DOI 10.1007/s00702-004-0253-5
J Neural Transm (2005) 112: 1083–1096

Journal of Neural Transmission
Printed in Austria

Salivary cortisol and aggression in a population-based longitudinal study of adolescent males

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Received July 16, 2004; accepted October 26, 2004
Published online December 7, 2004;

Summary. Chronic antisocial behaviour in youth has been associated with cortisol, a measure of stress reactivity. However, some studies have found low cortisol levels, while others have found elevated cortisol levels. The present study compared variously defined aggressive subgroups for differences in salivary cortisol. A population-based sample of boys was followed longitudinally from childhood to adolescence. Assessments of different forms of antisocial behaviour were obtained from various informants at several points in time, and cortisol was collected at age 13. Higher cortisol levels were found in boys with conduct disorder (CD) than in boys without CD. In addition, boys with an aggressive form of CD had higher cortisol levels than boys who showed a covert form of CD. Furthermore, reactive aggression was strongly correlated with elevated cortisol. Adolescent boys with chronic reactive aggression and those who scored high on aggressive CD symptoms seem to have a more active hypothalamic-pituitary-adrenal system.

Keywords: Salivary cortisol, aggression, longitudinal study, males.

Introduction

Among the many factors that contribute to individual differences in antisocial behaviour, stress-regulating mechanisms appear important (Kerr et al., 1997; McBurnett et al., 2000; Mezzacappa et al., 1997; Vanyukov et al., 1993). The

hypothalamic-pituitary-adrenal (HPA) axis is sensitive to physical and psychological stressors. Activity of the HPA axis can be estimated using measures of its end products, glucocorticoids. The primary glucocorticoid in humans is cortisol, and it can be assessed in saliva (Dettling et al., 1999).

Most psychobiological investigations of antisocial adults have observed an inverse relationship between the magnitude of behavioural deviation and cortisol level (King et al., 1990; Virkkunen, 1985; Woodman et al., 1978). Similar results have been obtained in children and adolescents: Cortisol levels have been reported to be negatively related to hostility towards teachers (Tennes and Kreye, 1985), to conduct problems (McBurnett et al., 2000; Pajer et al., 2001; Shoal et al., 2003; Van Goozen et al., 1998, 2000; Vanyukov et al., 1993), and to deviant behaviours in sons of fathers with a psychoactive substance use disorder (PSUD) (Moss et al., 1995). As in adults, in these younger age groups low levels of HPA axis activity have been interpreted as indicators of stress hyporesponsivity or fearlessness (Van Goozen et al., 1998, 2000). Although the cause of these patterns of cortisol hypoactivity or hyporesponsivity is as yet unknown, it may be the result of differences in genetic makeup or early modifications of the developing brain following pre- or postnatal stressful conditions of life (Van Goozen et al., 2000). However, the findings for children and adolescents are not as clear-cut as those for antisocial adults, in that positive relationships between cortisol and antisocial behaviour have also been found in some studies. In normal healthy adolescents a positive relationship was found between aggression and cortisol response level during an experimentally induced aggression task (Gerra et al., 1997) and during an emotion-arousing and painful procedure (Susman et al., 1997). Moreover, McBurnett et al. (1991) reported higher levels of cortisol in conduct-disordered (CD) children, but only when they had a comorbid anxiety disorder. Finally, some studies have found no relationship between cortisol and antisocial behaviour (Klimes-Dougan et al., 2001; Kruesi et al., 1989; Scerbo and Kolko, 1994; Schultz et al., 1997; Stoff et al., 1992; Susman et al., 1999). The mixed cortisol findings for children and adolescents may be due to important methodological differences among these studies. First, the label ‘antisocial’ has been used for behaviours as different as physical aggression, running away from home, stealing, and drug use (Coie and Dodge, 1998; Tremblay, 2000, 2003). Furthermore, studies, which specifically assess physical aggression, have generally not taken into account whether they are of the reactive or proactive type. Reactive and proactive aggression has been observed in children, adolescents and adults (Brendgen et al., 2001; Dodge et al., 1997; Pulkkinen and Tremblay, 1992; Vitaro et al., 1998). Reactive aggression is impulsive, often accompanied by disinhibition and affective instability, but not necessarily by antisocial tendencies; it is characterised by high levels of bodily arousal. On the other hand, proactive aggression is nonimpulsive and controlled, and occurs in the context of persistent antisocial behaviour. Proactive aggressive individuals are less likely to have unstable affects, their aggression is goal-directed, and the level of arousal is usually low (Vitiello and Stoff, 1997). Therefore, one could predict theoretically that proactive aggression is more likely to be associated with low levels of cortisol whereas reactive aggression will co-occur with elevated levels of

cortisol. Differences in assessments of cortisol may also explain the diversity of results. Some studies measured cortisol under resting conditions (Kruesi et al., 1989; McBurnett et al., 2000; Shoal et al., 2003; Tennes and Kreye, 1985), while other studies measured cortisol before the occurrence of an anticipated stressful event (Dawes et al., 1999; Moss et al., 1995), during an aggression provoking task (Gerra et al., 1997), or under highly stressful conditions (Van Goozen et al., 1998, 2000). Moreover, the majority of studies on children's cortisol concentration involved measurements varying over the day, without controlling for the clear circadian rhythm in cortisol secretion or for the kinds of activity that participants had done before the start of the study (McBurnett et al., 2000); only a few studies measured cortisol concentration at specific time points, having kept the conditions for all participants equal (Susman et al., 1997; Van Goozen et al., 1998, 2000). Finally, some studies used clinical samples (McBurnett et al., 2000; Scerbo and Kolko, 1994), some compared clinical samples to non-clinical samples (Kruesi et al., 1989; Pajer et al., 2001; Stoff et al., 1992; Van Goozen et al., 1998, 2000), while others used community samples (Gerra et al., 1997; Klimes-Dougan et al., 2001).

We report results of a study with a population-based sample of boys, followed over a 9-year period for the development of their aggressive behaviour. The aim was to investigate whether variously defined aggressive subgroups would differ in salivary cortisol level. We examined this in four different ways. First, we compared participants with and without a CD diagnosis. In a second analysis, we distinguished between aggressive and covert CD symptoms and examined the effect of this classification on cortisol. Third, we classified all participants according to the frequency of their use of physical aggression as assessed from age 6 to age 15, and related that to cortisol. And in a last analysis, we took into account the boys' use of reactive and proactive aggression and related this to cortisol. We specifically predict that the more aggressive groups would have lower levels of cortisol. Like in most studies on cortisol we used a single saliva sample and considered this to be the participants' baseline or resting level.

To our knowledge this is the first study that has used longitudinal data from a population-based sample comparing cortisol levels of physically aggressive and non-aggressive cases.

Material and methods

Participants

The participants involved in the present study (n = 194) were participating in a longitudinal study from kindergarten onwards (n = 1,161). The selection and reduction procedure of the larger sample had been described previously (Séguin et al., 1995, 1996; Tremblay et al., 1991b, 1994, see also Fig. 1). To obtain a high base rate of boys at risk for disruptive behaviour, the 53 schools in an urban area with the lowest socio-economic index were chosen. Teachers were asked to rate each boy in their classes. Ratings were returned by 87% of the teachers, and 1,161 boys had been rated. To control for cultural effects, boys were only included in the study if both biological parents were born in Canada and were French speaking. To ensure that the sample would be from families of low socio-economic background, we also eliminated boys when either

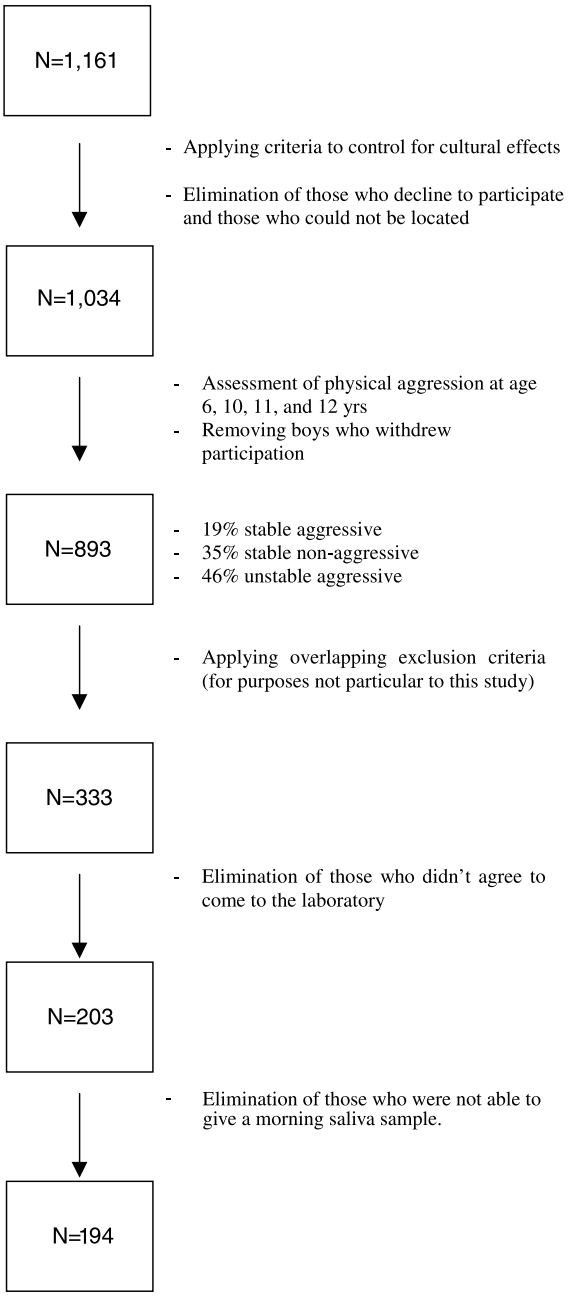

Fig. 1. Selection of participants (n = 194) from large community sample (n = 1, 161)

of the parents had more than 14 years of schooling. The sample was reduced to 1,034 boys after applying these criteria and eliminating those who declined to participate and those who could not be located (Tremblay et al., 1991b, 1994).

Physically aggressive behaviour had been assessed at ages 6, 10, 11, and 12 years by means of the fighting subscale of the teacher form of the French Canadian version of the Social Behavior Questionnaire (SBQ; Tremblay et al., 1991a). After eliminating the boys who withdrew their

participation from the longitudinal project (n = 116) and those who had more than one missing value on physical aggression at follow-up (n = 28), we determined stability and severity of physical aggression for 893 boys from assessments at ages 6, 10, 11, and 12 (see also Séguin et al., 1995, 1996). Those who fell above the 70th percentile at age 6 and at least at two or more assessment points were classified as stable aggressive boys (19% of the sample). Nonaggressive boys had scores that fell below the 70th percentile at all assessment points (35% of the sample). Those who did not meet the above criteria were classified as unstable aggressive boys (46% of sample). Compared to another sample of boys (n = 882) representative of the whole province, physically aggressive behaviour is over-represented in this urban community sample of low socio-economic status (Séguin et al., 1996).

For logistical reasons, we were able to invite approximately 200 13-year old boys to come to the laboratory for various observational and experimental procedures. Several overlapping criteria were used to select this subsample. Exclusion criteria (for purposes not particular to this study) were applied as follows: 234 boys who could not be classified as stable anxious or stable nonanxious were eliminated, as well as 326 boys who did not meet priority criteria such as (a) stability of physical aggression or nonaggression, (b) a history of going to the laboratory since age 6, or (c) a pattern of late onset physical aggressive or anxious behaviour. Thus, some moderate or unstable anxious boys remained in the selected sample because they met some of these priority criteria (n = 138). Those who met priority criteria (b) or (c) but not (a) were classified as unstable aggressive boys. When all these criteria were applied, the selected sample consisted of 333 boys who were 13 years old at the time of our assessment. Of these, 203 boys agreed to come to the laboratory (Séguin et al., 1995, 1996). It turned out not to be possible to take a morning saliva sample in 9 boys, leaving us with a total subsample of 194 boys. We considered this subsample representative of the larger sample (n = 843), because when we compared our subsample of 194 boys to the remainder of the sample on a number of important variables (e.g. SES, physical aggression trajectory group (Nagin and Tremblay, 1999), proactive and reactive aggression), no differences were observed with the exception of proactive aggression. The subsample was slightly more proactive aggressive ($t = -2.23$, $p = .03$).

Procedure and instruments

Assessments of conduct disorder (CD) and physical aggression at 14–16 years of age

The Diagnostic Interview Schedule for Children (DISC-2.25) (Shaffer et al., 1991) was administered to the participants and their parents (mostly their mothers) when the boys were 14–16 years old. A letter followed up by telephone contacts served to solicit participation (Séguin et al., 1999). The DISC could not be administered to 15 of the participants, and 7 participants had only a child or a parent report. A participant was attributed to the CD subgroup when he met three or more of the thirteen CD criteria based on combined reports of parent and child. In this way a CD subgroup consisting of 20 boys and a normal control (NC) subgroup of 159 boys were created. Additionally, and following McBurnett et al. (2000), we divided the 13 diagnostic criteria for CD into items describing aggressive behaviour (i.e. often initiates physical fights; has used a weapon; has been physically cruel to people; has been physically cruel to animals; has stolen while confronting a victim; has forced someone into sexual activity) and covert (non-aggressive) behaviour (i.e. has set fires; has destroyed property; has broken into someone else's house, building or car; tells lies; has stolen; has run away from home overnight; is truant from school). Two subgroups were created on the basis of the number of items checked for aggression and/or non-aggression. A participant belonged to the aggressive subgroup (A) when he exhibited one or more of the 6 aggressive behaviours, regardless of whether he exhibited covert behaviours (n = 22), and to the covert subgroup (C) when he exhibited one or more of the 7 covert behaviours, but not any of the aggressive behaviours (n = 39).

Assessments of physical aggression trajectories from 6 to 15 years of age

The estimation of developmental trajectories for repeated measures of physical aggression from kindergarten to mid-adolescence is based on teacher reports. The boys' classroom teachers rated physical aggression in the spring of each year using a French Canadian version of the Social Behavior Questionnaire (Tremblay et al., 1991a). This questionnaire was administered when the boys were age 6, 10, 11, 12, 13, 14, and 15 years. Physical aggression was assessed with three items; ''kicks, bites, hits''; ''fights''; and ''bullies or intimidates other children''. The range of possible values of the physical aggression score was 0 through 6. The internal consistency scores (Cronbach's alpha's) for the physical aggression scale ranged from .78 to .87 with a mean reliability score of .84 for assessments between 6 and 15 years.

Nagin and Tremblay (1999, 2001) identified four distinct groups when they estimated the developmental trajectories of physical aggression for the total Montreal sample with a semi-parametric, group-based method. When applied to our sub-sample the criteria led to the following four groups: a never physical aggression trajectory group (never agg, $n = 40$), a low level desister physical aggression trajectory group (low agg, $n = 78$), a high level near desister physical aggression trajectory group ($n = 68$), and a chronic physical aggression trajectory group ($n = 8$). Because the latter group was so small, we combined the high level near desister and chronic trajectory groups into one high physical aggression trajectory group (high agg, $n = 76$).

Assessments of reactive and proactive aggression from 12 to 15 years of age

In addition, when the boys were 12, 13, 14 and 15 years old, their teachers completed three reactive aggression, and three proactive aggression items (Dodge and Coie, 1987). The reactive items were ''when teased or threatened he gets angry easily and strikes back''; ''when accidentally hurt by a peer he assumes that the peer meant to do it and then overreacts with anger and fighting''; and ''always claims that other children are to blame in a fight and feels that they started the whole trouble''. The proactive items were ''uses (or threatens to use) physical force in order to dominate other children''; ''threatens or bullies others in order to get his way''; and ''gets other children to gang up on a peer he does not like''. The 3-unit response scale for these items ranged from 0 ''does not apply'', and 1 ''applies sometimes'', to 2 ''applies often'' (Brendgen et al., 2001). Cronbach's alpha's varied between .82 and .86. For each year a reactive and proactive aggression score was calculated by summing the scores of the three respective items, resulting in an annual reactive and proactive score ranging from 0 to 6. Next, these four annual scores were averaged to form a mean reactive and proactive score. Ten boys did not participate in the analyses, as they had missing values on reactive aggression in more than two years. On the basis of this information we created three subgroups of reactive aggressive boys; a boy belonged to the low reactive aggressive subgroup (LRA, $n = 52$) when this mean score over the four successive years was 0, he belonged to the moderate reactive aggressive subgroup (MRA, $n = 92$) when the mean score ranged from $0 < x \leq 2$, whereas boys who had a higher mean score (>2) belonged to the high reactive aggressive subgroup (HRA, $n = 40$).

Assessment of anxiety trajectories from 6 to 15 years of age

Because anxiety is expected to have a stimulating effect on the activity of the HPA axis in general, and the secretion of cortisol in particular, we used an assessment of anxiety as a control variable. Using the Nagin and Tremblay approach (Nagin and Tremblay, 1999), the developmental trajectories of anxiety were identified based on school teacher's ratings from 6 to 15 years of age. An annual score was obtained through the aggregation of three items from the teacher rated Social Behavior Questionnaire (Tremblay et al., 1991a; ''worries''; ''tends to be fearful''; and ''easily cries''). Two participants had missing data. The possible scores for the annual assessments ranged from 0 to 6. Crohnbach's aloha varied between .63 and .73 with a mean

Table 1. Classifications of aggression subgroups and their sample sizes

Conduct disorder symptoms	Normal control (NC) n = 159	Conduct disorder (CD) n = 20	
Covert vs. aggressive CD symptoms	covert (C) n = 39	aggressive (A) n = 22	
Reactive aggression	low (LRA) n = 52	moderate (MRA) n = 92	high (HRA) n = 40
Physical aggression trajectories	never agg n = 40	low agg n = 78	high agg n = 76
Anxiety trajectories	low anx n = 101	mod anx n = 33	high anx n = 58

NC: <3 CD symptoms, CD: ≥3 CD symptoms. C: ≥1 covert CD symptoms but no aggressive CD symptoms, A: ≥1 aggressive CD symptoms. LRA: mean reactive aggression score ≤ 0, MRA: mean reactive aggression score $0 < x \leq 2$, HRA: mean reactive aggression score > 2

reliability score of .68. The three developmental trajectories identified led to the following subgroups: a low level (low anx, n = 101), moderate level (mod anx, n = 33) and a high level (high anx, n = 58) subgroup.

An overview of the sample sizes of different subgroup classifications is given in Table 1.

Cortisol measurement

Cortisol level was assayed from a saliva sample, collected during the boys' one-day visit to the laboratory at 13 years of age. During that day, all boys were administered an extensive neuropsychological test battery and participated in different games (see for details Séguin et al., 2002). For all participants the cortisol sample was collected immediately upon arrival at the laboratory at approximately 9 h00 A.M., with the arrival time ranging from 8 h45 A.M. to 9 h55 A.M. (Mean ± SD: 9 h09 ± 0 h16). Although cortisol had a low inverse correlation with time of assessment ($r = -.14$, $p = .05$), the size of the correlation made us decide not to enter collection time as covariate. Participants were requested to donate saliva on paper strips (Stahl and Dorner, 1982). Salivary cortisol concentrations were determined using an adapted version of a commercially available radioimmunoassay kit (Coat-A-Count; Diagnostic Products Corp., Los Angeles, California). The interassay coefficients of variation were 5.2% and 6.4% respectively at 0.33 and 3.6 μg/dl. The Coat-a-Count Cortisol antiserum is highly specific for cortisol, with an extremely low crossreactivity to other naturally occurring steroids. Collection of saliva for cortisol determination is a stress-free approach that avoids potential confounds produced by venipuncture. Saliva cortisol levels have been shown to correlate highly with serum cortisol concentrations, and are thought to reflect the unbound fractions of circulation cortisol (Laudat et al., 1988).

Data analysis

Single isolated outlier values, with an outlier defined as an individual value more than 2.5 SD's above the mean value of the group, were removed from the analyses. One-factor analyses of variance (ANOVAs) were used to test for differences in cortisol levels between the various aggression groups. In case of significant group differences between more than two groups, post-hoc Bonferroni tests for multiple comparisons were conducted. Values are expressed as means (±SD). When scores or groups were unevenly distributed non-parametric tests were used.

In case there was a significant correlation between cortisol level and anxiety score at age 13, we controlled for anxiety. With respect to the teacher reported reactive and proactive aggression scores, correlations were calculated between these scores.

Finally, a continuous variable stepwise approach using multiple regression was applied to predict cortisol levels from assessments of aggressive behaviour. Continuous scores of proactive- and reactive aggression, the probability score (see Nagin and Tremblay, 2001) of being in the high physical aggression trajectory group, and aggressive and covert CD symptoms (as dichotomous variables) were used as predictors. Variables were entered if correlations between them were lower than $r = .90$ (multicollinearity assumption).

Results

First of all, we assessed whether anxiety had to be taken into account in any subsequent analyses on the relation between cortisol and aggression. Since no significant correlation was found between cortisol level and anxiety score at age 13 ($R = .08$, $p = .29$), we decided there was no need to control for anxiety.

A significant difference was found in cortisol between the CD and the NC group, with the CD group having a higher level of cortisol (Mean $\pm$ SD: NC $= 0.95 \pm 0.64$ µg/dl, CD $= 1.26 \pm 0.71$ µg/dl, $F(1, 170) = 3.85$, $p = .05$; see also Fig. 2).

In a further analysis, involving the covert (C) and aggressive (A) CD subgroups only, it was established that these subgroups differed significantly in cortisol (Mann Whitney U test; $Z = -2.31$, $p = .02$; Fig. 3).

Next we tested whether boys in the three teacher rated physical aggression trajectory groups differed in cortisol. Only a marginally significant difference in cortisol was found (Chi-square $= 5.12$, $p = .08$; see Fig. 4). Because, for the purposes of the present paper, we combined the high level near desister ($n = 68$) and the chronic trajectory subgroups ($n = 8$) into the high physical aggression trajectory group, we also examined whether these two subgroups differed in cortisol. This turned out not to be the case (Mann Whitney U test; $Z = -.71$, $p = .48$).

Next we analysed whether cortisol levels differed between the low reactive aggressive (LRA), the moderate reactive aggressive (MRA), and the high reactive aggressive (HRA) subgroups. A significant difference in cortisol was found between these three subgroups (Mean $\pm$ SD: LRA $= 0.83 \pm 0.59$ µg/dl, MRA $= 0.94 \pm 0.62$ µg/dl, HRA $= 1.31 \pm 0.78$ µg/dl, $F(2, 175) = 6.36$, $p < .01$; see also Fig. 5). Post hoc Bonferroni tests indicated that the difference in

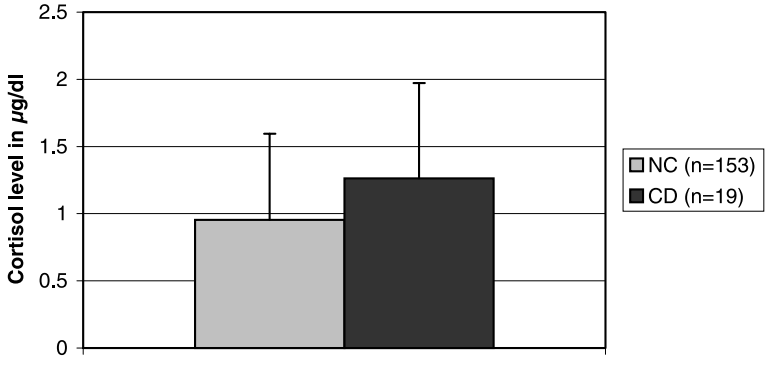

Fig. 2. Differences in cortisol concentration between normal control (NC, $n = 153$) and conduct disorder (CD, $n = 19$) subgroups ($F(1, 170) = 3.85$, $p = .05$

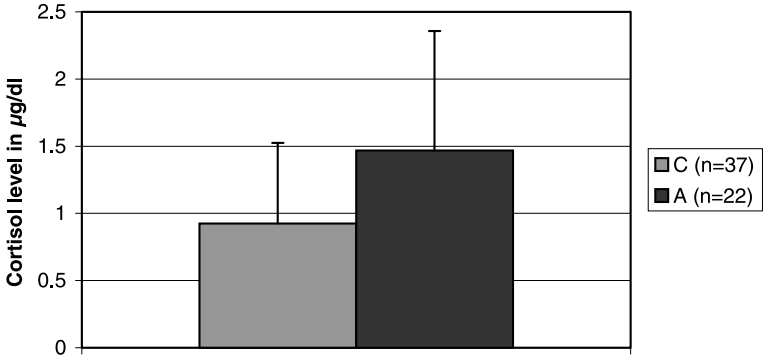

Fig. 3. Differences in cortisol concentration between covert (C, n = 37), and aggressive (A, n = 22) subgroups ($Z = -2.31$, $p = .02$), with the A subgroup having higher cortisol levels than the C subgroup

cortisol was mainly due to a significant difference between the HRA and both the MRA and LRA subgroups, with the HRA subgroup having higher levels of cortisol.

We did not find a difference in cortisol between the low, moderate, and high anxiety trajectory subgroups (ANOVA; $F(2, 184) = 0.75$, $p = .48$; see Fig. 6). Because some previous studies observed an interaction between CD and anxiety (e.g. McBurnett et al., 1991), we similarly calculated whether there was an interaction between the anxiety and aggression trajectory groups on cortisol. No significant interaction effect was found ($F(4, 183) = 0.45$, $p = .77$).

Finally, we used a stepwise multiple regression analysis to identify the aggression assessment that best predicted salivary cortisol level when controlling for each of the other assessments. We found that aggressive CD symptoms, together with reactive aggression score, best predicted cortisol level, accounting for 8.5% of the variance (with $beta = 0.21$, $p < .01$ and $beta = 0.16$, $p = .05$, respectively, $F(2, 165) = 7.62$, $p < .01$). Once aggressive CD symptoms and reactive aggression had been entered into the equation, covert CD symptoms, proactive aggression, and the aggression trajectory variables did not add significantly to the prediction.

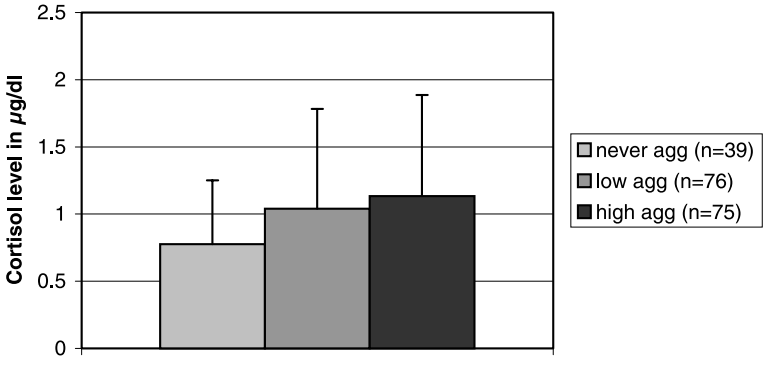

Fig. 4. Differences in cortisol concentration between never (never agg, n = 39), low (low agg, n = 76), and high (high agg, n = 75) physical aggression trajectory subgroups (Chi-square = 5.12, $p = .08$)

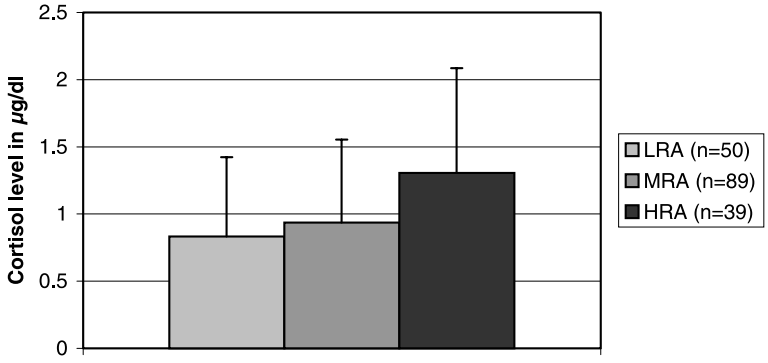

Fig. 5. Differences in cortisol concentration between low (LRA, n = 50), moderate (MRA, n = 89), and high reactive aggression (HRA, n = 39) subgroups ($F(2, 175) = 6.36$, $p < .01$), with HRA having higher cortisol levels than both the MRA and LRA subgroups (LRA = MRA < HRA)

Discussion

The aim of the present study was to investigate in early adolescent boys the relationship between cortisol and different types of aggressive and antisocial behaviour. In contrast to our prediction, the results suggest that teenage boys who have a history of relatively high levels of antisocial behaviour have higher levels of cortisol than similarly aged boys with less behavioural problems, and therefore could have a more active hypothalamic-pituitary-adrenal (HPA) axis. However, when different forms of antisocial behaviour were taken into account, the results indicated that it were specifically the boys who scored relatively high on aggressive CD symptoms that were the ones who had higher levels of cortisol (see Fig. 3). Moreover, high cortisol levels were also found to be related to reactive forms of aggression (see Fig. 5). These results confirm some previous findings that an association between cortisol with antisocial behaviour is most evident for aggression (McBurnett et al., 2000). Still, our pattern of findings is inconsistent with the results of some earlier studies which showed that

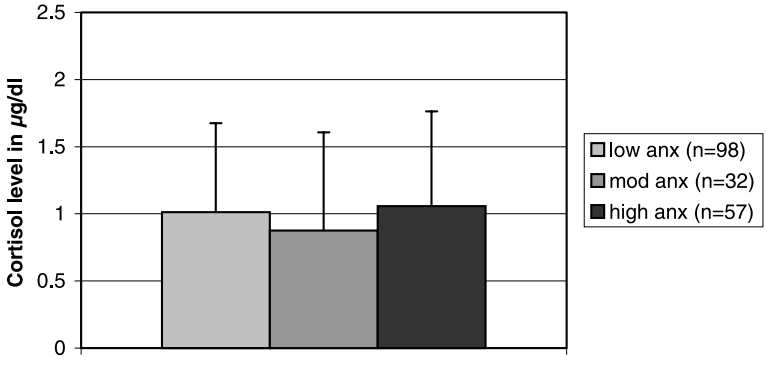

Fig. 6. Differences in cortisol concentration between low (low anx, n = 98), moderate (mod anx, n = 32), and high (high anx, n = 57) anxiety trajectory subgroups ($F(2, 184) = 0.75$, $p = .48$)

antisocial children have lower levels of cortisol (McBurnett et al., 2000; Pajer et al., 2001; Van Goozen et al., 1998, 2000).

This potentially important difference could be related to differences in the types of participants used in the studies. Previous studies, which observed lower levels of cortisol in antisocial children often assessed clinical cases whereas the current sample was drawn from a large population-based sample followed prospectively from kindergarten to adolescence. Thus, within clinical samples, participants with the highest levels of aggressive behaviour appear to have lower HPA axis activity; whereas in population samples the results suggest that the more aggressive individuals have elevated HPA axis activity. This phenomenon could be explained by the fact that there is not only a restriction of range in clinical samples, but also that the more severely aggressive cases are most likely to be found in clinical rather than population samples. Moreover, because our results with a population-based sample show that the aggression – cortisol association in particular concerns reactive aggression, clinically aggressive boys with low cortisol levels would theoretically be expected to engage more in proactive aggression. To our knowledge, studies addressing the cortisol – aggression relationship in clinical samples have not yet differentiated between proactive and reactive aggression. The previously established inverse association between cortisol and aggression in clinical samples could thus be dependent on the type of aggression measure used as well as the mix of proactively and reactively aggressive participants. Although the results of the present study indicate that in a community sample of early adolescent boys, those who tend to use reactive physical aggression may more frequently have a more active HPA axis, we could not determine with the available data whether this is a cause or a consequence of their chronic aggressive behaviour.

Limitations

The present study had also some methodological limitations. First, the population-based sample was limited to young Caucasian males from lower socioeconomic areas in a large North American city. It will be important to replicate these results with other population-based samples. Second, like the majority of studies on cortisol (McBurnett et al., 1991, 2000; Scerbo and Kolko, 1994; Shoal et al., 2003; Vanyukov et al., 1993), this study aimed to measure baseline cortisol concentration, and we therefore only took one sample which was collected on the participants' entrance to the laboratory. However, it has become clear that in order to be sure to measure the individual's baseline or resting cortisol level it is advisable to take more than one sample of saliva while keeping the participant in a relaxed state. Third, we collected salivary cortisol in 1991, using the filter paper method of Stahl and Dorner (1982), which is now considered to be a less optimal method. This might be an explanation for the rather high cortisol levels that we observed. Future research should use other collection methods, such as the salivette sampling device (Sarstedt Inc., Rommelsdorf, Germany). Fourth, since no special dietary restrictions were placed on the boys the night before the laboratory visits in 1991, we were not able to examine the effect of possible stimulants on the relationship

between cortisol and aggression. Fifth, another issue that is potentially important and that the current study could not address, is that it is unclear which cortisol parameter has the most reliable relationship with aggression. At present, it is not clear whether differences between aggressive and non-aggressive groups exist for baseline cortisol levels or for changes in cortisol reactivity during stress. Only a few aggression – cortisol studies have measured changes in cortisol due to stress (Gerra et al., 1997; Klimes-Dougan et al., 2001; Moss et al., 1995; Susman et al., 1997; Van Goozen et al., 1998, 2000). Clearly, future studies should within each individual take repeated measurements of cortisol under varying conditions, while keeping the timing for all participants equal. Moreover, because of its strong diurnal rhythm with lower afternoon values, the influence of external stimulation on cortisol can be more reliably assessed in the afternoon. Also, it is clear that studies need to assess antisocial behaviour and aggression in ways that would be comparable (Tremblay, 2000, 2003). Finally, future studies should examine assessments of proactive and reactive aggression from different sources of information.

Acknowledgements

This study was made possible by grants from Québec's CQRS and FCAR funding agencies, Canada's NHRDP and SSHRC funding agencies, the Molson foundation, the Canadian Institute for Advanced Research, and the LNJ Kamp foundation. We thank H. Beauchesne for supervising the data collection, and L. Desmarais-Gervais, P. McDuff, and M. Rorive for managing the data bank.

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