Low Salivary Cortisol and Persistent Aggression in Boys Referred for Disruptive Behavior

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Background: Persistent antisocial behavior in adulthood is often preceded by childhood-onset aggressive conduct disorder. Aggressive syndromes in both children and adults have previously been associated with abnormalities in peripheral responses to stress. One peripheral measure, salivary cortisol concentration, may reflect individual differences in the hypothalamic-pituitary-adrenal axis that underlie propensities for aggression, socialization, and adaptation to stress.

Methods: The relationship between salivary cortisol levels and aggression was tested in 38 clinic-referred school-aged boys. Persistent aggression was measured by collecting disruptive behavior disorder symptoms in 4 annual clinical evaluations and peer nominations of aggression in the first 2 annual evaluations. Salivary cortisol levels were measured during years 2 and 4 of the study.

Results: Low cortisol levels were associated with persistence and early onset of aggression, particularly when measures of cortisol concentrations were pooled. Boys with low cortisol concentrations at both time points exhibited triple the number of aggressive symptoms and were named as most aggressive by peers 3 times as often as boys who had higher cortisol concentrations at either sampling time.

Conclusions: This suggests that low hypothalamic-pituitary-adrenal axis activity is a correlate of severe and persistent aggression in male children and adolescents. A restricted (low) range of cortisol variability may be more indicative of persistent aggression than a low concentration of cortisol at any single point in time.

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Conduct disorder (CD) refers to a pattern of antisocial behavior in childhood or adolescence that lasts at least 6 months but which in some cases becomes a lifelong personality style. Two types are operationally distinguished by whether the first symptom appears before or after age 10 years. In many cases, adolescent-onset CD is confined to a stage of dalliance with delinquent behavior (often with the encouragement or coparticipation of peers) and remits with continued socialization and maturation. The more severe childhood-onset CD is typically marked by more aggressiveness, more severely disturbed peer relations, and a lengthier course than seen with adolescent-onset CD. Childhood-onset CD is notoriously difficult to treat. Children who exhibit this pattern often sustain severely antisocial behavior into the fourth or fifth decade of life, along the way accounting for a hugely disproportionate percentage of total crimes committed and acts of victimization on others.

As yet, persistent aggressive CD cannot be reliably identified except in retrospect, and the mechanisms underlying this pattern of behavior remain poorly understood. Biobehavioral studies often find this pattern to be associated with abnormal baseline levels of psychophysiological measures or abnormal reactivity to experimental stressors. One measure that responds to certain stressors is the concentration of cortisol in the saliva, which reflects activity in the malleable hypothalamic-pituitary-adrenal axis and mirrors the level of free (unbound) cortisol in the blood that can engage brain receptors. We have found that boys with CD with comorbid anxiety (who are probably highly reactive to stress) have very high concentrations of cortisol, whereas boys with CD whose behavior is unconstrained by comorbid anxiety have low concentrations of cortisol in the saliva, are more aggressive, and are more often rejected by their peers. Boys with CD and low cortisol concentrations were found to exhibit aggression at younger ages than boys with CD and cortisol concentrations in the upper ranges. We report a follow-up of the
SUBJECTS AND METHODS

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Subjects came from one site (University of Georgia, Athens) of a longitudinal study (the Developmental Trends Study\(^2\,3\)) of CD in males. Boys were referred for problem behavior. Of 80 Georgia Developmental Trends Study participants, 38 completed an auxiliary study\(^4\) by providing saliva samples in years 2 and 4 of the study. (Some eligible boys did not participate because they were unavailable, were residing far from the University of Georgia, or participated in the Developmental Trends Study before final institutional review board approval for the auxiliary study was secured.) The mean (SD) age at entry for subjects in this subsample was 9.73 (1.7) years (age range, 7-12 years); mean (SD) full-scale IQ score, 103 (16.9) (score range, 69-151). Subjects' socioeconomic status ranged across all 5 Hollingshead\(^5\) social status categories but was overrepresented in the lower 2 categories. Sixteen percent of subjects were African American. The remaining 84% were non-Hispanic white. Parents provided informed consent and boys provided oral assent to study participation.

METHODS

Aggression and Disruptive Behavior

Two methods of assessing aggression over time were used. The first measure was the total aggressive CD symptoms from the 4 annual child psychodiagnostic evaluations. Symptoms of childhood disorders described in DSM-III-R\(^6\) were determined from structured interviews (The NIMH Diagnostic Schedule for Children, Version 2\(^6\)) of children, parents (all mothers), and teachers. Independent diagnosticians (clinical psychology graduate trainees) observed 23% of the interviews through 1-way mirrors. Interrater agreement (\(k\)) exceeded 0.65 for each CD symptom. Aggressive CD symptoms included (1) threatens or intimidates others, (2) often initiates physical fights, (3) has used a weapon, (4) has been physically cruel to people, (5) has been physically cruel to animals, (6) has stolen while confronting a victim, and (7) has forced someone into sexual activity, plus the symptom of bullies described in DSM-IV.\(^7\) Other symptoms were summed to measure covert (nonaggressive) CD symptoms. Diagnostic plots of the fitted models (Q-Q-plots and plots of standardized residuals vs the fitted linear predictor portion of the models) visually confirmed that the assumed functional forms for the mean and variance adequately reflected those in the data and fully accounted for heteroscedasticity and skewness. Models were compared via F tests for the observed change in deviance using a computer program (S-plus; MathSoft Inc, Seattle, Wash). All tests of hypotheses were 2-tailed with a significance level of \(\alpha = .05\). Secondary tests were conducted to determine whether cortisol concentrations were more directly related to aggressive CD, covert CD, or hyperactivity.

The correlates of persistently low cortisol concentrations were examined by grouping the subjects by cortisol concentration range. Cortisol concentrations measured at years 2 and 4 of the study were dichotomized at the medians. Subjects were assigned to 1 of 3 cortisol groups: (1) persistently low, cortisol concentrations below the median in both years (\(n = 12\)); (2) variable, cortisol concentrations above the median in 1 year and below in the other (\(n = 15\)); or (3) persistently high, cortisol concentrations above the median in both years (\(n = 11\)). The groups did not differ significantly in age (mean ages: persistently low, 9.7 years; variable, 9.9 years; persistently high, 9.6 years; full-scale IQ (mean scores: persistently low, 96; variable, 109; persistently high, 104); socioeconomic status; or ethnicity.

Cortisol

A single saliva sample was gathered from each child during the clinic visit in years 2 and 4 of the study. Samples were stored at \(-80^\circ\)C until all samples for the year were collected, at which time they were centrifuged and assayed (procedures detailed elsewhere\(^8\,9\)) using a commercially available competitive radioimmunoassay kit (Coat-a-Count; Diagnostic Products Corp, Los Angeles, Calif). As seen in Table 1, the time of day of saliva collection could not be controlled and was allowed to vary across the day. However, as found in another study,\(^10\) the time of collection did not affect the relationship between cortisol concentrations and the dependent measures. Similarly, subject age played no significant role in any of the models, either as predictors or as confounders. (Terms for these potential confounds were tested and discarded from the statistical models.)

STATISTICAL ANALYSIS

Because limitations on sample size and measurement intervals precluded formal longitudinal analyses, the primary outcomes were cumulative measures of psychopathology (by symptom and peer nomination counts), each analyzed separately. The primary explanatory variable was salivary cortisol concentrations (originally in micrograms per deciliter) transformed to the natural logarithm scale to render the distributions more symmetric, and presented here as the average \(\log(cortisol)\) value from years 2 and 4 of our study. Similar results (not shown) were obtained using \(\log(cortisol)\) values from each year as independent predictors in the model. Relationships among variables were initially assessed using plots and graphical smoothing techniques.\(^11\) With count data, we expect right-skewed distributions and for the variance to grow with the mean. To account for these 2 features, responses were analyzed using log-linear (Poisson distribution) regression models with overdispersion,\(^12\) for example: \(\log(\text{mean aggression}) = \beta_0 + \beta_1 \times \log(\text{cortisol})\). Diagnostic plots of the fitted models (Q-Q-plots and plots of standardized residuals vs the fitted linear predictor portion of the models) were found in another study,\(^13\) the time of collection did not affect the relationship between cortisol concentrations and the dependent measures. Similarly, subject age played no significant role in any of the models, either as predictors or as confounders. (Terms for these potential confounds were tested and discarded from the statistical models.)

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RESULTS

SALIVARY CORTISOL

The mean (SD) log(cortisol) values for years 2 and 4 of the study were −3.48 (0.75). The mean log(cortisol) value in year 4 of the study was marginally higher than in year 2 (paired t test, t17 = 1.8, P = .07). The correlation of log(cortisol) values in study years 2 and 4 was 0.20, and the regression of year 4 log(cortisol) values on year 2 log(cortisol) values was not statistically significant (r2 = 0.23). Squaring the correlation (0.2 × 0.2) yields an R2 value of 0.04 (ie, 4% of year 4 variance is explained by year 2), indicating considerable within-subject variability over time. This suggests that the average of the 2 measures provides a more valid assessment of subjects’ true underlying cortisol tuning and of the relationship between subjects’ true log(cortisol) concentrations and the observed psychopathologic traits.

All distributions of psychopathologic traits were skewed right except ODD symptoms. Figure 1 shows that salivary cortisol concentrations are strongly and inversely related to aggressive CD, peer aggression nominations, and ODD and also related (less clearly) to covert CD. The concordance between fits by smooth curves and the log-linear regression model confirms that this model is of appropriate functional form. The change in log(mean behavioral counts) per SD unit increase in mean log(cortisol) value is stated above each plot. Inverting these values provides a relative rate of symptoms for a 1-SD decrease in log(cortisol). For example, a decrease of 1 SD in log(cortisol) corresponds to an increase in the mean number of aggressive CD symptoms by a factor of (1/0.47) = 2.13 (95% confidence interval [CI], 1.59-2.84). The estimated effect for peer aggression is virtually identical. A decrease of 1 SD in the average log(cortisol) value corresponds to a 1.44-fold increase in covert CD symptoms (95% CI, 0.98-2.13) and a 1.28-fold increase in ODD symptoms (95% CI, 1.14-1.45). The use of log(cortisol) values for study years 2 and 4 as separate effects, rather than as averages, never significantly improved the model fit (F tests for separate vs averaged log(cortisol) values for the 4 responses: F1,34 = 0.0096, P = .92; F1,30 = 0.204, P = .65; F1,34 = 0.433, P = .52; and F1,34 = 2.34, P = .13). Relationships were flexibly adjusted for the time of cortisol collection, and no difference in the cortisol- psychopathology relationship was found (F tests for the time of collection as predictors of the 4 responses: F4,31 = 1.112, P = .37; F4,27 = 1.002, P = .42; F4,31 = 0.438, P = .78; and F4,31 = 0.740, P = .57).

SPECIFICITY OF CORTISOL–AGGRESSIVE CD RELATIONSHIP

After adjustment for the linear and quadratic effects of log(cortisol + 1), the association of the mean log(cortisol) value to aggressive CD was −0.55 and still...
The variable and persistently high cortisol groups did not differ on aggressive CD symptoms, peer aggression nominations, covert CD, or ODD symptoms (Figure 2). The persistently low cortisol group (Table 2) was significantly worse than the remainder of the sample on all 4 of these variables, incurring roughly 3 times as many aggression counts (5.2 vs 1.5 aggressive CD symptoms, 33.5 vs 10.5 peer aggression nominations, 6 vs 2 covert CD symptoms, and 28.6 vs 16.8 ODD symptoms).

### LOW CORTISOL CONCENTRATIONS AND THE AGE OF ONSET CRITERION

In DSM-IV, the criterion that distinguishes childhood-onset CD from adolescent-onset CD is whether the first CD symptom emerged before the age of 10 years. Boys who met this criterion (n = 23) compared with those who did not (n = 15) had lower total cortisol concentrations (standardized log[cortisol] value of −0.33 vs 0.51, F = 7.6, P = .009).

Boys whose cortisol concentrations were above the median range in at least 1 study year were roughly evenly distributed between early emergence of the first symptom (n = 12) and late emergence (n = 14). However, all but 1 of the subjects in the persistently low cortisol group (n = 11, 92%) developed the first CD symptom by age 10 years. The cross-tabulation was significant (2-tailed Fisher exact test, P = .012).

The relationship of low cortisol to early emergence of the first symptom was examined separately for aggressive vs covert CD symptoms in log-linear models. The average log(cortisol) value was related to the age of emergence of the first aggressive CD symptom (estimate = 0.15, z = 2.62, P < .009). Exponentiation suggests that every SD increase in the log (cortisol) value is associated with a 16% increase in mean age of onset. Following adjustment for log-linear and log-quadratic effects of covert CD, salivary cortisol concentrations remained a significant predictor of aggressive CD symptom emergence (estimate = 0.12, z = 2.09, P = .037). Cortisol concentrations failed to significantly predict the age of the first covert CD symptom, regardless of whether the age of emergence for the first aggressive CD symptom was included. These analyses imply that differences in cortisol concentration between DSM-IV types of CD occur chiefly because low cortisol concentrations are directly associated with early emergence of aggression.

### COMMENT

We found that salivary cortisol concentrations sampled over time were inversely associated with several measures of aggression and disruptive behavior collected roughly over the same interval. Cortisol concentration was directly linked to aggression and indirectly to covert CD and hyperactivity via comorbidities of these syndromes with aggression. The age at which the first ag-
gressive symptoms appeared was positively associated with cortisol concentration. Boys with early emergence (by age 10 years) of the first CD symptom had lower cortisol concentrations than those with late emergence.

Some earlier studies, including one that focused on aggression, produced dissimilar results. However, accrual of other direct and indirect evidence linking life-course-persistent aggression to low cortisol concentrations attests that the current findings are not isolated. A retrospective study of adults found that aggressive antisocial prisoners and prisoners with childhood histories of undersocialized aggressive CD (the comparable version of early-onset CD in DSM-III) have lower cortisol concentrations than other prisoners and normal volunteers. A study with adolescents found an inverse relationship of salivary cortisol concentrations to the number of early personality CD symptoms and to paternal history of lifelong CD and/or antisocial personality disorder. Children with early-onset ODD (a frequent antecedent of CD) were reported to have lower cortisol concentrations than normal controls. A preliminary study (K.M., unpublished data, 1999) with a small sample size found that boys with childhood-onset CD as described in DSM-IV have significantly lower salivary cortisol concentrations than boys with adolescent-onset CD and clinic controls. Finally, a study of children with comorbid attention-deficit/hyperactivity disorder and ODD and/or CD found that those individuals whose attention-deficit/hyperactivity disorder diagnosis persisted over time had lower cortisol concentrations at rest and in response to stress. Together, this literature and the current findings suggest that the features most linked to low cortisol concentrations are childhood-onset CD, persistence through adulthood, and prominent aggressiveness. Some evidence also points to familial patterns and the absence of comorbid anxiety. The personality features of psychopathy were not evaluated in this study, but this outcome should be examined in future research.

The mechanism linking persistent aggression and low cortisol concentrations is not known. Animal models have shown that prenatal and early developmental stress can cause long-lasting or even permanent alteration of the hypothalamic-pituitary-adrenal axis by affecting steroid receptors situated in the hippocampus and frontal cortex. It may be of interest in future studies to examine whether certain lifestyle correlates of antisocial families (eg, maternal use of tobacco or exposure to other teratogens during pregnancy; incompetent and/or inadequate parenting; chaotic, unpredictable social environment; and abuse, threats, or deprivations might be associated with dysregulation of children’s hypothalamic-pituitary-adrenal axis.

Several methodological limitations should be acknowledged. The sample was relatively small and consisted only of males. The collection of saliva to measure cortisol levels was limited to 2 samples without controlling for time of day, and the concentrations were affected by substantial extraneous variability. The design and analyses were not truly longitudinal, ie, the key variables were not measured methodically at the outset so that temporal relationships could be specified.

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Table 2. Salivary Cortisol Concentration and Behavioral Variables

<table>
<thead>
<tr>
<th>Variables, Total</th>
<th>Minimum</th>
<th>25th Percentile</th>
<th>Median</th>
<th>75th Percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive CD</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Peer aggression nominations</td>
<td>0</td>
<td>4</td>
<td>11</td>
<td>35</td>
<td>66</td>
</tr>
<tr>
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<td>2</td>
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<tr>
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<td>20</td>
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<tr>
<td>Hyperactivity-impulsivity</td>
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<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Anxiety</td>
<td>5</td>
<td>11</td>
<td>16</td>
<td>21</td>
<td>34</td>
</tr>
</tbody>
</table>

*The study population is equal to 38. CD indicates conduct disorder; and ODD, oppositional defiant disorder. Behavioral variables are symptom counts summed over 4 annual evaluations, except for peer aggression nominations, which is peer nominations for fights most and meanest summed over 2 annual evaluations. Four subjects had missing values of peer aggression.

REFERENCES


