Mood and Hormone Responses to Psychological Challenge in Adolescent Males with Conduct Problems

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Background: *Relations between stress hormones and antisocial behavior are understudied.*
Methods: *A subsample (n = 335) of at-risk males recruited in first grade for a longitudinal study were recruited at approximately 16 years of age for a laboratory study, including two psychological challenges: describing their worst experience on videotape, and a task in which a loud tone could be avoided. Measures of affect, urine, and saliva were collected multiple times before and after challenges.*
Results: *Negative affect increased following the worst-event challenge and decreased following the avoidance challenge. Mean conduct problems (CP) across ages 7–17 years were positively related to negative affect and inversely related to positive affect. CP were inversely related to post-challenge urinary epinephrine (E) levels when baseline E and potential confounds were controlled. Cortisol concentrations in saliva collected soon after the first challenge were positively related to CP in a post hoc subset of youths with extreme CP.*
Conclusions: *Key findings A) associated persistent CP with more negative affectivity and less positive affectivity, B) replicated and extended prior findings of an inverse association of CP and urinary E, and C) suggested provocative hypotheses for future study relating CP, trauma history, trauma recall, and cortisol reactivity.*

Key Words: Antisocial behavior, conduct disorder, cortisol, HPA, epinephrine, mood

Researchers have long searched for relationships between hormones and antisocial behavior. Two hormones in particular reflect the activity of arousal systems that are hypothesized to be associated with the development of CP and impulsiveness (Lahey et al 1995; McBurnett 1992; Quay 1988a, 1988b). These are epinephrine, which is released from the adrenal medulla rapidly during sympathetic nervous system (SNS) activation, and cortisol, which is released from the adrenal cortex as the end product of a less rapidly-responding arousal system, the hypothalamic-pituitary-adrenal (HPA) axis. A lengthy literature substantiates that the SNS and HPA respond to threatening stimuli and activate during fearful states, as well as during strong appetitive or competitive states (Cannon 1932).

Previous studies have suggested that antisocial behavior in children and adolescents might be associated with underarousal of the HPA (Lahey et al 1993; McBurnett and Lahey 1994). Several studies have found low cortisol concentrations in antisocial individuals, or have linked low cortisol to history or family history of aggressive, antisocial behavior (Pajer et al 2001; Vanyukov et al 1993; Virkkunen 1985). An inverse relationship between cortisol response direction and oppositional behavior has been reported in two studies (King et al 1998; van Goozen et al 1998). Our own research group has found an interaction between aggression and anxiety in two studies, one with adolescent inpatients (McBurnett et al 2000) and the other with child outpatients (McBurnett et al 1991), reflecting low cortisol when aggression was not accompanied by significant anxiety. We failed to replicate the interaction in a follow-up study of the child sample, but we identified an association between persistently low cortisol and persistent aggression (McBurnett et al 2000). The opposite relationship—a positive association between cortisol and aggressive behavior—has sometimes been found, most often in nonclinical, population-based samples (Dettling et al 1999; Gerra et al 1997; Gerra et al 1998; McBurnett 1998; Salvadora et al 1999).

Turning to peripheral epinephrine, this hormone also has been shown to vary inversely with the trait of aggressiveness. In a sample of 58 15- through 17-year-old male adolescents selected from the community, a significant inverse correlation was found between urinary epinephrine levels following a classroom test and teacher ratings of aggressiveness (Olweus 1986). This finding was replicated in a similar Swedish sample of 86 13-year-old males selected from a larger population-based sample (Klinteberg and Magnusson 1989).

We report here on hormone-behavior relationships in a population-based sample that was selected to over-represent male youths at high risk for CP. This study examined salivary cortisol and urinary catecholamines in baseline samples and following two psychological challenges. The subjects were drawn from a larger study of antisocial behavior development, the Pittsburgh Youth Study (PYS; Loeber et al 1998). Participating families provided informed consent. The study was approved by the University of Pittsburgh School of Medicine Institutional Review Board.

Methods and Materials

Subjects

The PYS consists of three cohorts of male youths who were in grades 1, 4, and 7 at study entry. In 1987–1988, potential participants were randomly selected from all males in these grades in Pittsburgh public schools (at the time, 72% of Pittsburgh students attended public schools); 84.6% gave informed consent and participated in initial screening (wave S). Participants' grade 1 achievement test scores and the proportion of African-American subjects were similar to district means, suggesting that nonparticipation did not bias the sample.

Present analyses were based on the first-grade cohort only. At

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Received August 26, 2003; revised September 24, 2004; accepted January 24, 2005.

0006-3223/05/$30.00
doi:10.1016/j.biopsych.2005.01.041

wave S (screening), a risk score based on caretaker, teacher, and youth report of 21 serious antisocial behaviors was used to select (from a sample of 868) all consenting first-grade males scoring at or above the 70th percentile of the sample (n = 256), and an equal number of males (n = 247) randomly selected from the remainder of sample. At wave S, the 503 participants in the first-grade cohort averaged 6.8 years. The primary adult caretaker classified 57% of the males as African-American and 43% as white. Half the sample was receiving welfare, and the biological father was still a member of 38.5% of the households. For more information about sample acquisition, see Loeber et al (1998b).

Longitudinal Assessment of Youth Conduct Problems

Data were collected every six months through wave H (when youths averaged 10.9 years of age), then annually through wave T (average 17.0 years). Assessments consisted of interviews/questionnaires with the youth and primary adult caretaker (biological mother in 94% of cases), and a questionnaire completed by the teacher. Items were then used to construct a multiple-informant conduct problem score based on 11 CD behaviors—bullying, fighting, lying, cruelty to animals, running away from home, firesetting, theft without confrontation of the victim, truancy, breaking and entering, and vandalism—by assigning each item the highest rating obtained from any informant, per wave. During screening (S) and waves A-G, CP was based on a combination of the caretaker-completed Child Behavior Checklist (CBCL: Achenbach 1991a) and teacher-completed Teacher's Report Form (TRF;Achenbach 1991b). From wave H (when youths averaged 10.9 years) through wave T, data on CP were also obtained from the youth using the Youth Self-Report (Achenbach 1991c). In wave T, information was not obtained from teachers, because many youths had dropped out of school, and teachers spent little time with youths still in school.

Recruitment of Participants for the Laboratory Study

Participating families provided informed consent. The study was approved by the University of Pittsburgh School of Medicine Institutional Review Board.

Families in the first-grade cohort who refused further participation (n = 45), moved out of the area (n = 31), or were incarcerated (n = 20) were excluded, leaving 407 potential participants for the laboratory study. Of these, 72 (17.7%) did not participate in this study because of nonconsent (n = 35), or refusal at scheduling, repeated cancellation, or failure to attend appointments (n = 37). The remaining 335 participated in the laboratory study (a 66.6% participation rate).

The protocol required a single day of laboratory assessments. For most participants, this occurred in either wave P or R (a minority were in wave T), when their average age was 16.15 (range = 14.17–18.75). Participants were paid $60 and a bonus of $10 if the participant did not break his first appointment. Caretakers were paid $30 for transporting participants. If a responsible adult could not do this, the study paid for taxi service.

Procedure

Laboratory assessments were scheduled for 8:00 AM to 2:00 PM. Participants were instructed not to take acetaminophen 48 hours before the study; not to consume dairy products, brush or floss teeth, or smoke after 7:00 AM; not to urinate after 7:30 AM if possible; and not to consume caffeine the morning of laboratory visit. Compliance (except for urination) was queried. Violations were treated as covariates in data analyses.

Median start time was 8:16 AM (range 7:55 AM–9:11 AM for 90% of participants). Clock times were recorded at designated events. Average times urine and saliva collection reported below were estimated based on these reference times.

Affect During Protocol. Youth affect was assessed using the Positive and Negative Affect Schedule (PANAS; Watson et al 1988). This self-report measure combines items into positive affect (active, alert, attentive, determined, enthusiastic, excited, inspired, interested, proud, and strong) and negative affect (afraid, ashamed, distressed, guilty, hostile, irritable, jittery, nervous, scared, and upset) scales.

Baseline Urine. Baseline urine was collected in a vial 2 minutes into the protocol, following assessment of vital signs. Ten ml of urine was transferred to a polypropylene tube containing 200 μl of 6N HCl as preservative. If the pH was 3.0 or above, one drop of HCl was added until the pH was between 1.0 and 3.0. The sample (with an additional 10 ml for drug testing) was frozen at –70°C until analysis. Urinary drug screen revealed recent use of amphetamine (n = 1), cocaine (n = 1), barbiturates (n = 1), and tetrahydrocannabinol (THC) (n = 69). Recent use of alcohol or other drugs was not detected in any subject. The three youths who tested positive for amphetamine, cocaine, or barbiturates were excluded from further analyses.

Saliva. The schedule of seven stimulated saliva samples included two baselines and five samples tracking the cortisol response to experimental stressors (a curve which occurs predominantly 8–30 minutes post-stressor, peaking around 20 minutes post-stressor; Kudielka et al 2004). Beginning at a median time of 8:27 AM, 2 ml of saliva were collected. Flow was stimulated by chewing Carefree Sugarless Bubblegum.[1] Each collection took approximately 3 minutes. Participants were then given bottled water to facilitate post-challenge urination. Youths completed the PANAS for the first (Baseline 1, B1) of five times. After a 3-minute rest, they participated in a 5-minute tone-orienting task for a psychophysiological study. The second saliva sample was collected 44 minutes after the first, at median 9:11 AM.

Participants then completed a second PANAS (Baseline 2, B2) and participated in the first of two challenge procedures. The Trier Social Stress Test (Kirschbaum et al 1993) was adapted for this population, primarily by shortening its duration and increasing its relevance to participants. Youths were asked to think for 2-min about the worst thing that ever happened to them, attempting to recall what it felt like, as if they were re-experiencing the event. Then they were asked to describe that event out loud for 2-min while being videotaped, followed by a third PANAS (Post-challenge 1, P1). If a participant did not speak for the full 2-min, standard probes were used to encourage speaking.

The second challenge presented numbers on a video screen, counting down from 12 to 0, one per second. Participants were told a loud noise would sound when the numbers reached 0, but that the loud noise would occur unpredictably at other times. After five trials they were told they could prevent the loud noise from coming on if they pressed a button after 1 appeared but before 0, but if they pressed the button before 1 or after 0 they

[1]Although we have used this method in other studies (McBurnett et al 1991, 2000) without complication, the possibility exists that gum or flavoring may in some unknown way increase variability in cortisol concentration values. We refer future investigators to recommended methods for gathering sufficient saliva volume for assays (e.g., Schwartz et al 1998).

would hear the loud tone. Following this challenge, the fourth PANAS was administered (P2) and a third saliva sample was collected (median 9:32 AM).

Following another bottled water break, the fourth saliva sample was collected (median time 9:40 AM). After an evoked potential procedure (an auditory discriminant reaction time task), the fifth saliva sample was collected (median 9:52 AM). The sixth saliva sample was taken following a continuous performance task (median 10:08 AM). The participants rested for 3-min, then completed the fifth PANAS (P3) and seventh saliva sample (median 10:26 AM).

Thus, the first two stimulated saliva samples preceded the challenge protocols. Median times from the first of the two challenges (which took approximately 13 min to complete) were approximately 15 min for the third sample, 23 min for the fourth, 45 min for the fifth, 51 min for the sixth, and 69 min for the seventh. Besides the three youths who were eliminated because of recent drug use, three youths elected to discontinue after the first sample, leaving 328 youths with all seven saliva samples.

Salivary cortisol concentrations were assayed in solid phase radioimmunoassay using a modification of Diagnostic Product Corporation's plasma cortisol method. Because saliva cortisol levels are lower than in plasma, calibrators were diluted 1:10 in water. Range of the standard curve was .1 μg/dl – 5.0 μg/dl. Limit of detection for this method is .05 μg/dl. The test requires 200 μl of saliva for each replicate. Samples were tested in duplicate. Intra-assay coefficient of variation (CV) for duplicates ranged from .01% to 4.38% (mean = 1.19% CV). The inter-assay variation ranged from 13.75% CV (mean = .29 μg/dl) to 9.38% (mean = 3.62 μg/dl). Cortisol values were logged prior to analyses using the formula log C_n = log (1+C_n). Two extreme outliers were eliminated to avoid possible artifacts of blood contamination.

Post-Challenge Urine. Following the seventh saliva sample, participants gave their second urine sample for analysis of post-challenge catecholamines. Given our focus on arousal/stress systems, we were primarily interested in epinephrine (E). However, other catecholamines—dopamine (DA) and norepinephrine (NE)—have been inconsistently linked to aggressive behavior (Castellanos et al 1994; Miczek et al 2002; Placidi et al 2001; Serova and Naumenko 1996; van der Vegt et al 2003; van Goozen et al 1999), and we elected to include them in urinalysis.

Statistical Treatment of Data

All planned analyses used aggregate CP as a predictor in regression models. Preliminary analyses were conducted to determine if CP at different ages (or reported by different combinations of informants) were related to hormones in different ways. Three separate cross-sectional models, with means of CP during waves S-C (reported by parent and teacher), D-G (reported by parent and teacher), and H-T (reported by parent, youth, and teacher) used as predictors, produced qualitatively similar results. Therefore, we used the mean of the multiple-informant CP scores across all ages to represent individual differences in CD behaviors. Most relationships to criterion variables were modeled using Ordinary Least Squares (OLS) regression. Effects of extraneous variables that were related to criteria were controlled simultaneously by including them in joint OLS models. The complex pattern of changes in affect over time was examined using repeated-measures linear regression in general estimating equations (GEE; Zeger and Liang 1986). GEE is an extension of the generalized linear model that can accommodate serially-correlated data (as occurs in repeated observations within subjects).

Results

Association of CP with Positive and Negative Affect

Among the 335 youths who began the protocol, 330 completed the PANAS on all five occasions. The three hard-drug-positive participants were excluded so that subjects would be the same as in the cortisol analyses. PANAS scores are shown in Figure 1 (by quartile for clarity; continuous scores were used in statistical analyses).

Significant decreases occurred in both positive ($z = -5.96$, $p < .0001$) and negative affect ($z = -4.23$, $p < .0001$) between the first (B1) and second (B2) baseline PANAS. From the second baseline to immediately after the worst-event challenge (P1), no significant change in positive affect occurred ($z = 2.04$, $p = .15$), but negative affect increased ($z = 13.31$, $p < .0005$). From P1 PANAS to post-count-down-challenge PANAS (P2), positive affect increased ($z = 2.69$, $p < .01$) and negative affect decreased ($z = -6.12$, $p < .0001$). From the first (P2) to second (P3) PANAS following count-down, positive affect marginally declined ($z = -1.87$, $p = .06$) and negative affect slightly declined ($z = -2.25$, $p < .03$).

After accounting for linear, quadratic, and significant cubic components of the time dimension for positive and negative affect in GEE (which occurred at introduction and removal of challenges), youths with higher CP exhibited more negative affect ($z = 2.17$, $p = .03$) and less positive affect over time ($z = -2.22$, $p < .03$). Tests for interactions between CP and time found two significant interactions for positive affect change: from P1 to P2 ($z = -2.28$, $p < .03$) and from P2 to P3 ($z = 2.07$, $p < .04$). Positive affect of youths with more CP increased less from

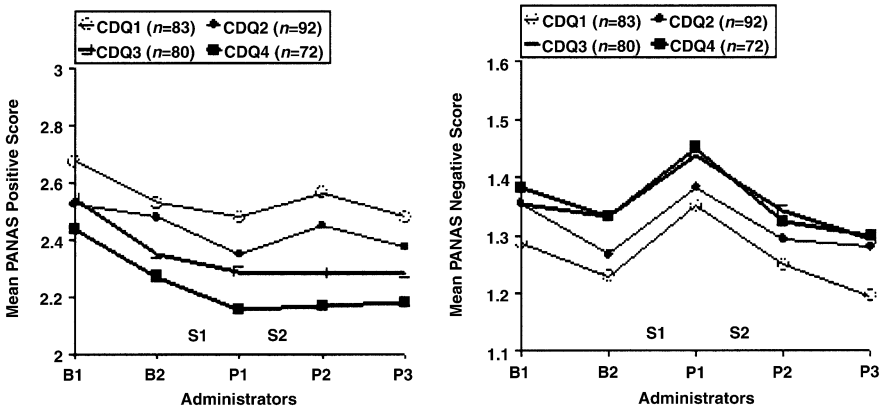

Figure 1. PANAS scores for current positive and negative affect measured before (B1 and B2) and after (P1-P3) the worst incident challenge (S1) and the count-down challenge (S2) in four groups based on quartiles of the sample distribution of conduct problem scores over waves. PANAS, Positive and Negative Affect Schedule; CDQ, conduct disorder quartile.

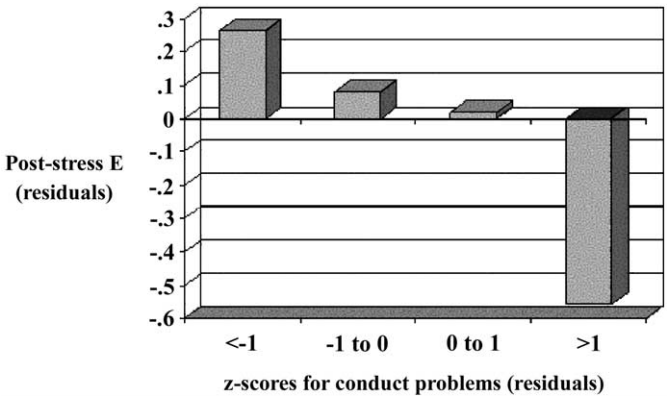

Figure 2. The mean post-stress urinary epinephrine values for boys whose CP scores were at least −1 SD below the mean (< −1. SD) , below the mean of the distribution but less than −1 SD below the mean (−1 to 0), above the mean of distribution but less than 1 SD above the mean, or 1 SD above the mean. Both epinephrine and conduct problem scores are residualized for recent marijuana use, race-ethnicity, and consumption of dairy products during the morning prior to the laboratory session. CP, conduct problems; E, epinephrine.

before to after the second challenge (the count-down task), and then decreased less after the second challenge.

Association of CP with Urinary Catecholamines

Of the 335 youths at entry, 42 were excluded from catecholamine analyses because they did not urinate at baseline and/or after the challenges. Additionally, the three youths who tested positive for recent drug use were dropped. The 290 youths whose urine was analyzed were compared to the 178 nonparticipants. Participants had lower family incomes than nonparticipants ($20,647 vs. $23,935) ($t$ (184) = 2.28, $p <$ .03) but did not differ on race-ethnicity, age in first grade, or CP over waves.

CP were not significantly related to either baseline or post-challenge urinary DA and NE, but were inversely related to post-challenge E. Youths' ages at entry, family income, tobacco use during the past 24 hours and morning of the protocol, consumption of products containing caffeine, and time of protocol start were not significant covariates in any model for E. Because neighborhood crime, race-ethnicity, consumption of dairy products preceding the protocol, and recent THC use were significant correlates of E or NE/E ratio, they were used as covariates in models for E and NE/E.

In a joint OLS regression model, baseline urinary E was significantly related to race-ethnicity (higher in African-Americans), (β = 3.19, t = 2.88, df = 1, $p <$ .005) and to recent THC use (β = 2.16, t = 2.62, df = 1, $p <$ .04); but not to CP (β = −3.68, t = −1.37, df = 1, p = .17), consumption of dairy products (β = −2.48, t = −1.62, df = 1, p =.11), or neighborhood crime (β = −.00, t = −.05 , df = 1, p = .96). In contrast, post-challenge urinary E levels were significantly related to CP (β = −10.52, t = −3.67, df = 1, $p <$ .0003), THC (β = 4.01, t = 3.10, df = 1, $p <$ .003), consumption of dairy products that morning (β = −3.65, t = −2.23, df = 1, $p <$ .03), and marginally to race-ethnicity (β = 2.29, t = 1.94, df = 1, p = .05); but not neighborhood crime (β = .04, t = .62, df = 1, p = .53). The inverse relation between post-challenge E and CP is illustrated in Figure 2. For this plot, both post-challenge E and CP scores were residualized for significant covariates (THC, dairy products, race-ethnicity) and converted to z-scores for plotting. The plot shows that the significant inverse association between CP and post-challenge E is almost entirely due to an inverse relation at the extremes of CP scores, with little variation in the broad middle of the distribution (±1 SD). None of the youths with an E z-score of 1 or more (1 SD above the mean) had a CP z-score over 1 SD above the mean. Conversely, none of the youths with a CP z-score of 1 or more had a post-challenge E z-score of 1 or more.

When baseline E was controlled to assess change from baseline, post-challenge E was positively related to baseline E (β = .47, t = 8.08, df = 1, $p <$ .0001), inversely related to CP (β = −8.08, t = −3.40, df = 1, $p <$ .001), and positively related to recent THC use (β = 2.78, t = 2.37, df = 1, $p <$ .02), but not to race-ethnicity (β = .80, t = .74, df = 1, p = .46), dairy products (β = −2.48, t = −1.68, df = 1, p = .09), or neighborhood crime (β = .04, t = .72, df = 1, p = .47). The significant inverse association between CP and pre-post change in urinary E again reflected an inverse association at the extremes of each distribution.

Association of CP with Salivary Cortisol

A total of 333 youths provided all seven saliva samples. Two were dropped from analyses because their scores were extreme outliers. Three were dropped because of recent drug use. The remaining 328 were compared to the 175 nonparticipants. There was a trend toward participants to have lower family incomes than nonparticipants ($21,663 vs. $23,965)($t$ (177) = −1.89, p = .06). There were no differences ($p <$ .10) in race-ethnicity, age in first grade, or CP.

Several variables were tested as possible covariates. Differences in age at study entry, family income, recent use of marijuana, tobacco use in past 24-hours or morning of the protocol, consumption of acetaminophen, consumption of dairy products, and flossing in the morning before the protocol were insignificantly related to cortisol. Consumption of caffeine before and start time of the protocol were each related to cortisol in some models and were therefore included as covariates in all models. OLS regressions were conducted for each of the seven cortisol samples. When the CP score was treated as a continuous variable, CP were not related to any of the seven cortisol values. We conducted two post-hoc categorical analyses to explore this unexpected finding. Youths in the top quartile of CP did not differ significantly in cortisol levels from the remainder of the sample. We then narrowed the extreme group to include only the top 5% of the 290 youths for whom lab data was available. When the 15 youths with extreme CP (≥95th sample percentile) were compared to the remainder of the sample, they had higher cortisol concentrations at P2 (t = 2.47, $p <$ .02), and P3 (t = 2.91, $p <$ .005), collected approximately 23 and 45 minutes after start of the worst-experience challenge, but not on other cortisol samples (Figure 3).

To further characterize this group (top 5% of CP), we compared their baseline and post-challenge E to the remaining subjects, controlling for significant covariates. The extreme group had lower E at baseline (t = −2.03, $p <$ .05) and at post-challenge (t = −2.24, $p <$ .03). When baseline E was controlled, the direction of effect remained but the difference became nonsignificant (t = −1.51, p = .13).

Upon finding this response divergence between cortisol and E in the extreme group, we changed the direction of analysis and used these hormones as continuous predictor variables in multiple regression with CP as the criterion, controlling for extraneous variables. Baseline E had virtually no association with CP. Post-challenge E was negatively associated (t = −3.73, $p <$ .001),

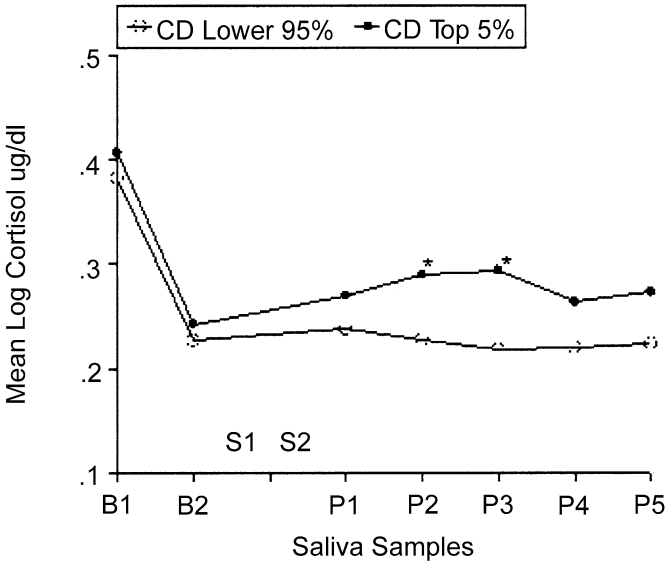

Figure 3. Comparison of logged cortisol values in each of the seven saliva samples between youths with conduct problem scores in the top 5% of the sample distribution and the remainder of the sample. Two baseline saliva samples (B1 and B2) were obtained at approximately 8:27 am and 9:11 am prior to the worst-event challenge (S1) and the count-down challenge (S2) and five post-challenge samples (P1-P5) were obtained approximately 15, 23, 45, 51, 69 minutes after the initial of the worst-event challenge, respectively. Asterisk indicates difference in log cortisol concentrations between the groups for samples P2 and P3. CD, conduct disorder.

and the mean of post-challenge cortisol concentrations (no. 3–6) was positively associated ($t = 2.19$, $p < .03$) with CP.

Exploratory Analyses of Relations of Affect and Urinary Catecholamines

Preliminary bivariate correlations and partial correlations suggested that neither NE nor DA were significantly related to PANAS scores. Post-stress E was correlated with both positive and negative affect, however. Means of positive and negative affect (and the sum of positive and negative affect) across the five PANAS administrations were found to represent PANAS scores as well as, or slightly better than, individual PANAS scores. In a joint model, post-challenge urinary E was positively related to mean positive affect ($\beta = 1.43$, $t = 5.16$, $df = 1$, $p < .03$), but not mean negative affect ($\beta = 2.02$, $t = 2.14$, $df = 1$, $p = .14$), controlling for baseline E, dairy products, cannibis use, neighborhood, and race-ethnicity. Because the significant association of positive affect and insignificant association of negative affect with post-challenge E were both directionally positive, mean positive and negative affects were summed. Post-challenge urinary E was positively associated with mean total affect ($\beta = 1.55$, $t = 8.47$, $df = 1$, $p < .004$), controlling for baseline E, dairy products, cannibis, neighborhood, and race-ethnicity.

Because youths with higher CP reported lower positive affect, we examined the possibility that the inverse association of CP with post-challenge E was mediated by either positive affect or total affect. There was no evidence of mediation.

Exploratory Analyses with Affect and Salivary Cortisol

Cortisol was not correlated with PANAS positive affect. However, 9:00 AM (second baseline) cortisol was weakly but significantly correlated with all five negative affect scores (Table 1). The first post-challenge cortisol concentration predicted some of the subsequent negative affect scores, but none of the remaining concentrations were significantly correlated with negative affect. To summarize, CP were only associated with cortisol in the second and third post-challenge samples; negative affect was only associated with cortisol in the second pre-challenge baseline and first post-challenge samples.

Discussion

Relation of Affect and CP in Challenges

The challenge paradigms appear to have changed affect. However, a causal relationship cannot be proven without a control group that was administered the PANAS five times under similar circumstances, but without the challenges. Although it seems unlikely, it is possible that observed changes in affect over time reflect the effect of repeated measurement of affect in a restricted setting rather than the causal impact of the psychological challenges.

Results are consistent with these interpretations:

1. The worst-event challenge was stressful and induced higher negative affect.
2. The count-down protocol was not experienced as stressful in an aversive sense (it did not change negative affect). Rather, the increase in positive affect following this challenge suggests that it was experienced as an engaging (possibly enjoyable) challenge. Given the relative ease of responding within a one-second interval, the task may have functioned as an active avoidance task in which the aversive event was successfully avoided. Although this is a post hoc explanation in the context of this experiment, the increase in positive affect is consistent with the experience of mastery and relief that occurs with successful active avoidance (Gray 1987).
3. Across conditions, youths with more CP report more negative and less positive affect. Youths with higher CP appeared to respond to the count-down challenge with less increase in positive affect. A similar pattern of low positive

Table 1. PANAS Negative Affect Scores Partially Correlated With Corticol Concentrations

	PANAS Negative Affect Scores				
	1	2	3	4	5
	Pre-Challenge Cortisol Samples				
Sample 1	$r = .06$	.04	.06	.05	.03
	$p = .29$	.51	.26	.40	.56
Sample 2	$r = .18$	.13	.17	.14	.12
	$p = .001$	.02	.002	.02	.03
	Post-Challenge Cortisol Samples				
Sample 3	$r = .11$	.10	.13	.11	.06
	$p = .05$	.08	.02	.04	.24
Sample 4	$r = .09$	.07	.08	.05	.07
	$p = .12$	.24	.16	.34	.22
Sample 5	$r = .05$	.07	.07	.05	.10
	$p = .35$	.23	.24	.38	.08
Sample 6	$r = -.02$	.00	.02	−.02	.05
	$p = .75$	.96	.67	.79	.40
Sample 7	$r = -.01$	−.00	.03	.01	.08
	$p = .84$	.93	.54	.86	.14

Partial correlations between the five Positive and Negative Affect Schedule (PANAS) negative affect scores and concentrations of cortisol in the seven saliva samples (controlling for session start time and caffeine consumption during the morning before the laboratory assessment).

and high negative affect has been found to characterize both youths and adults with major depression (Chorpita 2002). CP and depression are often comorbid and co-dynamic in children and adolescents (Lahey et al 2002). High negative affect/low positive affect may be common to both CP and depression and may partially account for their comorbidity.

Relation of Post-Challenge Urinary E and CP

Post-challenge E (controlling baseline E) was inversely associated with CP. This association was not monotonic, but reflected differences at the extremes of the distributions (Figure 2). Youths with high peripheral adrenergic response to psychological challenges consistently refrained from severe CP from ages 7–17 years. Conversely, youths high on CP did not show high adrenergic responsiveness. These results match prior linkages of behavior disorders with low output of E (Anderson et al 2000; Klinteberg and Magnusson 1989; McCracken 1991; Mefford and Potter 1989; Olweus 1986; Pliszka et al 1994, 1996), and fit the general view that low SNS reactivity is associated with antisocial behavior (Fowles 2000; Fowles and Missel 1994; Lahey et al 1993; McBurnett and Lahey 1994; Raine et al 2000).

Relation of Salivary Cortisol and Conduct Problems

Exploratory statistical analyses (not reported here) found no significant associations of cortisol-anxiety relations or anxiety-by-CP interaction. In contrast to our prior studies, we found no evidence of an inverse relationship between cortisol and CP. Instead, a post hoc finding (subject to error and thus tentative) showed that the most antisocial youth exhibited higher cortisol after the worst-event challenge (although here again, a control group not exposed to challenge would be required to incontrovertibly demonstrate this relationship). As noted in the introduction, existing literature on cortisol-aggression relationships is replete with opposite directional findings (see also the positive relationship between basal cortisol and aggressive response to provocation in Gerra et al 1997). Across studies, we can infer that youth with antisocial behavior often have abnormalities in HPA activity, but we have yet to measure well the complex of factors that play a role in determining the precise nature of HPA differences. In general, however, there is a pattern for population-sampled antisocial behavior to show positive associations between cortisol output and aggression, and for clinic- or institution-sampled studies to show the inverse. This is consistent with speculation that aggression in relatively prevalent hostile-reactive individuals is associated with reactivity, whereas aggression in less prevalent primary psychopaths is associated with lack of reactivity (McBurnett et al 2003; Skeem et al 2003).

Dissociation of E and Cortisol Reactivity

Reactivity is temporally distinct between the SNS and HPA. Post hoc analyses in this study show that E and cortisol responses can be directionally unlinked as well and can proceed in opposite directions, in the same set of subjects. The post hoc nature of the counter-directional response finding renders it ungeneralizable but suitable for future hypothesis testing. The question immediately arises, why would two stress-reponsive systems vary in this way? Setting aside the question of unmeasured explanatory factors, we can speculate on why a fast-responding system might react differently than a slow-responding system. Situations requiring immediate behavioral activation or inhibition call for a fast system. Situations in which a difficult or antagonistic encounter is anticipated may be most successfully managed by a slower responding system that mobilizes resources in different ways. Such is the case in many competitive or combative situations—the context develops over the course of minutes, hours, or days—but not seconds. However, only a fast-responding system can deliver the physiological underpinnings needed for a conditioned emotional response to develop. Under-activity in the fast-responding SNS, therefore, is likely to be rather consistently associated with a lack of inhibition or self-constraint of punishable antisocial behaviors. On the other hand, excessive release of either epinephrine or cortisol strengthens memory consolidation and can lead to indelible recall of context-specific information (Charney 2004; McGaugh 2000).

Another possible explanation for directional uncoupling of stress-responsive systems in an extreme subsample may be that historical-environmental events may have affected the systems differently. The HPA – or the more inclusive limbic-hypothalamic-pituitary-adrenal axis; LHPA – is subject to vastly different control and feedback regulation than the SNS. The plasticity of the LHPA, and the vulnerability to environmental deprivations during critical periods of development, have been known for some time (Meany and Aitken 1985; Meany et al 1985). Humans may be protected from experimental stress, but it has become well-documented that naturalistic stressors and deprivations can dysregulate normal HPA diurnal rhythms and can leave durable abnormalities in stress reactions (Cicchetti and Rogosch 2001; Gunnar et al 2001; Gunnar and Vazquez 2001; Kellner et al 2002; Yehuda et al 2002, 2004).

Individual Differences in Arousal Systems: Theoretical Questions

Individual differences in the HPA are subject to a complex developmental interplay among genetic factors, temperament, developmental timing of deprivations and stressors, and competition for social rank (Lahey et al 2002; McBurnett et al 2003). The direction of HPA correlates of aggression are determined by biobehavioral factors in combination with contextual factors (King and Edwards 1999; King et al 2001). Such complexity presents staggering design challenges for a single study, but it should be possible for future studies to address a limited number of potential interactions. One intriguing speculation from this study is the possibility that recounting one's most traumatic event has the greatest impact, in terms of HPA stimulation, on the most seriously antisocial boys. In other populations, victimization or exposure to violence has been found to moderate the association of aggression and cortisol, leading to a pattern in which aggressive individuals with a history of victimization display strong HPA activation (Scarpa 1997; Scarpa et al 2000; Scarpa and Kolko 1996; Scarpa and Ollendick 2003). Our study did not have the a priori hypotheses or measures needed to investigate the role of trauma exposure, but this problem merits careful investigation in the future.

Another explanatory lode may lie in the multi-factorial nature of CP. Lorber (2004) recently reviewed the literature on heart rate and electrodermal activity and found clear indications that the relationship between these physiologies and antisocial behavior was moderated by how antisocial behavior was operationalized (coded as aggression, psychopathy, antisocial personality, or conduct problems). Even if we narrow our focus to aggression, it may be important to distinguish reactive/defensive aggression from proactive/instrumental aggression, in light of findings distinguishing cortisol patterns in primates who differ in these types of aggressive behavior (Kalin 1999).

Finally, scrutiny of temperament and personality may shed

light on how arousal systems and types of CP are related. We are intrigued by the findings herein that recall of worst experience induced negative affect and increased cortisol in high-CP youths. It is possible that the aggressive + anxious individuals in two of our prior studies (McBurnett et al, unpublished data; McBurnett et al 1991), as well as the extreme (top 5% on conduct problems) group in the present study, fit a kind of affectively hostile, emotionally dysregulated, reactively aggressive, and behaviorally explosive profile that is cognitively predisposed to make attributions of threat and hostility, and to react with HPA activation. In contrast, the individuals without anxiety in one previous study (McBurnett et al 1991), and the individuals with early-onset aggression in another study (McBurnett et al, unpublished data), may fit more of a primary psychopathic profile that is cognitively predisposed to minimize risk, and to react less frequently with HPA activation. This relatively simple theoretical portrait is not without its problems, but it does provide a framework for organizing what may otherwise seem to be overly descriptive and discrepant results across studies.

Points of view or opinions in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Justice, the National Institute of Mental Health, or the National Institute of Drug Abuse.

Work on this paper was supported by grant #96-MU-FX-0012 from the Office of Juvenile Justice and Delinquency Prevention, grant #50778 from the National Institute of Mental Health, and grant #411018 from the National Institute on Drug Abuse (to RL); and by Independent Scientist Award #K02 MH01114-01 and grant #RO1 MH51091-01A1 from the National Institute of Mental Health (to AR).

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