Effect of antenatal glucocorticoids on sympathetic nerve activity at birth in preterm sheep

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Effect of antenatal glucocorticoids on sympathetic nerve activity at birth in preterm sheep. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R160–R167, 1998.—Renal sympathetic nerve activity (RSNA) increases rapidly after delivery of term fetal sheep and parallels the rise in heart rate (HR) and arterial pressure. To examine the RSNA response at birth in immature lambs, experiments were performed in chronically instrumented preterm fetal sheep (118- to 125-day gestation, term 145 days) before and after delivery by cesarean section. HR remained unchanged from fetal values at 1 and 4 h after birth, whereas mean arterial blood pressure (MABP) decreased significantly (P < 0.05) by 4 h after delivery. RSNA significantly decreased after premature birth in all animals studied (n = 6), achieving only 39 ± 17% of fetal RSNA (P < 0.05; all results are mean ± SE). Because cardiovascular function after premature birth is improved by the use of antenatal corticosteroids, we also tested the hypothesis that corticosteroid administration would evoke a more pronounced sympathetic response in prenatally delivered lambs (n = 7, 118 to 125-day gestation). After maternal administration of dexamethasone (5 mg im, 48 and 24 h before delivery), RSNA increased after birth in six of seven fetuses to 166 ± 32% of the fetal RSNA value. Dexamethasone treatment also increased the sensitivity of baroreflex-mediated changes in HR in response to increases in MABP. Because the sympathetic response at birth is depressed in preterm compared with term lambs, we performed an additional study (n = 8) to determine if immature sheep are capable of mounting a sympathetic response to cold. In utero cooling produced rapid and sustained increases in MABP (20 ± 4%), HR (26 ± 6%), and RSNA (282 ± 72%) (all P < 0.05), consistent with a generalized sympathoexcitation. These results suggest that sympathoexcitation is absent after premature delivery despite the presence of functional descending autonomic pathways. Furthermore, exogenous corticosteroids appear to have a maturational effect on the sympathetic response at birth, which may be one mechanism by which maternal steroid administration improves postnatal cardiovascular homeostasis.

NUMEROUS CARDIOVASCULAR, pulmonary, and metabolic adjustments are required at birth to ensure successful adaptation to the extrauterine environment (20). Previous studies have suggested that activation of the sympathoadrenal system, including a surge in adrenal catecholamine release (12, 19) and stimulation of sympathetic nerve activity (25), participates in regulating these physiological responses. Interestingly, preterm animals and human infants have attenuated physiological adaptive responses at birth compared with those delivered at term, despite having greater increases in circulating catecholamine levels (18, 22). Immaturity of neurohumoral systems, receptor mechanisms, and end-organ function may all contribute to impairment of physiological adaptation at preterm birth.

Before birth, there are programmed increases in circulating levels of several neuroendocrine mediators, including cortisol (17). The increasing availability of endogenous glucocorticoids late in fetal development modulates the rate of differentiation of numerous tissues, the most well studied being the lung (3). The enhancement of fetal lung structural and functional maturity by administration of corticosteroids to pregnant women has been extensively reviewed (2). In addition, studies in premature animals (21, 32) and human infants (10) have documented that antenatal corticosteroid administration improves cardiovascular status at birth and lessens the incidence of as well as the morbidity and mortality related to hemorrhagic and ischemic complications (5). The mechanisms by which antenatal corticosteroids facilitate postnatal circulatory function are uncertain but may be related in part to augmented cardiac and peripheral adrenergic (32) and angiotensinergic (34) functions. Corticosteroids may also influence the development of other neurohumoral systems regulating cardiovascular function at birth, including noradrenergic activity (26).

We have recently shown in term fetal sheep that efferent sympathetic nerve activity increases several-fold at birth and likely contributes to the cardiovascular changes after the transition from fetal to newborn life (25). Absence or attenuation of this sympathoexcitatory response may in turn contribute to the impaired cardiovascular responses at birth in preterm lambs. Therefore, the present studies were designed to investigate the sympathetic response at birth in preterm sheep and to determine if the increase in renal sympathetic nerve activity (RSNA) is impaired compared with that seen in term animals. Furthermore, we examined the effects of antenatal glucocorticoid administration on birth-related changes in systemic hemodynamics and RSNA in preterm fetal lambs. Because the sympathetic response at birth is attenuated in preterm lambs, we performed an additional study to determine if immature sheep are capable of mounting a sympathetic response to other stimuli, namely in utero cooling. We compared our results with those previously published in term fetal sheep.
METHODS

Studies were performed in conscious, chronically instrumented fetal sheep at 118- to 125-day gestational age (term 145 days). Pregnant ewes of Dorset and Suffolk mixed breeding were obtained from a local source; gestational ages were based on the induced ovulation technique as previously described (9). Ewes were randomized to receive either saline or dexamethasone (Dex, 5 mg im) 48 and 24 h before delivery.

Surgical preparations. All procedures were performed within the regulations of the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Briefly, the ewe was fasted for 24 h before surgery and anesthetized using a mixture of halothane (1%), oxygen (33%), and nitrous oxide (66%). For the first series of studies, the uterus was opened under sterile conditions over the fetal head, and polyethylene catheters were placed into the fetal femoral arteries and veins bilaterally. A catheter for recording amniotic pressure was also secured to the fetal skin. The left kidney, renal artery, and renal nerves were exposed through a flank incision, and a plastic-coated copper wire, used as a ground wire, was secured in the paravertebral muscle. After isolation of a branch of the left renal nerve bundle, platinum electrodes were secured onto the nerve for recording of RSNA as described previously (30). Function of the renal nerve was tested by audible monitoring of pulse-synchronous bursts of neural activity and by examining oscilloscope tracings during bolus phenylephrine infusion. When function was demonstrated, electrodes were secured using Sil-Gel (Sil-Gel 604A and 604B, Wacker-Chemie, Munich, Germany) and the flank incision was closed in separate layers. Fetal skin incisions were closed, and the fetus was returned to the uterus. Maternal incisions were closed in separate layers. All catheters and tubing were exteriorized through subcutaneous tunnels and placed in cloth pouches on the ewe’s flank. Ampicillin sodium (Wyeth Laboratories) was administered to the ewe intramuscularly before surgery (2 g) and was infused into the amniotic cavity after surgery (2 g).

In the second group of fetuses, the uterus was opened under sterile conditions over the fetal head, and the head, forelimbs, and thorax were exteriorized. Approximately 5 m of tubing from a closed injectate delivery system (model 93–600, Baxter Healthcare, Irvine, CA) was wrapped around the fetal thorax and secured to the skin. A thermistor (Gould test and measurement group, Valley View, OH) was then inserted into the fetal esophagus and secured in place to the corner of the mouth. Fetal skin incisions were closed, and the fetus was returned to the uterus. Femoral arterial and venous catheters as well as a left renal nerve recording electrode were placed as described above. After surgery, pregnant ewes were returned to individual pens and allowed free access to food and water. Twenty-four hours were allowed for recovery from surgery before experiments were performed.

Physiological studies. Before the start of the experiments, the ewe was transferred to the laboratory in a small cart that was placed in a Faraday cage. The pregnant ewe was then sedated with diazepam (0.3 mg/kg), given an intravenous bolus infusion of vecuronium bromide (0.1 mg/kg), intubated, and ventilated to maintain venous blood gas values similar to those obtained during spontaneous respiration. Sedation with diazepam and paralysis has previously been shown to have no effect on heart rate (HR), arterial pressure, or plasma catecholamine concentrations in lambs (29). Diazepam (0.1 mg/kg estimated wt) and vecuronium (0.1 mg/kg estimated wt) were also administered to the fetus. Muscle paralysis was necessary to eliminate movements that interfere with nerve recording. Additional doses of vecuronium (0.01 mg/kg) were administered when movement was detected. During the experiments, a constant infusion of a solution of 5% dextrose and 0.2% sodium chloride was administered to the ewe at a rate of 125 ml/h and to the fetus at 100 ml·kg⁻¹·day⁻¹. After intubation, a 1-h stabilization period was allowed before the start of the experiment.

During each experiment, fetal mean arterial blood pressure (MABP) and amniotic pressure were recorded continuously using Statham P23 Db pressure transducers (Spectrarecord, Critical Care Division, Oxnard, CA) and a Grass 7–24P chart recorder (Grass Instruments, Quincy, MA). Fetal MABP was corrected relative to concomitant amniotic pressure. HR was monitored with a cardiotachometer triggered from the arterial pressure pulse waves. Temperature (when applicable) was measured continuously using a Gould 13–4615–47 temperature amplifier (Gould, test and measurement group, Valley View, OH).

With the ewe housed inside a Faraday cage, the renal nerve electrodes and ground wire were attached to a high-impedance probe (HIP5, Grass Instruments). The neural signal was amplified (×20,000) and filtered (low-frequency cutoff 100 Hz, high-frequency cutoff 3 kHz) using a Grass P15C amplifier (P15C). The output of the amplifier was visually displayed on an oscilloscope (511A, Tektronix, Beaverton, OR) and routed to a Grass AM8 audio monitor. The neural signal was integrated over 1 s using a Grass voltage integrator. The integrated voltage and neurogram signals were stored on videotape (Vetter 4000A PCM, Vetter Digital, Rebersburg, PA), displayed on the recorder, and simultaneously recorded on-line to a personal computer using Labtech Notebook (version 7.2; Laboratory Technologies, Wilmington, MA).

Experimental protocol. The first series of studies (n = 13) was designed to characterize the changes in system hemodynamics, RSNA, and baroreflex function at birth in preterm lambs and to determine the effects of prenatal corticosteroid administration on these autonomic responses. Fetal baseline values for HR, MABP, and RSNA were obtained by recording and averaging those values over a 30-min period. Baroreflex function in the fetus was then determined by producing ramp changes in MABP with a continuous intravenous infusion of progressive doses of phenylephrine (1–30 μg·kg⁻¹·min⁻¹) over a 3- to 5-min period using a Harvard infusion pump, while simultaneously recording HR and RSNA. A 30- to 40-min recovery period was allowed for MABP, HR, and RSNA to return to baseline values. After this recovery period, the amount of background noise in the nerve signal was assessed by inhibiting nerve activity using an intravenous infusion of the ganglionic blocking agent tetraethylammonium bromide (10 mg/kg). Integrated RSNA was corrected by subtracting the background noise level obtained in the presence of ganglionic blockade.

After the fetal studies were completed, the ewes were returned to the surgical area and mechanical ventilation was continued. Low spinal anesthesia (1% lidocaine, 10 ml) was administered to the ewe, after which the lamb was delivered by cesarean section. Tracheal intubation of the lamb and administration of exogenous surfactant (100 mg/kg Surfactant, kindly provided by Abbott Laboratories, Columbus, OH) was performed before cutting the umbilical cord. Lambs were placed on an infant warmer bed, dried, manually ventilated, and returned to the laboratory. Lambs were then transferred to a sling-frame assembly to maintain them in an upright position and were mechanically ventilated with a time-cycled, pressure-limited infant ventilator. Initial ventilator settings included fractional concentration of inspired O₂ of 1.0, a rate...
of 40 breaths/min, an inspiratory time of 0.5 s, positive end-expiratory pressure of 4 cm H2O, and peak inspiratory pressure of 26 cm H2O. Arterial blood gases were obtained at least every 20 min, and ventilator settings were adjusted to maintain a partial pressure of O2 in arterial blood of 80–100 mmHg and PCO2 40–50 mmHg. Diazepam and vecuronium were administered to the lambs in doses previously noted. One and four hours after delivery, resting HR, MABP, and RSNA were again recorded for 15 min. Baroreflex function studies were performed after the 1-h recording. At the end of the study, background noise in the nerve signal was again assessed as described above.

Arterial blood for determination of blood gases and pH and plasma catecholamine (norepinephrine and epinephrine), arginine vasopressin (AVP), angiotensin II (ANG II), and cortisol levels were obtained before each baroreflex function study. The volume of blood sampled from the fetus or newborn was replaced immediately with an equivalent volume of maternal blood in order to avoid any hemodynamic effects of sampling.

A second set of experiments (n = 8) was designed to examine whether preterm fetal lambs are capable of generating a sympathoexcitatory response to other stimuli. We have previously shown in late-gestation fetal lambs that cooling the fetus in utero produces a >300% increase in RSNA (14). Therefore, we investigated the effects of in utero cooling on HR, MABP, and RSNA in preterm fetal lambs at 118–122 days of gestation.

These experiments consisted of four study periods: 1) control, 2) cooling, 3) after rewarming of the fetus, and 4) after delivery by cesarean section. Baseline fetal HR, MABP, and RSNA were obtained initially and averaged over a 15-min period. In utero fetal cooling was performed as previously described (14). Water cooled to 16–20°C was continuously pumped through tubing wrapped around the fetal thorax using a Lauda refrigerating circulator, model RKS-20D (Brinkman Instruments, Westbury, NY). The pump speed was set at its maximum capacity of 15 l/min. The study period was begun 10 min after starting the water pump, at a time when fetal core temperature was decreased by no more than 2°C. Water temperature was then reduced as necessary to maintain the fetus at the desired temperature. Values for HR, MABP, and RSNA were obtained every 15 min, after which the fetus was rewarmed by increasing the temperature of the circulating water to 42°C. A 1-h equilibration period was then allowed before again obtaining fetal hemodynamic and RSNA values. Finally, the fetus was delivered by cesarean section as described for the first series of studies. Values for HR, MABP, and RSNA were obtained at 1 h after birth. At the completion of each study period, arterial blood was sampled for determination of plasma catecholamine, AVP, ANG II, cortisol, and arterial blood gas values. All blood samples were replaced with an equivalent volume of maternal blood. Background electrical noise in the RSNA signal was determined at the completion of the studies as described above.

Analytic procedures. Arterial blood for pH, PCO2, and PO2 was collected anaerobically in heparinized syringes, and measurements were immediately determined using a BGM 1302 pH/blood gas analyzer (Instrumentation Laboratory, Lexington, MA). All blood gas values were corrected for fetal temperature. Measurements of cortisol, AVP, ANG II, and catecholamines were performed by radioimmunoassay (University of Iowa Cardiovascular Center RIA Core Facility, Donna B. Farley, Director).

Computation and data analysis. RSNA was normalized for each animal and expressed as the percentage of activity observed in the fetus under control conditions. Statistical analyses of differences in HR, MABP, RSNA, plasma hormone concentrations, and arterial blood gas values during the described study periods were performed using a two-way repeated-measures analysis of variance (ANOVA), factoring for treatment group and time. If the F value for the interactive term (group × time) was found to be significant (P < 0.05), indicating that differences between groups depend upon what level of time (fetus vs. 1 h vs. 4 h) is present, pairwise multiple-comparison procedures were performed by the Student-Newman-Kuels method (7). For data exhibiting a lack of homogeneity of variances among groups, nonparametric analysis was applied using the Kruskal-Wallis test.

For determination of baroreflex control of HR, control values of HR were defined as 100%. HR values were collected at 5-mmHg increments from control MABP (–5, 10, and 15 mmHg) for data analysis. Linear regression analysis of the changes in HR (expressed as %control HR) with increasing MABP was performed, with the differences in the slopes of the regression lines determined by analysis of variance. Differences in changes in HR were also analyzed by three-factor ANOVA (factoring for group, age, and change in blood pressure). Differences were considered significant when P < 0.05. All results are expressed as means ± SE.

RESULTS

Effect of delivery on arterial blood values. Fetal and newborn arterial blood values before and after delivery are summarized in Table 1. Fetal arterial PO2, PCO2, and pH were similar in preterm control and Dex-treated animals. After birth, significant decreases in pH and increases in PO2 were seen in both groups of animals. Arterial pH was significantly higher in Dex-treated animals at 1 and 4 h after birth compared with control animals.

Table 1. Arterial blood values before and after delivery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fetus</th>
<th>Newborn, h of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.79</td>
<td>7.24</td>
</tr>
<tr>
<td>Dex</td>
<td>7.42</td>
<td>7.34</td>
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<tr>
<td>PCO2, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>25±1</td>
<td>108±37*</td>
</tr>
<tr>
<td>Dex</td>
<td>23±1</td>
<td>147±51*</td>
</tr>
<tr>
<td>PCCO2, mmHg</td>
<td>40±5</td>
<td>36±5</td>
</tr>
<tr>
<td>Control</td>
<td>41±2</td>
<td>33±4*</td>
</tr>
<tr>
<td>ANGI, pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20±3</td>
<td>100±13*</td>
</tr>
<tr>
<td>Dex</td>
<td>33±7</td>
<td></td>
</tr>
<tr>
<td>NE, pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>160±30</td>
<td>231±51†</td>
</tr>
<tr>
<td>Dex</td>
<td>128±33</td>
<td></td>
</tr>
<tr>
<td>Epi, pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>516±328</td>
<td>1,896±438*</td>
</tr>
<tr>
<td>Dex</td>
<td>979±328</td>
<td></td>
</tr>
<tr>
<td>Cortisol, µg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.69±0.27</td>
<td>3.58±0.44†</td>
</tr>
<tr>
<td>Dex</td>
<td>1.48±0.40</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 in control group, n = 7 in dexamethasone (Dex) group. AVP, arginine vasopressin; ANG II, angiotensin II; NE, norepinephrine; Epi, epinephrine. *P < 0.05 compared with fetus; †P < 0.05 compared with other group at similar time. Statistical differences determined by 2-way repeated-measures analysis of variance or Kruskal-Wallis.
animals, whereas no differences in newborn PO2 or PCO2 values were seen between the two groups.

No differences were detected in fetal AVP, ANG II, norepinephrine, epinephrine, or cortisol values between control and Dex fetuses. After birth, plasma AVP levels significantly increased in control animals, although no significant difference was seen between newborn control and Dex AVP values. ANG II levels tended to increase in control animals after birth (P = 0.06) and were significantly higher in control than in Dex newborns. Norepinephrine levels significantly increased in both groups after birth, whereas plasma epinephrine values increased only in the control group. Plasma cortisol concentration increased after birth in control but not Dex animals.

Effect of delivery on systemic hemodynamics and RSNA. The changes in HR, MABP, and RSNA occurring with delivery in term and preterm sheep are shown in Fig. 1. Data for the term sheep have previously been published by our group (25) and are presented for comparison with values obtained in preterm sheep. In contrast to term sheep, in which significant increases in HR and MABP occur after birth, preterm sheep failed to demonstrate increases in either HR or MABP after delivery. In fact, by 4 h after birth, HR was significantly less than that seen in the fetus (−31 ± 6%, P < 0.05). Large differences in the sympathetic response at birth were also seen between term and preterm sheep. One hour after delivery, RSNA increased by almost 250% (P < 0.05) in term fetuses, and remained at this level at 4 h after birth. However, in preterm fetuses, RSNA actually decreased after birth, (P < 0.05 at 1 and 4 h) being ~35–40% of the value obtained in utero.

Preterm fetuses receiving antenatal Dex had similar fetal HR and MABP values compared with preterm controls, although there was a tendency for HR to be lower and MABP higher in the Dex group (Fig. 1). However, unlike controls, Dex animals had significant increases in HR (+27 ± 6 beats/min) and MABP (10 ± 2 mmHg) 1 h after birth. The increase in HR was sustained at 4 h, whereas MABP returned to values similar to those in the fetus. Nonetheless, 4-h HR and MABP values were significantly greater in Dex animals than in controls. A large difference in the RSNA response at birth was also seen in the two preterm fetal groups (Fig. 1). RSNA after birth was significantly greater in Dex animals than in controls, the difference being present at both 1 and 4 h. Although the mean value for RSNA in the Dex animals at 1 h is not significantly different from the fetal value, it should be noted that five of seven Dex-treated animals showed an increase in RSNA at birth, whereas all seven of the control animals showed a decrease. The increase in RSNA after birth in Dex animals was significantly less than that previously seen in term sheep.

Effect of antenatal Dex on baroreflex control of HR before and after birth. Progressive intravenous administration of increasing doses of phenylephrine resulted in increases in MABP and pressure-related decreases in HR (Fig. 2). Values for HR after 10- and 15-mmHg increases in MABP were significantly lower in control animals, whereas no differences in newborn PO2 or PCO2 values were seen between the two groups.

Fig. 1. Changes in mean arterial blood pressure (MABP), heart rate (HR), and renal sympathetic nerve activity (RSNA) at birth in term, preterm, and dexamethasone (Dex)-treated preterm lambs delivered by cesarean section. Data for term fetuses from Segar et al. (25). bpm, Beats/min; d, days. *P < 0.05 compared with fetus in same group; †P < 0.05 compared with preterm at similar chronologic age; ¥P < 0.05 compared with other groups of similar chronologic age.

Fig. 2. Relationship between increase in MABP and reflex decrease in HR during intravenous infusion of phenylephrine. Data points for HR at each increment in MABP are expressed as means ± SE. Lines represent least squares linear regression equations fit to these points, including baseline data. *P < 0.05 for slopes of MABP-HR relationship in Dex animals before (fetus) and after birth (newborn) compared with those in control animals; †P < 0.05 compared with control values at similar changes in MABP.
animals than in those receiving Dex. Using linear regression analysis, we found significant differences in the slope of the relationship between MABP and HR in Dex and control fetuses (\(21.8 \pm 0.4\) vs. \(23.5 \pm 0.9\% / \text{mmHg}\), respectively). The slope of the baroreflex response for HR was also significantly greater (\(P < 0.05\)) in control (\(24.7 \pm 1.0\% / \text{mmHg}\)) than in Dex newborns (\(-1.9 \pm 0.3\% / \text{mmHg}\)). Within each treatment group, no differences were detected in the MABP-HR slope between fetal and newborn values.

In addition to evaluating baroreflex control of HR early during development, we also attempted to assess reflex control of RSNA. It was evident that in several fetuses, increases in MABP resulted in inhibition of RSNA (Fig. 3). However, these RSNA responses were not always reproducible and were not present in all animals. Specifically, in several animals, RSNA appeared dissociated from the reflex-mediated inhibition in HR. Therefore, we were unable to accurately determine measurement of the MABP-RSNA relationship.

Effects of in utero cooling on arterial blood values. Arterial blood values during control conditions and after fetal cooling, fetal rewarming, and delivery are shown in Table 2. Arterial PCO₂ remained unchanged throughout the experiment. Arterial pH was significantly lower after birth compared with all fetal conditions, whereas PO₂ was significantly higher after birth. Plasma AVP, ANG II, and cortisol concentrations remained unchanged throughout the study. Norepinephrine levels were increased (\(P < 0.05\)) during the fetal cooling and newborn periods, whereas epinephrine levels increased only after birth.

Table 2. Arterial blood values during fetal cooling and after birth

<table>
<thead>
<tr>
<th></th>
<th>Fetus Control</th>
<th>Fetus Cool</th>
<th>Fetus Rewarm</th>
<th>Newborn (1 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.38 ± 0.06</td>
<td>7.39 ± 0.07</td>
<td>7.36 ± 0.05</td>
<td>7.27 ± 0.08*</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>42 ± 3</td>
<td>40 ± 3</td>
<td>46 ± 3</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>21 ± 1</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
<td>21 ± 1²</td>
</tr>
<tr>
<td>AVP (µU/ml)</td>
<td>22.6 ± 3.9</td>
<td>18.4 ± 2.8</td>
<td>28.4 ± 9.2</td>
<td>20.5 ± 1.2</td>
</tr>
<tr>
<td>ANG II (pg/ml)</td>
<td>58 ± 7</td>
<td>60 ± 10</td>
<td>48 ± 9</td>
<td>70 ± 13</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>399 ± 80</td>
<td>723 ± 112†</td>
<td>372 ± 53</td>
<td>812 ± 147†</td>
</tr>
<tr>
<td>Epi (pg/ml)</td>
<td>226 ± 86</td>
<td>173 ± 22</td>
<td>179 ± 42</td>
<td>465 ± 99*</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>2.21 ± 0.19</td>
<td>2.62 ± 0.27</td>
<td>2.47 ± 0.19</td>
<td>3.61 ± 0.55</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 8\). *\(P < 0.05\) compared with all fetal values; \(1P < 0.05\) compared with control and Rewarm.

Effects of in utero cooling on systemic hemodynamics and RSNA. Ten to fifteen minutes after the beginning of in utero cooling of the fetus, during which time fetal core temperature decreased by \(-2^\circ\text{C}\), significant increases in HR (26 ± 6%), MABP (20 ± 4%), and RSNA (282 ± 72%) were demonstrated (Fig. 4). Notably, significant changes in systemic hemodynamics and RSNA occurred within minutes after cooling began, before any significant decrease in core temperature. Five minutes after the start of the water pump, at a time when fetal core temperature had decreased by 0.4 ± 0.1°C, HR, MABP, and RSNA had increased by 23, 17, and 259%, respectively. Rewarming of the fetus...
returned HR, MABP, and RSNA to values similar to those observed during the control period. The final intervention of the experiment consisted of delivering the fetus by cesarean section. During this period, HR remained similar to that observed during the fetal control period and after rewarming. However, in contrast to fetuses not previously cooled in utero (first series of studies), animals delivered after cooling and rewarming demonstrated significant increases in MABP (10 ± 2 mmHg) and RSNA (145 ± 62%) by 1 h after birth. This increase in RSNA was also significantly greater than that seen in Dex animals at birth.

DISCUSSION

In previous experiments in term fetal sheep, we demonstrated that sympathetic outflow, as measured in the renal nerve, increases dramatically at birth and parallels the increases in HR and arterial pressure (25). The present studies were designed to characterize the changes in RSNA at birth in preterm fetal lambs and to investigate the effects of antenatal glucocorticoid administration on the sympathetic response and baroreflex control of HR at birth. The results from these studies indicate that in contrast to lambs delivered at term, a sympathoexcitatory response is absent at birth in preterm sheep. However, preterm fetal sheep of ewes receiving antenatal glucocorticoids had improved cardiovascular and sympathetic function at birth compared with control animals of similar gestational age. Glucocorticoids also decreased the sensitivity of the cardiac baroreflex after birth. The impaired sympathetic response at birth seen in non-Dex-treated fetuses occurred despite the fact that the descending pathways of the sympathetic nervous system appear intact and functional at this stage of development, as demonstrated by the large pressor and sympathoexcitatory response to in utero cooling.

Postnatal cardiovascular and metabolic functions are attenuated after premature birth compared with those seen at term (22). These maturational differences cannot be attributed to adrenal unresponsiveness because postnatal circulating catecholamine levels are higher in preterm than term animals (22). Investigators have therefore speculated that immature or incomplete end-organ responses to adrenergic receptor stimulation in target tissues contribute to these impaired responses (21, 32) or that other neurohumoral mechanisms are involved in regulating the physiological adjustments at birth which are not fully developed in the preterm animal. In contrast to our previous observations in term sheep, the present results demonstrate that a sympathoexcitatory response is absent at birth in preterm sheep and that RSNA actually decreases after birth. We speculate that this impaired sympathetic response accompanying premature birth contributes to the attenuated postnatal cardiovascular responses seen with prematurity and suggest that autonomic modulation of circulatory function is of major importance in adaptation to the extraterine environment.

The effects of corticosteroids on fetal lung development and pulmonary function are well known (2). More recently, it has been appreciated that the use of antenatal glucocorticoids improves cardiovascular function after premature birth (10, 21, 32). Both maternal and direct fetal administration of glucocorticoids improve postnatal blood pressure, cardiac output, and cardiac contractility in prematurely delivered lambs, although the mechanisms regulating these responses are not clearly understood (21, 31, 32). Stein et al. (32) demonstrated that myocardial adenyl cyclase activity is augmented in corticosteroid-treated fetal lambs, although myocardial β-receptor density and affinity were similar in treated and nontreated fetuses. Exogenous cortisol has also been shown in immature fetal sheep to enhance the vascular responsiveness to ANG II, the levels of which increase at birth, but not to norepinephrine (6, 34). Finally, glucocorticoids may also regulate the synthesis of vasoactive compounds, such as prostaglandins (8) or nitric oxide (33), which in turn modulate peripheral vascular reactivity.

It may also be hypothesized that the effect of glucocorticoids on postnatal cardiovascular function is mediated by central mechanisms. In the rat brain, glucocorticoid receptors are present in the fetus and increase in number after birth (16). Studies in a number of species have shown that glucocorticoid receptors are preferentially concentrated within limbic system structures, the paraventricular nucleus of the hypothalamus (PVN), and the nucleus of the solitary tract (16), these latter two regions being strongly implicated as cardiovascular control centers (13). Maternal administration of Dex accelerates development of central noradrenergic function in newborn rats, as measured by norepinephrine turnover (26), and enhances noradrenergic synaptogenesis (27). At a biochemical level, glucocorticoids increase phenylethanolamine N-methyltransferase (PNMT) within the brain stem and hypothalamus of fetal and newborn rats (36). Increased PNMT activity within the central nervous system has been postulated to raise blood pressure by increasing sympathetic activity, although the data are not consistent (11). Interestingly, a number of studies suggest that in adult animals with glucocorticoid hypertension, sympathetic activity appears reduced or unchanged (40, 41). In addition, in adult rats, central administration of Dex exerts a blood pressure-lowering effect, whereas intra-cerebroventricular injection of a glucocorticoid antagonist increases blood pressure (35, 39). Whether similar effects of central glucocorticoids are present in the developing animal has not been investigated.

The finding that the gain of the HR baroreflex response was reduced in the Dex animals provides additional evidence that glucocorticoids exert an effect on the autonomic nervous system. It is not known whether the effects on the baroreflex were mediated by afferent (within the carotid sinus) or central or efferent (responsiveness of the sinoatrial node) mechanisms. Within the carotid sinus, glucocorticoids may alter the release of endothelial factors that have been shown to contribute to baroreceptor resetting (4). As previously mentioned, glucocorticoid receptors are present in high density within the nucleus of the solitary tract, the initial component of the central baroreflex pathway, as well as the PVN. Alteration of the baroreflex by glucocor-
ticoids may therefore be mediated by effects on neurotransmitters or neuronal activity within these regions. Finally, acute elevations in arterial pressures seen in the Dex-treated fetuses at birth may also contribute to resetting of the baroreflex (4). However, this latter mechanism appears unlikely because the sensitivity of baroreflex responses for HR and RSNA is unchanged immediately after birth in term fetuses despite a significant increase in resting blood pressure (24), and 2) acute (1–2 h) increases in blood pressure produced by phenylephrine do not reset baroreflex function in newborn lambs (24).

Unfortunately, we were not able to reliably assess the RSNA baroreflex. Several animals appeared to have functional baroreflex control of RSNA, whereas in other animals RSNA but not HR appeared dissociated from incremental changes in MABP. These responses are different from those we have previously described in term fetal lambs. In these studies, we found that the sensitivity of the baroreflex-mediated inhibition of RSNA (and HR) was greater in fetal sheep than in 1- and 6-wk-old lambs and parallels the developmental changes observed in the carotid sinus nerve activity. These findings suggest that development of baroreflex control of HR and RSNA are not simultaneous and that in fetal lambs reflex control of HR occurs before control of sympathetic outflow.

We noted that antenatal steroids attenuated the postnatal surge in plasma epinephrine but not in norepinephrine concentrations, whereas other investigators have found both epinephrine and norepinephrine levels to be lower in treated animals (21). Reasons for this difference are unknown but may be related to differences in the type or dosage of glucocorticoid administered or gestational age of the fetuses studied. Catecholamine release from the immature adrenal gland is primarily regulated by nonneurogenic mechanisms that disappear concurrently with the onset of neurogenic control (28). Maturation of neurogenic control may be stimulated by repeated maternal stress during late gestation, neonatal hyperthyroidism (28), and we speculate, exogenous corticosteroids. Thus the previously observed reduction in plasma epinephrine and norepinephrine levels may result from Dex-induced development of splanchnic nerve function and maturation of neurogenic control of adrenomedullary catecholamine release (28). If indeed neuronal competence is present (or nonneurogenic secretory capabilities are lost) in Dex fetuses, failure of the Dex-treated preterm lamb to generate a large sympathetic response at birth (relative to the term fetus) may therefore result in an impaired postnatal surge in catecholamine release.

Because a sympathoexcitatory response was absent after delivery of preterm lambs, we sought to determine if immature sheep are capable of mounting a sympathetic response to other stimuli. We have previously shown in late-gestation fetal lambs that cooling the fetus in utero by circulating cooled water through tubing wrapped around the fetal thorax produces a >300% increase in RSNA (14). Surprisingly, the immature fetus not treated with Dex displayed a similar pronounced increase in sympathetic outflow, suggesting that at this stage of development, the descending pathways of the sympathetic nervous system are intact and functional. This increase in sympathetic activity was accompanied by increases in HR, blood pressure, and plasma norepinephrine levels. Consistent with that seen in term fetuses, the increase in RSNA was observed with the onset of cooling before a significant decrease in core temperature occurred. In addition, fetal HR, MABP, RSNA, and norepinephrine levels decreased with subsequent rewarming, indicating that the sympathoexcitation was stimulus dependent and likely mediated by sensory input from skin thermal receptors.

The mechanisms regulating sympathoexcitation at birth are not known but may be related to hypoxemia, acidemia, and surface cooling (14, 38). The lack of a sympathoexcitatory response at birth in immature animals suggests that either 1) pathways carryingafferent signals mediating birth-related sympathetic activation are immature, 2) central integration of these signals is absent, or 3) there is a centrally mediated suppression of sympathetic outflow. Unexpectedly, we found that preterm lambs exposed to in utero cooling had augmented cardiovascular and sympathetic responses at birth compared with noncooled animals at a similar gestational age. The mechanism(s) for this apparent facilitative effect of in utero cooling on sympathetic activity at birth is not known. We speculate that as part of the physiological response to cooling, modulators of a centrally mediated suppression of sympathetic activity are in turn inhibited or downregulated. Acute cold stress alters the level and biosynthesis of a number of neuropeptides and neurotransmitters, including γ-aminobutyric acid, thyrotropin-releasing hormone, corticotropin-releasing hormone, and neuropeptide Y within the hypothalamus, including the PVN (1, 15, 23, 37, 42). Changes in the expression of these neuropeptidemakers may in turn augment the sympathoexcitatory response at birth.

Perspectives

The mechanisms regulating the numerous physiological adjustments at birth remain largely unknown. This study demonstrates that despite appearing to have intact peripheral adrenergic pathways, preterm sheep have an impaired sympathetic response at birth consistent with the attenuated postnatal changes in cardiovascular function. The maturational effect of glucocorticoids on the sympathetic response at birth may be one mechanism by which maternal steroid administration improves cardiovascular homeostasis after birth and lessens the incidence of complications associated with hypotension and disordered hemodynamic regulation.

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REFERENCES


