Decreased Cortisol Levels in Adolescent Girls With Conduct Disorder

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Background: Female adolescent antisocial behavior is increasing, but little is known about the neuroendocrinologic aspects of this disorder. On the basis of reports of decreased cortisol levels in antisocial males, we investigated morning plasma cortisol levels in adolescent girls with conduct disorder (CD).

Methods: Three plasma samples for cortisol levels were taken every 20 minutes between 8 and 9 AM in 47 adolescent girls with CD (mean±SD age, 16.5±0.9 years) and 37 normal control girls (mean age, 16.0±0.8 years). All blood was drawn within 72 hours after the onset of menstrual flow.

Results: Girls with CD had significantly lower cortisol levels than girls in the normal control group at all 3 sampling times. This finding was not due to procedural factors, demographic characteristics, or the use of medications. The girls with CD who had no other psychiatric problems had lower cortisol levels than girls with other disorders or those in the normal control group. In the multiple regression analysis, having CD predicted 10% of the variance in cortisol levels.

Conclusions: Morning plasma cortisol levels were significantly diminished in adolescent girls with CD. Decreased cortisol levels appear to be most strongly associated with antisocial girls who do not have other psychiatric disorders.

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Female adolescent antisocial behavior is prevalent, whether defined as conduct disorder (CD) or delinquency. Conduct disorder is the second most common diagnosis given to adolescent girls,1 and one third of adolescent female psychiatric patients receive the diagnosis.2 In the general population, nearly 10% of 15- to 17-year-old girls meet criteria for CD.3,4 The delinquency rate for adolescent girls and the proportion of arrests of females for violent crimes have increased dramatically in the past 2 decades.5,6 Adolescent antisocial behavior is financially and emotionally costly to the teens and their families, and to society.7 Antisocial girls become women with rates of criminal behavior up to 40 times greater than those of other women, a high risk of early death, complex psychiatric problems, a high rate of substance abuse, poor adult physical health, and intergenerational transmission of antisocial behavior.8

Unfortunately, current treatments for female antisocial behavior are ineffective.9-15 Treatments may fail, in part, because little is known about biological correlates of female antisocial behavior. All previous research on neuroendocrinologic function in antisocial disorders has studied males.

One of the most provocative neuroendocrinologic abnormalities reported in antisocial males is decreased cortisol secretion. Low plasma cortisol levels in response to experimental stressors was first described among adult male criminals referred to a maximum-security hospital.16 These men were more violent than a criminal control group, who responded normally to the stressors with an increase in cortisol levels. Ninety-three percent of these hyporesponders had histories of repeated physical violence. Sixty-seven percent of the hyporesponders had committed murder, compared with only 14% of the criminal control group. Similarly, Virkkunen17 found that violent male criminals had decreased cortisol secretion, but that cortisol levels were normal in nonviolent criminals and noncriminal violent men.

Antisocial behavior in boys has also been associated with low resting cortisol levels, especially in boys exhibiting physical aggression.16-20 Similarly, both resting salivary cortisol levels and the cortisol response to psychological stimuli were nega-
SUBJECTS AND METHODS

SUBJECTS

We recruited 13- to 17-year-old girls to ensure that as many girls as possible would be in Tanner stage V and yet not out of adolescence. Girls were recruited predominantly from the community, through newspaper ads and posters. The ads sought girls with behavior problems such as “truvancy, fighting, stealing, and lying.” The ads also indicated that we were interested in girls without these problems. Three hundred fifty-four girls were contacted through ads and posters, through friends already in the study (n = 7), or through contacts in clinics (n = 6).

Intake appointments were made after a brief telephone screening and explanation of the project. One hundred fifty-one girls completed intake appointments. Each girl was interviewed concurrently with a parent or guardian who knew her history (the majority of adult informants were mothers or fathers). The intake appointment was used to determine eligibility for the study and to collect baseline nonbiological data.

In addition, a structured psychiatric interview (see below) was used to classify girls into the conduct disorder (CD) or normal control (NC) group. Exclusion criteria for both groups were age outside the 15- to 17-year range, pubertal development less than Tanner stage V, history of head trauma with loss of consciousness for more than 15 minutes, serious medical illness that could affect hypothalamic-pituitary-adrenal (HPA) axis function (e.g., diabetes, thyroid disorders, renal disease), IQ less than 65, or a history of psychosis. Additional exclusion criteria for the NC group were the presence of any lifetime psychiatric disorder or any symptom of CD. Since we were trying to recruit a sample that would be as representative as possible of girls in the general population who matched girls with CD, we did not exclude girls in the NC group who smoked cigarettes or had experimented with alcohol, marijuana, or other drugs. We defined experimentation as not having used these substances more than 5 times throughout a girl’s life and having no social or physical sequelae of use; no NC girl exceeded these criteria.

Ninety-three girls (52 in the CD group and 41 in the NC group) were found eligible and agreed to participate. We dropped 5 girls (3 in the CD group and 2 in the NC group) from the neuroendocrine protocol because we could not obtain a blood sample within 4 menstrual cycles of the baseline data collection. Four girls (2 in the CD group and 2 in the NC group) refused to give blood either initially or after an adverse event (vein collapsing, fainting). Thus, the final sample size for the neuroendocrine data presented in this article was 84 (47 in the CD group and 37 in the NC group).

ASSESSMENTS

After explaining both stages of the study, we obtained written, informed consent from each girl and her parent or guardian. The intake interview was conducted separately with each girl and her parent. Data were collected about the following topics: family demographics, medical and developmental history of the girl, school experiences, psychosocial history, family function, family psychiatric history (parent only), and Tanner pubertal stage self-report (girl only). Psychiatric diagnosis was determined from the computerized version of the NIMH (National Institute of Mental Health) Diagnostic Interview Schedule for Children, Version 2.3 (DISC), Parent and Youth Versions. All interviewers held bachelor’s or postgraduate degrees. They were trained by the principal investigator (K.P.), who in turn had been trained by the developers of the DISC.

As indicated above, the DISC was used to classify girls into 2 groups. On the basis of concerns regarding the validity of using the aggression and age at onset DSM-IV criteria to diagnose female CD, 1 we modified the DISC slightly. First, we rephrased the question on fighting to read “Do you often get into fights?” rather than “Do you often start fights?” Second, our diagnostic algorithm required that antisocial behaviors be demonstrated for at least 1 year before the interview, but onset before age 13 years was dropped from the items to which it is attached in the DSM-IV criteria.

Subjects were given a diagnosis based on meeting criteria from either the youth or the parent report. It is difficult to know who is the more accurate informant at this age because some adolescents may share their feelings and activities with their parents, and others may not. Moreover, some adolescents exaggerate reports of what they do, while others minimize them. Therefore, we considered data from all sources to be equally valid.

RESULTS

SUBJECT CHARACTERISTICS

The girls in the CD group were, on average, 6 months older than the NC girls (Table). Because all girls were in Tanner stage V, we doubt that this finding is clinically significant. The CD group included slightly higher proportions of African American girls and girls of lower socioeconomic status. Rates of smoking, alcohol, and drug experimentation were also higher among girls with CD, particularly experimentation with drugs other than marijuana (primarily hallucinogens). Girls with CD had high rates of comorbid substance abuse, internalizing, and oppositional defiant disorders, although few of the girls with CD had comorbid attention-deficit/hyperactivity disorder.
PROCEDURES

If a girl met criteria for either group, she was asked to participate in neuroendocrinologic testing. To control for any menstrual cycle effects on HPA axis activity, we drew all blood within the first 72 hours after the onset of menstrual flow.22-28 Each girl participating in stage 2 was asked to call the study office as soon as she started her next menstrual period. When contacted, we arranged for an early-morning phlebotomy appointment within 72 hours of when she had begun her period. Fifty-two percent of the subjects had their blood drawn in their homes or at another designated meeting place (eg, school). The others were done at the study office. We inserted an indwelling catheter into the antecubital fossa of one arm. Samples were taken immediately on insertion (time 0), 20 minutes later (time 1), and 40 minutes later (time 2). Time 2 samples were used in the analyses. They were likely to be the most reliable estimates of morning basal secretion, since 40 minutes should have allowed the HPA axis to recover from the stress of catheter insertion. The catheter was kept patent with a saline flush between samples. Sixty-seven percent of the blood draws occurred before 9 AM (this time, however, was not significantly associated with CD vs NC status). However, because of the unpredictable schedules and behavior of these subjects, some girls had their blood drawn later than 9 AM, with starting times ranging to 11:15 AM. Most girls were studied within 1 menstrual cycle of baseline data collection, but erratic menses and scheduling difficulties resulted in some subjects having their blood drawn 2 to 4 cycles later.

All cortisol assays were performed without regard to diagnosis status. Plasma cortisol levels were determined with radioimmunoassay by means of a commercially available kit (Nichols Institute, San Juan Capistrano, Calif.). Interassay variability, intra-assay variability, and assay sensitivity were 6.1%, 4.7%, and 1.4 nmol/L, respectively. 29

Ten girls were taking medroxyprogesterone acetate and 5 were using oral contraceptives. One girl in the NC group signed up for the study and was pregnant by the time she was ready for neuroendocrinologic testing. Since the menstrual cycle in all 16 of these girls was suppressed, we drew blood when it was convenient for them, and as close as possible to the intake interview. We then tested for effects of these variables in the data analysis.

Because hypocortisolemia in antisocial men may be particularly related to violence, we categorized the girls with CD as aggressive or nonaggressive on the basis of the presence of 1 or more aggressive behaviors in either the parent or youth DISC report (fighting, cruelty to animals or people, carrying or using a weapon, fire-setting, stealing with confrontation, police arrests for assault). Each behavior described by the girl or her parent was assigned 1 point, and the final score for aggressive CD was the sum of these points. Girls with CD were also divided into 3 groups defined by the presence of psychiatric comorbidity: CD and no comorbidity, CD and oppositional defiant disorder only, and CD and multiple diagnoses.

The protocol was approved by the institutional review boards at Allegheny General Hospital and the University of Pittsburgh Medical School, Pittsburgh, Pa. The girls and their parents were paid $20 for baseline testing, and each girl received $50 on completion of the neuroendocrinologic testing.

DATA ANALYSIS

Any variable not displaying a normal distribution was log-transformed, including plasma cortisol level. Comparisons between the 2 groups were tested for statistical significance by using unpaired t tests, univariate and repeated-measures analyses of variance, correlation coefficients, or χ², depending on whether the variables were interval or nominal. All tests were 2-tailed, and statistical significance was set at P<.05.

Cortisol levels are associated with many factors, including the time of day, time of year, and place when blood was drawn, 29 demographic factors, 31,38-40 taking oral contraceptives, 41,42 being pregnant, 38-40 and other psychiatric conditions, including posttraumatic stress disorder (PTSD). 31 Statistically controlling for all of these factors would absorb more degrees of freedom than we could afford with our sample. Therefore, we calculated propensity scores 42 (ie, the probability of CD vs NC membership) by logistically regressing group status on the above covariates. We then controlled for CD vs NC differences on the covariates by calculating a linear regression of cortisol on group status and the propensity score. By including the propensity score in the regression, we controlled for that information in the covariates that increased the likelihood of group membership. SPSS (SPSS Inc, Chicago, Ill) was used to conduct the analyses.

To control for NC vs CD differences on other variables that might affect cortisol level, we estimated propensity scores to summarize these differences (see above). We then computed a linear regression of time 2 cortisol level on CD vs NC group status and the propensity score. In this regression, belonging to the CD group lowered cortisol level by 185.1 nmol/L (t81 =2.53; P = .01), despite our having controlled for the group differences represented by the propensity score.

PLASMA CORTISOL: AGGRESSION AND PSYCHIATRIC COMORBIDITY

Thirty-seven girls met the criteria for aggressive CD, with the number of aggressive behaviors ranging from 1 to 10 (mean and median, 3 behaviors). The mean of the plasma levels of cortisol in the aggressive CD group (n=37) was
This is the first study, to our knowledge, of cortisol levels in antisocial girls. It confirmed our hypothesis that adolescent girls with CD would have lower morning cortisol levels than girls without any psychiatric disorder. This, it appears that adolescent antisocial girls may have HPA axis dysregulation similar to that found in antisocial boys and men.

The difference in mean cortisol levels between the CD and NC groups is much larger than those reported in previous studies on males. Not all researchers have found a negative correlation between cortisol levels and antisocial behavior in boys. Mean plasma cortisol levels drawn for morning baseline levels before a fenfluramine challenge were not different in a mixed group of prepubertal and adolescent boys who had “disruptive behavior disorders” compared with normal controls. In 2 samples of boys with attention-deficit/hyperactivity disorder, the majority of whom had comorbid CD or oppositional defiant disorder, urinary free cortisol levels and plasma cortisol levels were no different than levels in NC subjects. However, all of these samples were composed of boys spanning the age range from latency to adolescence and who had been selected on the basis of disruptive behavior or attention-deficit/hyperactivity disorder, rather than repeated acts of antisocial behavior. Our more robust effect may be due to our sampling strategy, which selected a homogeneous group of late-adolescent girls who were in Tanner stage V and who had exhibited a pattern of repeated antisocial acts for at least 1 year. Our design further limited intersubject variance secondary to sampling time and menstrual cycle effects by standardizing these factors across subjects. However, it is also possible that the difference in cortisol levels between antisocial and normal subjects may be more pronounced in females because normal women have higher basal and reactive levels of cortisol than do normal men.

The girls with CD displayed substantial psychiatric comorbidity, a finding consistent with other reports in the literature. Nonetheless, we were able to demonstrate that the relationship between cortisol level and CD was not simply a reflection of severity of psychopathology, because subjects without other diagnoses actually had the lowest mean cortisol levels.

The finding that antisocial females have low cortisol levels raises important clinical questions. First, low cortisol levels may put these girls at risk for later autoimmune diseases, some types of atopilinesses, increased inflammation, and infections with extracellular pathogens. This may partially explain the poor adult health of women who had been antisocial adolescents. Second, low cortisol level may be a diagnostic marker for subtypes of girls with CD. To determine whether a biological correlate of a behavioral syndrome is a diag-

### Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CD (n = 47)</th>
<th>NC (n = 37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>16.5 ± 0.9</td>
<td>16.0 ± 0.8</td>
<td>.01</td>
</tr>
<tr>
<td>Lower SES†</td>
<td>41 (87.2)</td>
<td>26 (70.3)</td>
<td>.06</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>28 (59.6)</td>
<td>26 (70.3)</td>
<td>.31</td>
</tr>
<tr>
<td>African American</td>
<td>19 (40.4)</td>
<td>11 (29.7)</td>
<td>.31</td>
</tr>
<tr>
<td>Nicotine use‡</td>
<td>35 (74.5)</td>
<td>22 (59.5)</td>
<td>.15</td>
</tr>
<tr>
<td>Alcohol experimentation§</td>
<td>42 (89.4)</td>
<td>31 (83.8)</td>
<td>.45</td>
</tr>
<tr>
<td>Marijuana experimentation§</td>
<td>31 (66.0)</td>
<td>19 (51.4)</td>
<td>.39</td>
</tr>
<tr>
<td>Other drug experimentation</td>
<td>15 (31.9)</td>
<td>1 (2.7)</td>
<td>.001</td>
</tr>
<tr>
<td>ODD</td>
<td>20 (42.6)</td>
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<td>NA</td>
</tr>
<tr>
<td>ADHD</td>
<td>3 (6.4)</td>
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<td>NA</td>
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<td>5 (10.6)</td>
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<td>Anxiety disorders</td>
<td>12 (25.5)</td>
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<tr>
<td>Posttraumatic stress disorder</td>
<td>2 (4.3)</td>
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<tr>
<td>Alcohol abuse/dependence</td>
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</tr>
<tr>
<td>Marijuana abuse/dependence</td>
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<td>NA</td>
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<tr>
<td>Other drug abuse/dependence</td>
<td>1 (2.1)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Diagnosis other than CD/ODD</td>
<td>29 (61.7)</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

*All variables except age are expressed as number (percentage).
CD indicates conduct disorder; NC, normal control; SES, socioeconomic status; NA, not applicable; ODD, oppositional defiant disorder; and ADHD, attention-deficit/hyperactivity disorder.
†The SES categories 4, 5, and 6.
‡Girls were defined as nicotine users if they reported daily smoking.
§Experimentation was defined as not having used these substances more than 5 times throughout a girl’s life.
||The NC girls were recruited on the basis of absence of these disorders.

336.9±173.5 nmol/L compared with a mean of 386.5±102.6 nmol/L in the nonaggressive CD group (n=10). However, this difference was not statistically significant (t_{45}=1.92; P = .07).

To determine whether decreased cortisol level was simply a correlate of severe psychopathology, as manifested by multiple diagnoses, we examined the mean cortisol levels in the following groups: NC (n=37) (467.4±212.2 nmol/L), CD with no comorbidity (n=12) (291.9±131.6 nmol/L), CD with ODD only (n=6) (338.8±103.2 nmol/L), and CD with multiple diagnoses (n=29) (372.2±179.1 nmol/L). It is clear that the girls with CD who had no comorbidity, not the girls with multiple comorbid diagnoses, had the lowest mean cortisol level. Only 2 girls had comorbid PTSD, but their cortisol levels were the lowest 2 in the sample. However, these 2 girls also met criteria for major depression, separation anxiety disorder, and generalized anxiety disorder.
nostic clinical marker, one must first show that the rela-
tion between the two is not due to a confounding
factor. We have extended the previous work in males by
demonstrating that the association between cortisol and
CD is not due to factors such as season or race. The next
step is to demonstrate that the biological finding dis-
criminates between subgroups within the spectrum of a
diagnostic category.67,68 We did find that girls with ag-
gressive CD had lower mean cortisol levels than girls with
nonaggressive CD did, but this difference was not sta-
tistically significant. However, only 10 girls with CD were
not aggressive. Thus, the question of whether low cor-
tisol level is a marker for aggressive CD in girls remains
unanswered.

On the basis of a study reporting a high rate of PTSD
in a sample of incarcerated adolescent females69 and data
suggesting low cortisol levels in subjects with PTSD,70
we also investigated whether low cortisol level was a
marker for antisocial girls with PTSD. The girls with CD
with comorbid PTSD had the lowest cortisol levels in our
sample, but there were only 2 of them and they also met
criteria for several other diagnoses. Furthermore, the NC
vs CD differences remained when these 2 cases were de-
eloped from analyses. This important issue should be ad-
dressed in a study with larger numbers of antisocial girls
with PTSD.

Our design permitted us to examine many alterna-
tive explanations for the group difference in cortisol lev-
els. One limitation of our study, however, is that we could
not exclude sleep disturbance as an explanation for the
difference between the CD and NC groups. If the girls
with CD are less well supervised and their lives more cha-
ocatic, it is possible that, as a group, they may routinely go
to sleep later than midnight. This could shift their cir-
cadian rhythm such that the 8 to 9 AM blood-drawing time
may still be in the prepeak phase of their HPA axis cycle.70
This question merits further research.

To date, there have been no studies of the mecha-
nisms underlying decreased cortisol levels in antisocial
subjects. However, animal and human data suggest that
the ratio of corticotropin-releasing hormone to arginine
vasopressin may be lower than normal in aggressive sub-
jects, particularly in animals who have been bullied and
become bullies themselves.71,72 This explanation may ac-
count for both the aggression and PTSD aspects of our
findings. Although this research has focused on males,
one clinical study of pregnant teens did report that the
girls with CD had lower plasma levels of corticotropin-
releasing hormone than the pregnant controls without
CD.73

Future studies on HPA axis function in these girls
should focus on describing the basal state of cortisol se-
cretion throughout the rest of the 24-hour cycle, deter-
miming if their HPA axis responds normally to experi-
mentally induced stressors, and methodically investigating
the function of each component of the HPA axis (eg, re-
sponse to corticotropin-releasing hormone, assessment of
glucocorticoid receptor function). Research on poten-
tial mechanisms may result in more effective pharmaco-
logical interventions or facilitate the identification of sub-
groups of girls with CD who respond to different types of
treatments.

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REFERENCES

1. Zoccolillo M. Gender and the development of conduct disorder. Dev Psycho-
opathol. 1993;5:65-78.
gland: Longerman; 1970.
3. Kashani JH, Orvaschel H, Rosenberg TK, Reid JC. Psychopathology in a com-
munity sample of children and adolescents: a developmental perspective. J Am
4. Fergusson DM, Horwood J, Lysneky MT. Prevalence and comorbidity of DSM-
III-R diagnoses in a birth cohort of 15 year olds. J Am Child Adolesc Psy-
chiatry. 1993;32:1127-1134.
5. Total arrest trends, sex, 1993-1997 [Table SS]. In: Department of Justice. Crime
tice, Office of Justice Programs, Office of Juvenile Justice and Delinquency Pre-
8. Pajer KA. What happens to “bad” girls? a review of the adult outcomes of anti-
9. Campbell M, Gonzalez NM, Silva RR. The pharmacologic treatment of conduct
Loeber M, Green S. Serious conduct problems in the children of adolescent
58:840-844.
11. Bourdou C, Mann B, Cone L. Multisystematic treatment of serious juvenile of-
fenders: long-term prevention of criminality and violence. J Consult Clin Psy-
13. Haddad J, Barocas R, Hollenbeck A. Family organization and parent attitudes
14. Huizinga D, Loeber R, Torrente T. Urban Delinquency and Substance Abuse:
Research Summary. Washington, DC: Office of Juvenile Justice and Delin-
quency Prevention; 1994.
15. Mendel R, Prevention or Parke? A Hard-Headed Look at Youth-Oriented Anti-
16. Woodman DD, Hinton JW, O’Neill MT. Cortisol secretion and stress in maxi-
18. McCrumben K, Lahay BB, Rathouz PJ, Loeber R. Low salivary control and persis-
tent aggression in boys referred for disruptive behavior. Arch Gen Psychiatry.
2000;57:38-43.
England H. Salivary cortisol and cardiovascular activity during stress in opposition-
20. McCormack K, Lahay BB, Capasso L, Loeber R. Aggressive symptoms and sali-
toms in preadolescent boys and in their parents: associations with cortisol. Psy-
22. Moss HB, Vanyukov MM, Martin CS. Salivary cortisol responses and the risk for
23. Vinson GP, Whitehouse BJ, Hinson JP. Structure and function of the adrenal cor-
well Science; 1998:401-430.


