP revealed a significant decrease at midgestation and a dramatic further fall at end gestation. HR increased, to reach maximum values immediately prior to parturition. In addition, MAP and HR rebounded quite quickly after delivery, to become similar to prepregnant values by postpartum day 2. Previous research indicates that nitric oxide contributes to the systemic vasodilation, hypotension, and tachycardia, since nitric oxide levels are increased and blockade of nitric oxide synthase increases MAP, decreases systemic vascular conductance, and decreases HR, more in pregnant than in virgin animals (5, 14, 50). Nitric oxide may increase secondarily to elevations in estrogen and/or relaxin (25). The present results, showing rapid normalization of MAP and HR following delivery, are consistent with this proposal, since these hormones rapidly decrease postpartum (48).

Table 1. Sigmoidal baroreflex parameters before, during, and after pregnancy

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>P12</th>
<th>P18</th>
<th>P20</th>
<th>PP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. rats</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Maximum HR, beats/min</td>
<td>502±18</td>
<td>449±7</td>
<td>476±20</td>
<td>438±21</td>
<td>469±19</td>
</tr>
<tr>
<td>Minimum HR, beats/min</td>
<td>263±7</td>
<td>282±12</td>
<td>273±14</td>
<td>270±14</td>
<td>256±7</td>
</tr>
<tr>
<td>MAPso, mmHg</td>
<td>100±5</td>
<td>109±1</td>
<td>95±6</td>
<td>96±7</td>
<td>106±2</td>
</tr>
<tr>
<td>Width, mmHg</td>
<td>14.5±1.3</td>
<td>15.3±1.3</td>
<td>13.4±1.3</td>
<td>19.6±2.5*</td>
<td>9.8±1.5</td>
</tr>
<tr>
<td>Range, beats/min</td>
<td>239±15</td>
<td>167±14</td>
<td>204±22</td>
<td>168±29</td>
<td>213±19</td>
</tr>
</tbody>
</table>

NP, nonpregnant; P12, P18, P20, days 11 or 12, 17 or 18, and 19 or 20 of pregnancy, respectively; PP3, postpartum days 3 or 4; HR, heart rate; MAP, mean arterial pressure. *P < 0.05, P20 vs. PP3 (ANOVA, P = 0.015).
Table 2. Changes in MAP, HR, and sBRS during labor and delivery in conscious rats

<table>
<thead>
<tr>
<th></th>
<th>Predelivery Hours</th>
<th>Delivery Hours</th>
<th>Postdelivery Hours</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1  2</td>
<td>1  2</td>
<td>1  2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84±5 80±6</td>
<td>85±7 85±8</td>
<td>89±8 88±7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>434±13 435±19</td>
<td>439±26 448±21</td>
<td>463±19 460±13</td>
</tr>
<tr>
<td>sBRS, ms/mmHg</td>
<td>1.22±0.09 1.28±0.10</td>
<td>1.56±0.09* 1.69±0.15*</td>
<td>1.32±0.05 1.20±0.11</td>
</tr>
</tbody>
</table>

sBRS, spontaneous baroreflex sensitivity. *P < 0.05 compared to prelabor.
Delivery Increased BRS

Another novel finding of the present study was that sBRS increased rapidly but transiently during labor and delivery. While we did not investigate the mechanism, one potential contributor is oxytocin. Oxytocin is released in brain, as well as into the blood stream, during parturition (36), and oxytocin receptors are increased during late pregnancy and delivery in key brain stem cardiovascular regions, such as the nucleus tractus solitarius (NTS) and ventrolateral medulla (34). Moreover, oxytocin has been shown to increase baroreflex gain, potentially by an action in NTS to presynaptically enhance the release of glutamate from baroreceptor afferents (21, 23, 43, 46). Nevertheless, given the behavioral and endocrine complexity of parturition, it remains possible that other factors are also involved.

Perspectives

Recent evidence suggests that brain insulin levels are the link between systemic insulin resistance and baroreflex impairment during pregnancy. More specifically, we hypothesize that the factors that contribute to pregnancy-induced insulin resistance also retard insulin transport into the brain. Since brain insulin supports BRS (40, 44), decreases in insulin would cause BRS to fall. In support of this hypothesis, we have found that cerebrospinal fluid insulin levels are half of normal in late pregnant rabbits (15), and, more importantly, intracerebroventricular insulin infusion normalizes BRS in late pregnant rats (2). Therefore, we speculate that the oscillation of insulin sensitivity is a contributor to the oscillation of BRS during rat pregnancy and the BRS normalization postpartum, by altering brain insulin levels.

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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