Effects of prenatal betamethasone exposure on regulation of stress physiology in healthy premature infants

Elysia Poggi Davisa,*, Elise L. Townsendb, Megan R. Gunnarb, Michael K. Georgieffb,c, Sixto F. Guiangc, Raul F. Ciffuentesd, Richard C. Lusskyd

aDepartment of Psychiatry and Human Behavior, University of California Irvine, City Tower, 333 City Boulevard West, Orange, CA 92868, USA
bInstitute of Child Development and the Center for Neurobehavioral Development, University of Minnesota, 51 East Rover Road, Minneapolis, MN 55455, USA
cDepartment of Pediatrics, D-136 Mayo, 420 Delaware St SE, University of Minnesota, Minneapolis, MN 55455, USA
dDepartment of Pediatrics—867B, Hennepin County Medical Center, 701 Park Avenue South, Minneapolis, MN 55415, USA

Received 18 October 2002; received in revised form 4 September 2003; accepted 20 October 2003

KEYWORDS
Cortisol; Stress; HPA axis; Prematurity; Prenatal experience; Betamethasone

Summary The objective of this study was to examine the effects of prenatal exposure to betamethasone, a corticosteroid, on postnatal stress regulation, particularly activity of the hypothalamic-pituitary-adrenocortical (HPA) axis. Effects were assessed by measuring salivary cortisol production at baseline and in response to two potentially stressful events, a heel-stick blood draw and a physical exam, in infants born at 33–34 weeks gestation. Subjects included 9 infants with antenatal betamethasone treatment (2 doses of 12 mg of betamethasone administered intramuscularly to the mother twelve hours apart) and 9 infants without such treatment. Testing took place 3–6 days after delivery. Measures of behavioral distress confirmed that both events were stressful to these premature infants. Infants with betamethasone exposure, however, failed to exhibit increases in cortisol to either stressor. In contrast, infants without betamethasone exposure displayed elevated cortisol to the heel-stick blood draw but not the physical exam. These findings suggest that antenatal corticosteroids suppress infants’ HPA response to a stressor typically encountered in a neonatal intensive care situation.

© 2003 Elsevier Ltd. All rights reserved.

Work with rodents and nonhuman primates has illustrated that prenatal exposure to elevated levels of glucocorticoids has life long consequences for the regulation of the hypothalamic-
pituitary-adrenocortical (HPA) axis (Levitt et al., 1996; Matthews, 2000; Muneoka et al., 1997; Takahashi, 1998; Uno et al., 1994; Welberg and Seckl, 2001). The HPA axis produces cortisol, often described as a stress hormone. Cortisol has a multitude of physiological effects and when released during stress enhances an organism’s ability to adapt (McEwen, 1998; Sapolsky et al., 1996; Matthews, 2000; Muneoka et al., 1997; Takahashi, 1998; Uno et al., 1994; Welberg and Seckl, 2001). The HPA axis produces cortisol, which is a stress hormone. Cortisol has numerous physiological effects and when released during stress enhances an organism’s ability to adapt (McEwen, 1998; Sapolsky et al., 1996; Matthews, 2000; Muneoka et al., 1997; Takahashi, 1998; Uno et al., 1994; Welberg and Seckl, 2001).

Regulation of the HPA axis has critical implications for health and development. Despite evidence for a lasting impact of prenatal exposure to corticosteroids on the functioning of the HPA axis in animals, few studies of this phenomenon have been undertaken in human populations (Ward and Phillips, 2001). This issue is critical given the widespread use of corticosteroids in pregnant women at risk for premature delivery. Antenatal corticosteroid administration is a standard of care for women at risk of premature delivery and has been shown to reduce mortality and respiratory distress among preterm infants born at less than 34 weeks gestation. Betamethasone, one of the two preferred corticosteroids used for antenatal treatment, crosses the placenta and initiates surfactant production in fetal lungs. Surfactant production in premature infants facilitates respiratory function with less pharmacological and mechanical support (ACOG Committee Opinion, 2002; Maher, Cliver, Goldenberg, Davis, & Copper, 1994; Murphy, 2001; NIH Consensus Development Conference, 1995). Additionally, betamethasone crosses the blood brain barrier and thus, may impact central nervous system development (Antonow-Schlorke et al., 2003; Trenque et al., 1994). The short-term side effects of synthetic corticosteroids, including betamethasone, seem to be well tolerated by preterm infants (Bettendorf et al., 1998). However, long-term effects of steroid administration on the HPA axis are unknown (Bakker, van Bel and Heijnen, 2001; Bettendorf et al., 1998).

Research examining the effects of antenatal corticosteroid treatment on HPA axis regulation in humans has focused primarily on baseline cortisol production. Baseline cortisol levels are suppressed for 2–7 days after prenatal corticosteroid treatment and subsequently return to normal levels (Ballard et al., 1980; Dorr et al., 1989; Kauppila et al., 1978; Parker et al., 1996; Wittekind et al., 1993). These studies, however, failed to examine the HPA response to a stressor. Only one study has assessed the effect of antenatal corticosteroid administration on HPA axis activation in humans. This study employed the human corticotrophin releasing hormone (hCRH) stimulation test. The cortisol response to the hCRH test was suppressed in infants who received antenatal corticosteroid treatment indicating that exposure impairs activation of the HPA axis (Ng et al., 2002). These data raise the possibility that antenatal corticosteroids may suppress the HPA axis responsivity to environmental stressors experienced in the neonatal intensive care unit (NICU). The effect of antenatal corticosteroids on regulation of the HPA response to stressors, however, has not been examined.

The goal of the current study was to evaluate the impact of antenatal betamethasone exposure on cortisol levels at resting baseline and in response to stressors typical of the NICU environment. Previous work indicates that both full term infants (Gunnar, 1992; Gunnar et al., 1992) and preterm infants (Magnano et al., 1992) without antenatal corticosteroid treatment display increases in cortisol to two stressors; a physical exam and a heel-stick blood draw. Infants, however, respond differently to these events. Full term infants cry less and habituate more quickly to physical exams than heel-stick blood draws across daily presentations, suggesting that the blood draw is more aversive than the physical exam (Gunnar et al., 1992).

Physical exams and heel-stick blood draws were used as stressors to examine whether antenatal betamethasone suppresses cortisol production. Infants who did and did not receive antenatal betamethasone treatment were assessed between postnatal days three and six after stabilization of birth-related fluctuations in basal cortisol (Stahl et al., 1979). To determine whether antenatal betamethasone effects were specific to the cortisol response, behavioral state and heart rate responses to these stressors were also examined.

1. Methods

1.1. Participants

Subjects included 18 healthy premature infants (33–34 weeks gestational age at birth). Verbal and written consent was obtained prior to each infant’s enrollment in the study. Consent was obtained from 90% of parents solicited for participation. Subjects represented two groups: a no treatment group, 9 infants (4 girls and 5 boys) without antenatal betamethasone exposure and a treatment group, 9 infants (4 girls and 5 boys) whose mothers had received a single course of antenatal betamethasone prior to delivery. Participants were recruited from two large metropolitan hospitals with neonatal intensive care units: Fairview University Medical Center (n = 10) and
Hennepin County Medical Center (n = 8) with 550 and 250 admissions per year respectively.

Only healthy infants appropriate for gestational age were included. All infants met the following criteria: within 2 standard deviations of the mean length and weight for gestational age (determined by plotting the measurements on standardized growth curves), and absence of chromosomal or other genetic anomalies (e.g., trisomy 21), congenital infections, chronic lung disease, mechanical ventilation over 24 hours, interventricular hemorrhage, neonatal illness (e.g., sepsis), maternal history of adrenal illness or endocrine problems (e.g., diabetes), major maternal illness, and maternal substance use during pregnancy (e.g., alcohol).

1.1.1. Antenatal betamethasone
The decision to administer antenatal betamethasone rested entirely on the clinical judgment of the attending obstetrician. Antenatal betamethasone was not administered to mothers of infants in the no treatment group because delivery was imminent1. Infants in the treatment group were exposed to a single course of betamethasone. Each course consisted of two doses of 12 mg of betamethasone delivered intramuscularly to the mother twelve hours apart. The second of the two doses was administered between one and twenty-one days prior to delivery (M = 8.3).

1.1.2. Clinical characteristics
Clinical information for infants in both groups is described in Table 1. Mothers of the two groups of infants did not differ in age at time of delivery, marital status, race, reported cigarette use, or whether they received prenatal care from an obstetrician during the first half of pregnancy. Infants in the two groups did not differ in one or five-minute Apgar scores, birth weight, length, or head circumference, method of delivery, whether they received prenatal care from an obstetrician during the first half of pregnancy. Infants in the two groups did not differ in one or five-minute Apgar scores, birth weight, length, or head circumference, method of delivery, whether they were a singleton or fraternal twin.

1.2. Procedure
Medical history was obtained through chart review. On two days between postnatal days three and six, resting baseline and responses to a heel-stick blood draw and a physical exam (order counterbalanced) were examined. Duration of each stressor and the number of prior heel-sticks or exams were recorded. Infants’ postnatal age on the heel-stick blood draw day (M = 4.1 days vs. M = 4.3 days), t(16) = 0.29, p = 0.77, and physical exam day (M = 5.2 days vs. M = 4.4 days), t(16) = 1.84, p = 0.09, did not differ between groups.

Each two hour testing period began one hour after the feeding that occurred between 0400 and 0700 hours. First, infants were monitored continuously for one hour and behavioral state was recorded at five-minute intervals. Infants were not handled and all infants were observed to be in either quiet or active sleep during this hour. Resting baseline cortisol, heart rate, and behavioral state were then assessed. Thus, these measures were taken two hours after the infant’s last feeding and after the infant was observed to be sleeping for an hour.

During the five minute resting baseline, heart rate was collected and the infant was videotaped for behavioral observations. Resting baseline salivary cortisol was then assessed, followed by the event period consisting of either a physician’s physical exam or a physician ordered heel-stick blood draw2. Heart rate recording and videotaping continued during the event and recovery periods. Salivary cortisol samples were obtained 20–25 minutes and 40–45 minutes after the start of the event.

1.3. Measures

1.3.1. Salivary cortisol
Saliva was collected without waking the infants by placing a Q-tip® cotton swab in the infant’s mouth, for 5 minutes, until the cotton was saturated3. Salivary cortisol reflects the unbound or active fraction of cortisol and is highly correlated with plasma cortisol in premature and full term newborns, children, and adults (Calixto et al., 2002; Gunnar, 1989; Kirschbaum and Hellhammer, 1989; Woodside et al., 1991). The Q-tip was then placed in a salivette® from Sarstedt, centrifuged (10 mins at 4000 Hz) to extract saliva and stored in a freezer at −20 °C until assayed. Saliva samples were assayed for cortisol determination employing a competitive solid phase time-resolved fluorescence immunoassay with fluoroemeric end

---

1 Management guidelines for starting antenatal betamethasone therapy in women between 24 and 34 weeks gestation were: 1) threatened preterm labor, 2) antepartum hemorrhage, 3) premature rupture of membranes, and 4) any condition requiring elective premature delivery.

2 Blood draws were performed for clinically indicated reasons and not for the purposes of this study.

3 To ensure that the cotton on the Q-tip was not interfering with the assay saliva samples were collected from 7 adult volunteers. Participants spit directly into the salivette to collect a ”pure” sample. A Q-tip was then placed into the saliva sample until saturated and then placed into a second salivette. Salivettes were placed into a centrifuge to extract saliva and then stored in a freezer at −20 °C until assayed. Q-tip samples were highly correlated with pure samples, r(7) = 0.985, p = 0.0001.
point detection (DELFIA) (Dressendorfer et al., 1992). All samples from one infant were included in the same assay batch to eliminate within subject inter-assay variance. Each batch contained both control and betamethasone subjects. Volume permitting, samples were assayed in duplicate and averaged. Sixty-eight percent of the samples were assayed in duplicate. The inter-assay and intra-assay coefficients of variance were 11.99 and 4.30, respectively.

1.3.2. Heart rate
Heart rate was recorded continuously from premature infants in the NICU using either a Space Labs or an Air Shields monitor. Heart rate was retained for the purposes of this study at 30-second intervals during a five-minute resting baseline period just prior to the event, during the event, and during the 5-minute recovery period. Mean heart rate was calculated for each of the 3 periods.

1.3.3. Behavioral coding
Videotapes obtained for this purpose were coded for behavioral state during the resting baseline period, the event, and the recovery period. Behavioral state was assessed using a modified version of a coding system designed for premature infants (Als et al., 1988). This scheme was used to categorize each infant’s state on a scale of 1 to 6: quiet sleep, active sleep, quiet awakeness/drowsy, awake and alert, awake and fussy, and crying. Tapes were coded in 10-second epochs. Codes recorded represented the highest level of state or activity noted during each 10-second epoch. Percent agreement for state codes, obtained on 20% of the tapes, was 92.9%. Infants’ average state score during the resting baseline, event and recovery periods were calculated.

1.3.4. Analysis plan
The physical exam and heel-stick blood draw events were assessed to ensure that they did not differ between treatment groups. Distributions were examined and measures found to be positively skewed were log transformed. Next, dependent measures were examined to determine if they were affected by treatment group, event, or trial.
Cortisol data were log transformed to normalize the distribution. Cortisol measures were examined to determine whether cortisol levels were affected by treatment group (No treatment, Treatment), event (Heel-stick, Physical exam), or trial (Resting baseline, 20 min post event, and 40 min post event) using an ANOVA with repeated measures on the last 2 factors, with Greenhouse-Geisser corrections as necessary. Planned contrasts to examine linear and quadratic effects were utilized to test the hypothesis that infants with antenatal betamethasone would not increase cortisol to either event, but that infants without antenatal betamethasone would respond to the heel-stick blood draw.

Next, infants’ heart rate and behavioral state scores were examined to determine whether they were affected by treatment group (No treatment, Treatment), event (Heel-stick, Physical exam), or trial (Resting Baseline, Event, and Recovery) using separate ANOVAs with repeated measures on the last 2 factors, with Greenhouse-Geisser corrections as necessary. The hypothesis that heart rate and behavioral state would be higher during the event relative to baseline and recovery was tested utilizing planned contrasts to test for quadratic trends.

2. Results

2.1. Assessment of heel-stick and physical exam

Duration of the heel-stick and physical exam events did not differ between infants with or without antenatal betamethasone; heel-stick ($M = 3.6$ minutes vs. $M = 3.8$ minutes), $t(16) = 1.88$, $p = 0.08$, physical exam ($M = 3.8$ minutes vs. $M = 3.7$ minutes), $t(16) = 0.23$, $p = 0.82$. Likewise, the groups did not differ on number of previous heelsticks ($M = 7.2$ vs. $M = 7.5$), $t(15) = 0.23$, $p = 0.52$, or physical exams ($M = 4.4$ vs. $M = 5.2$), $t(16) = 1.84$, $p = 0.09$. Additionally, to ensure that the inclusion of twins did not alter the pattern of results the mean cortisol levels at baseline, response and recovery on the physical exam and heel-stick event days in twins and non-twins were examined and found not to differ ($p’s > 0.20$).

2.2. Cortisol

Two subjects were missing sample 3 on the physical exam day due to a failure to collect a sufficient volume of saliva. For the 2 subjects with missing sample 3 data the mean of their standard scores for sample 1 and 2 on the physical exam day was used to estimate sample 3. There was a main effect of trial, $F(2,15) = 6.4$, $p = 0.01$, but not treatment group, $F(1,15) = 2.3$, $p = 0.38$, or event, $F(1,15) = 0.28$, $p = 0.71$. There was, however, a significant three-way interaction between treatment group, event, and trial, $F(2,15) = 5.38$, $p = 0.017$. This finding remained when the two subjects with missing sample 3 data were excluded $F(2,13) = 8.2$, $p = 0.002$.

To explore the 3-way interaction, separate treatment 2 (group) by 2 (trial) ANOVAs with repeated measures on the last factor were run for each event. For the physical exam there was a main effect of trial, $F(2,15) = 5.26$, $p = 0.019$, but no interaction between treatment group and trial, $F(2,15) = 1.27$, $p = 0.20$. Planned contrasts for the main effect of trial revealed a significant linear trend such that cortisol decreased from resting level to 20 and 40 mins post exam $F(1,16) = 9.6$, $p = 0.007$ (see Fig. 1).

For the heel-stick blood draw, both the main effect of trial, $F(2,15) = 7.5$, $p = 0.005$, and the interaction between treatment group and trial, $F(2,15) = 4.03$, $p = 0.04$, were significant. Polynomial contrasts for this interaction yielded significant quadratic trends, $F(1,16) = 7.35$, $p = 0.015$. Infants with antenatal betamethasone treatment displayed decreases in cortisol at 20 and 40 minutes post event relative to their resting baseline levels, whereas infants in the no treatment group showed increases in cortisol at 20 minutes post event (See Fig. 2).

Betamethasone was administered between one and twenty-one days prior to delivery. Days post betamethasone administration was not correlated with cortisol levels ($p’s > 0.1$) using Spearman rank-order correlation. Thus infants treated three weeks prior to testing exhibited cortisol suppressions that were no different than suppression
exhibited by infants who were treated within one week of testing.

2.3. Heart rate

Heart rate data were not obtained from one subject on the heel-stick day due to technical problems. There was a main effect of trial, $F(2,14) = 24.99, p = 0.0001$, but not treatment group, $F(1,15) = 0.062, p = 0.81$, or event, $F(1,15) = 0.007, p = 0.93$. Planned contrasts for quadratic trends showed that infants display higher heart rates during the event relative to the resting baseline or recovery period, $F(1,15) = 52.2, p = 0.0001$. There was also a treatment group by trial interaction, $F(2,14) = 5.65, p = 0.016$, indicated that infants with antenatal betamethasone treatment displayed a significant increase in heart rate from the baseline to the event period, $t(8) = 9.8, p = 0.0001$, whereas the increase in heart rate seen in infants without treatment did not reach traditional levels of significance, $t(8) = 1.97, p = 0.08$ (see Table 2).

2.4. Behavioral state

The main effects of trial, $F(2,15) = 21.8, p = 0.0001$, and event, $F(1,16) = 12.67, p = 0.003$, but not treatment group, $F(1,16) = 1.11, p = 0.31$, were significant. Quadratic trends testing the hypothesis that infants would be in a more aroused behavioral state during the event as compared to resting baseline and recovery were significant, $F(1,16) = 46.13, p = 0.0001$. There was also an event by trial interaction, $F(2,15) = 9.58, p = 0.002$, indicated that, consistent with the literature on full-term infants, these premature infants were in a more aroused behavioral state during the heel-stick event period as compared to the physical exam event period, $t(17) = 6.4, p = 0.0001$ (see Table 3).

3. Discussion

Infants exposed to antenatal betamethasone failed to mount a cortisol response to a painful stimulus, a heel-stick blood draw. In fact, these

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of heart rate changes in response to heel-stick blood draw and physical exam in infants with and without antenatal betamethasone exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N$</td>
</tr>
<tr>
<td><strong>Heel-stick</strong></td>
<td></td>
</tr>
<tr>
<td>Resting baseline</td>
<td>8</td>
</tr>
<tr>
<td>Event</td>
<td>8</td>
</tr>
<tr>
<td>Recovery</td>
<td>8</td>
</tr>
<tr>
<td><strong>Physical Exam</strong></td>
<td></td>
</tr>
<tr>
<td>Resting baseline</td>
<td>8</td>
</tr>
<tr>
<td>Event</td>
<td>8</td>
</tr>
<tr>
<td>Recovery</td>
<td>8</td>
</tr>
</tbody>
</table>

Heart rate is reported in beats per minute.
infants exhibited a significant decrease in cortisol in response to this event. Notably, infants with antenatal betamethasone treatment reacted to the heel-stick with increases in both behavioral state and heart rate indicating that they were responding to this event. In contrast, infants who did not receive prenatal betamethasone treatment displayed an increase in cortisol in response to the heel-stick stressor. This cortisol response, also displayed by full term infants, is considered appropriate and supports adaptation to challenge (Gunnar, 1992). Consistent with previous research demonstrating that baseline cortisol is suppressed only for the first two to seven days after prenatal treatment, these two groups of infants did not differ in their resting baseline cortisol levels. These data suggest that infants with betamethasone treatment are able to maintain appropriate levels of baseline cortisol but their ability to mount a cortisol response to stress is impaired even beyond the first week after treatment.

Similar to healthy full term infants, neither group of premature infants displayed cortisol increases in response to a physical exam, which they had experienced previously (Gunnar et al., 1992). Infants in this study experienced multiple exams on a daily basis and may have habituated to this event. In support of this explanation, infants displayed smaller behavioral responses to the physical exam than the heel-stick blood draw.

An inability to secrete cortisol in response to stress, such as the painful stimulation of a heel-stick blood draw, may reflect dysregulation of the HPA axis (McEwen, 1998). Failure to respond to stressful events with an increase in cortisol may have implications for infant functioning. Patients with cortisol deficiency manifest hypotension, which is resistant to the effects of volume expansion and vasoressors (Helbock et al., 1993), a frequent concern in this patient population. A hypoactive HPA axis is also related to autoimmune disorders such as rheumatoid arthritis and asthma (Stratakis and Chrousos, 1995). The ability to mount a cortisol response is necessary to survive even moderate challenges. The failure to produce sufficient cortisol may limit infants’ capacity to manage challenge. Infants who received antenatal corticosteroids displayed a greater heart rate response as compared to infants who did not receive treatment. This may be an indication that these infants are less able to regulate stress responses. Research has demonstrated that a single dose of antenatal betamethasone reduces mortality among premature infants (Crowley, 1995). These data do not suggest that clinical administration of corticosteroids is contraindicated. However, this work demonstrates that just one course of antenatal corticosteroids affects postnatal regulation of the HPA axis. There is a population of infants who receive antenatal betamethasone, but are not delivered until after 34 weeks gestation, when lungs are sufficiently mature. Understanding the effects of antenatal corticosteroids may have implications for the treatment of these infants.

Researchers have proposed that glucocorticoid exposure prenatally has a programming effect on CNS development (Welberg and Seckl, 2001) and that early experiences that impact the HPA axis may create vulnerability for the development of mood and anxiety disorders (Pine and Charney, 2002). The long-term implications of prenatal exposure to corticosteroids in humans are poorly understood. These data indicate that prenatal exposure to corticosteroids may have implications for the treatment of these infants.

### Table 3: Comparison of behavioral state changes in response to heel-stick blood draw and physical exam in infants with and without antenatal betamethasone exposure

<table>
<thead>
<tr>
<th></th>
<th>No Betamethasone</th>
<th>Betamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>M</td>
</tr>
<tr>
<td><strong>Heel-stick</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting baseline</td>
<td>9</td>
<td>1.5</td>
</tr>
<tr>
<td>Event</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Recovery</td>
<td>9</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Physical exam</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting baseline</td>
<td>9</td>
<td>1.3</td>
</tr>
<tr>
<td>Event</td>
<td>9</td>
<td>1.9</td>
</tr>
<tr>
<td>Recovery</td>
<td>9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Behavioral state is scored on a scale of 1–6 (see text for details).
Acknowledgements

This research was supported by grant from the Minnesota Medical Foundation (643-7051). The authors wish to give a very special thank you to the families who participated in this research and to the nurses, lab technicians, and physicians at Fairview University Medical Center and Hennepin County Medical Center, Minneapolis, MN. Thanks also to the many undergraduates at the University of Minnesota that assisted with data collection and to Andrea Geiben at the University of Trier for careful analysis of the salivary cortisol data.

References


Levitt, H.S., Lindsay, R.S., Holmes, M.C., Seekl, J.R., 1996. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 64 (6), 412–418.


