**Effect of Prenatal Alcohol and Cigarette Exposure on Two- and Six-Month-Old Infants’ Adrenocortical Reactivity to Stress**

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Examined the effect of prenatal alcohol and cigarette exposure on infant adrenocortical reactivity to stress at 2 and 6 months of age. Cortisol response (pre-to poststressor increase) at 2 months was lower for the exposed than nonexposed infants, whereas cortisol response at 6 months did not differ between the exposed and nonexposed infants. The 2-month group difference in cortisol response reflected a higher prestressor cortisol level in the exposed infants.

KEY WORDS: adrenocortical reactivity; alcohol; cigarettes; cortisol; inoculation; prenatal exposure.

There is a large literature on adverse developmental effects of prenatal exposure to alcohol and cigarettes (Streissguth, 1986). Follow-up studies of infants and children on the adverse effects of prenatal alcohol and cigarette exposure typically have used various measures of physical growth, motoric functioning, attention, cognition, and language (e.g., Barr, Streissguth, Darby, & Sampson, 1990; Brown et al., 1991; Coles et al., 1991; Fried & Watkinson, 1990). To further advance our knowledge on these effects, Coles (1992) indicated the importance of conducting theoretically based studies in specific areas of functioning using relatively discrete measures of outcome. In this regard, Shoemaker,
Kehoe, and Baker (1992) found that prenatal alcohol exposure in the rat was associated with hyporesponsivity of the opioid system, as indicated by the release of β-endorphin in specific brain regions, in response to the stress of uncontrollable footshock. There is evidence that β-endorphin and adrenocorticotropic hormone (ACTH), the hormone secreted by the anterior pituitary gland that stimulates cortisol release from the adrenal cortex, are formed by the cleavage of a single precursor molecule and secreted concurrently (Asterita, 1985; Cooper, Bloom, & Roth, 1986). This mechanism is supported in a study of surgery patients in which rises in cortisol are accompanied by increased β-endorphin levels (Cohen, Pickar, & Dubois, 1983).

Nicotine has been shown to be a potent vasoconstrictor of placental vessels (Manning & Feyerabend, 1976). Fetuses whose mothers smoke cigarettes have been found to suffer decreased breathing movements and increased heart rate within 10 to 45 minutes after the inhalation of tobacco smoke. This effect, which has been attributed primarily to nicotine, lasts an average of 90 minutes (Johnston, 1981). In addition, increased levels of carboxyhemoglobin from carbon monoxide exposure in the blood of fetuses of cigarette smoking mothers indicates chronic fetal hypoxia (Astrup, Olsen, Trolle, & Kjeldsen, 1972; Haddon, Nesbitt, & Garcia, 1961; Longo, 1976; Spira, Spira, Goujard, & Schwartz, 1975). The increased levels of physiological stress imposed by this intrauterine environment may have long-term effects on adaptive adrenocortical responsiveness (Magnano, Gardner, & Karmel, 1992).

The purpose of the present study was to determine whether prenatal alcohol and cigarette exposure in human infants is related to hyporesponsivity of the adrenocortical system. A standard stressor, routine inoculation, was used to examine adrenocortical reactivity.

Our previous normative work on infants' salivary cortisol response to inoculation indicated a developmental shift in adrenocortical functioning between 2 and 6 months of age (Lewis & Ramsay, 1995a, 1995b; Lewis & Thomas, 1990; Ramsay & Lewis, 1994). Findings for this developmental shift included an age-related decrease in cortisol response during the 2- to 6-month period. Moreover, there was a decline in the amount of cortisol found in infants' saliva during this period. Our research (Lewis & Ramsay, 1995a; Ramsay & Lewis, 1995) and that of others (Gunnar, Porter, Wolf, Rigatuso, & Larson, 1995) pointed to a change in the meaning of a high cortisol response depending upon the age of the subject. A high cortisol response may indicate more optimal functioning at 2 months, but less optimal functioning by 6 months of age.

The evidence that a vigorous cortisol response indicates more optimal functioning in young infants, the results of Shoemaker et al. (1992) on differences in stress response as a function of alcohol exposure, as well as the hypothesized impact of cigarette smoking on response to stress, led us to predict that prenatal alcohol and/or cigarette exposure would be associated with a lower cortisol
response to inoculation at 2 months of age. In light of the findings for a developmental shift in adrenocortical functioning between 2 and 6 months, we were interested in determining whether a lower cortisol response in the exposed infants would continue to be present at the older age.

METHOD

Participants

The cross-sectional sample seen at 2 and 6 months consisted of 26 infants (15 girls, 11 boys). They were recruited from a larger sample of infants enrolled in a prospective study on the adverse developmental effects of prenatal exposure to various drugs (Bendersky, Alessandri, Gilbert, & Lewis, 1996). Subjects were included in the larger sample if they were full-term, relatively healthy, had no congenital anomalies, had oxygen therapy for no more than 24 hours, and if the mother was at least 15 years old and was not HIV positive. Infants came from predominately minority, lower class families. Extensive medical and environmental risk information has been collected on these subjects as part of the larger prospective study. There were no exposure group differences in birth weight, neonatal medical risk (Hobel, Hyvarinen, Okada, & Oh, 1973), sex, ethnicity, level of the mothers’ education, or main source of income. As in our previous work (e.g., Lewis & Ramsay, 1995a), there were no sex differences in any adrenocortical measure. Informed consent was obtained from each infant’s mother.

Procedure

Prenatal Alcohol and Cigarette Exposure

Prenatal alcohol and cigarette exposure was determined by an in-depth, structured substance-use interview with the mother within 1 month of the infants’ birth. The substance-use interview was administered by a staff member trained in interview techniques by a risk reduction specialist and a clinical psychologist. The interview included questions about the frequency of use of prescription and nonprescription medications, the frequency, amount, and trimester of use of alcohol, cigarettes, marijuana, cocaine, opiates, phencyclidine, and other street drugs, as well as tranquilizers, amphetamines, and barbiturates. For the exposed infants in the present sample, during the first, second, and third trimester of pregnancy, respectively, the proportion of mothers admitting to any alcohol use was .75, .67, and 0, while the proportion of mothers admitting to any cigarette use was .83, .75, and .58. Level of alcohol consumption was characterized, in
all but one case, as not more than one drink per month. Cigarette consumption ranged between one-quarter and one-half pack of cigarettes per day.

To provide independent evidence for prenatal exposure to substances other than alcohol and cigarettes, the meconium of the infants was collected and analyzed by American Medical Labs, Chantilly, VA. The meconium samples were screened by radioimmunoassay for metabolites of cocaine, amphetamines, phencyclidine, opiates, and cannabinoids. Positive screens were verified by gas chromatography/mass spectrometry. This analysis provides reliable documentation of substance use from 20 weeks of gestation through the end of pregnancy.

These data were used to assign subjects to the prenatal alcohol- and/or cigarette-exposed group and the prenatal nonexposed group. The criterion for inclusion in the exposed group was maternal admission of alcohol and/or cigarette use at any time during the pregnancy along with documentation of no maternal use of any other substance (i.e., by admission or positive meconium screen). As described above, the substance-use interview data suggested moderate prenatal exposure to alcohol and/or cigarettes. The criterion for inclusion in the nonexposed group was documentation of no maternal use of any substance at any time during the pregnancy (by both maternal interview and meconium screen).

**Adrenocortical Reactivity**

Infants' cortisol responses to routine inoculation were observed at 2 and 6 months of age. The cortisol data were collected in the waiting and examining rooms of the pediatric clinic of the participating hospital in this study (Mercer Medical Center, Trenton, NJ). An observer from the Institute met the mothers and their infants at the clinic at the scheduled appointment times for their well-baby visits (ranging between 9:00 a.m. and 5:30 p.m.). The observer collected a baseline and an inoculation salivary cortisol sample. As in our previous work (e.g., Lewis & Ramsay, 1995a, 1995b), the baseline salivary cortisol sample was collected soon after the infant's arrival at the clinic, while the inoculation salivary cortisol sample was collected 20 minutes after the inoculation. This 20-minute interval reflects the point that the peak cortisol response is known to occur (Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Lewis & Ramsay, 1995a, 1995b). Cortisol response was indexed by the difference between the baseline and inoculation cortisol level (inoculation minus baseline).

To collect each sample, an absorbent dental cotton roll was applied to the tongue, cheeks, and gums of the infant. A syringe was then used to express the saliva into labeled test tubes. The samples were immediately refrigerated and then transferred to a freezer for storage. Samples were shipped in dry ice to
Corning Hazleton Laboratory, Vienna, VA for radioimmunoassay for unbound salivary cortisol (see Lewis & Thomas, 1990, for details).

Five time-of-day measures were obtained: (a) wake-up on the morning of the pediatric clinic visit ($M = 7:30$ a.m., $SD = 1.2$ hours across age); (b) last feeding prior to the clinic visit ($M = 10:06$ a.m., $SD = 2.5$ hours); (c) nap duration, if any, between morning wake-up and arrival at the clinic ($M = 0.7$ hours, $SD = 0.8$ hours); (d) arrival at the clinic ($M = 12:06$ p.m., $SD = 2.0$ hours); and (e) inoculation ($M = 1:12$ p.m., $SD = 2.3$ hours). Two-way ANOVAs (exposure group, age) indicated no exposure group difference in any time-of-day measure. Two-way ANOVAs also indicated no exposure group difference in the intervals between inoculation and either morning wake-up, last feeding, or arrival at the clinic. The absence of differences in these ANOVAs indicates that these measures were not confounding factors in the results reported below.

The cortisol data were inspected for outliers. With one exception (an exposed 6-month-old), no baseline or inoculation cortisol level was more than $2 SD$ above the respective mean. The data for this subject were excluded from the data analyses reported below.

RESULTS

Table I shows baseline and inoculation cortisol level and cortisol response at 2 and 6 months for the exposed and nonexposed infants. Inspection of the cortisol response data suggests a greater cortisol response in the nonexposed infants that was present primarily at 2 months of age. To test this difference, a repeated measures (baseline and inoculation cortisol sample) ANOVA with two between-subjects factors (exposure group, age) was performed. This ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Inoculation</th>
<th>Response</th>
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<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$M$</td>
<td>$SD$</td>
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<tr>
<td>2 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonexposed</td>
<td>9</td>
<td>0.58</td>
<td>0.32</td>
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<tr>
<td>Exposed</td>
<td>5</td>
<td>1.16</td>
<td>0.63</td>
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<tr>
<td>6 months</td>
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</tr>
<tr>
<td>Nonexposed</td>
<td>5</td>
<td>0.47</td>
<td>0.16</td>
</tr>
<tr>
<td>Exposed</td>
<td>6</td>
<td>0.73</td>
<td>0.59</td>
</tr>
</tbody>
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*Values given are in micrograms per deciliter. Each subscript indicates a pair of means that differ significantly ($p < .05$ or better) by $F$ or $t$ test (see text).
yielded a significant cortisol sample by exposure group interaction, $F(1, 21) = 5.61, p < .03$, as well as a significant cortisol sample by exposure group by age interaction, $F(1, 21) = 4.39, p < .05$. To clarify these interactions, separate repeated measures (cortisol sample) ANOVAs were performed to examine baseline to inoculation change in cortisol level for each exposure group at each age. The nonexposed group at 2 months showed a significant baseline to inoculation cortisol increase, $F(1, 8) = 11.00, p < .02$. Neither the exposed group at 2 months nor either exposure group at 6 months showed a reliable baseline to inoculation change in cortisol level.

Post hoc $t$ tests were used to identify differences by exposure group and by age in cortisol response as well as in baseline and inoculation cortisol levels. For cortisol response, the nonexposed group showed a greater response than the exposed group at 2 months ($p < .02$), whereas the nonexposed and exposed groups did not differ in cortisol response at 6 months of age. Both the trend for a decline in cortisol response between 2 and 6 months in the nonexposed infants and the trend for an increase in cortisol response between 2 and 6 months in the exposed infants were not significant. For baseline cortisol level, the exposed group showed a higher baseline cortisol level than the nonexposed group at 2 months of age ($p < .04$). There was a nonsignificant trend for a comparable exposure group difference in baseline cortisol level at 6 months of age. Neither the exposed nor the nonexposed infants showed reliable change in baseline cortisol level between 2 and 6 months of age. For inoculation cortisol level, the nonexposed infants showed a decline with age in inoculation cortisol level ($p < .03$). The exposed infants did not show reliable age change in inoculation cortisol level. There was no significant exposure group difference in inoculation cortisol level at either age.

**DISCUSSION**

This study examined the effect of prenatal alcohol and/or cigarette exposure on cortisol response (pre- to poststressor cortisol increase) to inoculation at 2 and 6 months of age. At 2 months, while the nonexposed infants showed a reliable cortisol response, the exposed infants did not. This exposure group difference reflected a higher prestressor cortisol level in the exposed infants. At 6 months, the exposed and nonexposed infants did not differ in cortisol response. There was a trend for a higher prestressor cortisol level in the exposed than nonexposed infants at this age. Finally, only the nonexposed infants showed an age-related decline in poststressor cortisol level.

The present findings indicate that prenatal alcohol and/or cigarette exposure is associated with hyporeactivity of the adrenocortical system to stress at 2 months, but that this effect is no longer present by 6 months of age. Stated
differently, at 2 months, the exposed infants did not show the vigorous cortisol response that was shown by the nonexposed infants and which has been associated with more optimal functioning in newborn and young infants (Gunnar et al., 1995; Ramsay & Lewis, 1995).

There was a trend for the exposed infants to continue to have high prestressor cortisol levels at 6 months of age. Moreover, while there was a trend for a decrease with age in cortisol response in the nonexposed infants, there was a trend for an increase with age in cortisol response in the exposed infants. These trends suggest that there may be a continued long-term effect of exposure on adrenocortical functioning that might have proved significant in a larger sample and would become more apparent at older ages. It is of interest to identify the effect over time of prenatal exposure on both basal cortisol level and cortisol response.

Our previous normative findings indicated a developmental shift in adrenocortical functioning during the 2- to 6-month period. The findings for this developmental shift included age-related declines in cortisol response and cortisol level. The findings in the present study for a vigorous cortisol response at 2 months by the nonexposed infants as well as the decline in their poststressor cortisol level between 2 and 6 months provide additional evidence for this developmental shift.

Since the present sample was relatively small, it was not possible to determine whether prenatal exposure to alcohol as opposed to cigarettes had comparable effects on adrenocortical functioning at 2 and 6 months of age. Inspection of the data for individual subjects (collapsing over age) suggests that prenatal exposure to alcohol but not to cigarettes is likely to involve high baseline cortisol levels and baseline to inoculation cortisol decreases. Such a finding would provide direct confirmation for the Shoemaker et al. (1992) finding for hyporesponsivity to stress in infant rats as a function of prenatal alcohol exposure, and would implicate high basal cortisol levels as one factor in this hyporesponsivity. We are currently addressing this issue by assessing adrenocortical reactivity to stress in separate groups of infants whose prenatal exposure was limited to alcohol or cigarettes. In addition, we will examine level of exposure to these substances as potential discriminating variables.

REFERENCES


Ramsay, Bendersky, and Lewis


