The development of antisocial behavior and hypothalamic-pituitary-adrenal axis activity in adolescence

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Chapter six

Testosterone and cortisol in relation to aggression in a large general population sample of boys and girls

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Abstract

Testosterone and cortisol have been proposed to jointly regulate aggressive behavior. However, only few empirical studies actually investigated this joint relation in humans, and reported inconsistent findings. Moreover, samples in these studies were rather small and/or specific, and consisted largely of males. Therefore, in the current study testosterone and cortisol in relation to aggression was investigated in a large general population sample of 254 boys and girls (mean age 17.26). A positive ratio of testosterone to cortisol, that is, high testosterone relative to cortisol, was found to be associated with aggressive behavior, explaining 6.5% of the variance. Gender differences were not found. The ratio may reflect an imbalance leaving the individual more prone to rewarding aspects, than fearful of negative implications of aggressive behavior. Current findings indicate that this relation can be generalized to aggression in general population adolescents. The ratio of testosterone to cortisol could serve as a useful basis for further theories and studies to increase our understanding of aggression.

Introduction

Testosterone and cortisol have been proposed to jointly regulate aggressive behavior (Terburg et al., 2009; Montoya et al., 2012; van Honk et al., 2010). However, only few empirical studies actually investigated this relation in humans, and reported inconsistent findings (Dabbs et al., 1991; Glenn et al., 2011; Popma et al., 2007a; Scerbo and Kolko, 1994). Moreover, samples in these studies were rather small and/or specific (e.g., delinquent adolescents or clinic-referred disruptive children), and consisted largely of males. Therefore, the aim of the current study was to investigate the joint relation of testosterone and cortisol with aggression in a large general population sample of adolescent boys and girls.

It has been posed that aggression is specifically linked to high testosterone levels in combination with low cortisol levels (Terburg et al., 2009; Montoya et al., 2012; van Honk and Schutter 2006). Testosterone and cortisol are the end products of the hypothalamic-pituitary-gonadal (HPG) and the hypothalamic-pituitary-adrenal (HPA) axes, respectively. Independently, high levels of testosterone are only weakly associated with aggression in humans, whereas in animal studies this relation is well-established (meta-analysis by Book et al., 2001). Similarly, in humans low levels of cortisol have been reported to correlate with aggressive and antisocial behavior (e.g. de Vries-Bouw et al., 2012; McBurnett et al., 2000; Platje et al., 2013b; Popma et al., 2007b; Shoal et al., 2003), but not consistently (see meta-analysis by Alink et al., 2008). However, the HPG and HPA axes mutually inhibit one another (Viau, 2002); testosterone inhibits HPA axis activity at the level of the hypothalamus, and cortisol inhibits HPG axis activity at all levels (Johnson et al., 1992; Tilbrook et al., 2000; Viau, 2002). On a psychological level, HPG axis activity is related to approach in rewarding situations, whereas HPA axis activity is related to withdrawal from fearful situations (Schulkin, 2003). Together they thus maintain a healthy balance between reward and fear. As such, it has been hypothesized that when this balance is disturbed, with high levels of testosterone and concurrent low levels of cortisol, cortisol does not inhibit testosterone sufficiently, and an individual may be more prone to rewarding aspects, than fearful of negative implications, of aggressive behavior (Denson et al., 2012; Montoya et al., 2012; Terburg et al., 2009; van Honk and Schutter, 2006).

This theory has largely been based on the finding from a study by Dabbs, Jurkovic, and Frady (1991), who reported that cortisol moderated the correlation between testosterone and violence of crime in 113 late-adolescent male offenders. Specifically, as cortisol concentrations increased, the magnitude of the correlation between testosterone and violent behavior dropped. In the two decades that followed, only few studies actually investigated this relation, with inconsistent results. In a similar sample of 103 delinquent male adolescents, Popma et al. (2007a) also found a positive relationship between testosterone and overt aggression in adolescents with low cortisol levels, but not in those with high cortisol levels. Other studies however, reported less consistent

results. In 40 clinic-referred disruptive children, no interaction between testosterone and cortisol was found in relation to a general aggression measure (Scerbo and Kolko, 1994). In 53 female students a different direction of the interaction, that is, concurrent high levels of testosterone and cortisol, was associated with provoked reactive aggression (Denson et al., 2012).

Recently, it has been put forth that the ratio between testosterone and cortisol may better reflect the interconnectedness of the HPG and HPA axes as compared to an interaction term (Glenn et al., 2011; Terburg et al., 2009). The ratio reflects the relative level of testosterone to cortisol within each individual, whereas the interaction treats testosterone and cortisol as two distinct variables. Indeed, an increased ratio of testosterone to cortisol was found in 178 general population adults in relation to psychopathic traits, associated with aggression and a lack of fear (Glenn et al., 2011). Although the direction of the relation between the two hormones and aggression was similar to the findings of Dabbs et al. (1991) and Popma et al. (2007a) in (late) adolescents, no interactions between testosterone and cortisol were found. Comparable results were found for girls with conduct disorder (CD), where aggressive CD symptoms were related to a decreased ratio of cortisol to DHEA (a testosterone precursor), that is, low cortisol relative to high DHEA (Pajer et al., 2006).

In females, the relation between testosterone, cortisol and aggression may be different than in males. Testosterone is not only lower in girls compared to boys, it is also produced differently. Whereas in boys testosterone is produced mainly by the HPG axis, in girls approximately half of testosterone is produced by the adrenal cortex, which is part of the HPA axis (Burger, 2002). To date, boys and girls have not been compared on the joint relation of testosterone and cortisol with aggression. Although Glenn et al. (2011) and Scerbo and Kolko (1994) investigated mixed samples, the large majority in these studies (88% and 92% respectively) was male, and gender comparisons could not be made. Moreover, the two studies in female samples (Denson et al., 2012; Pajer et al., 2006) yielded contrasting results.

As such, although the proposed interplay between testosterone and cortisol in relation to aggression is likely to aid in understanding its underlying mechanisms, more research is required before further inferences can be drawn. More specifically, the generalizability of the finding of concurrent high testosterone and low cortisol in relation to aggression, to general population and female samples needs to be elucidated. Therefore, in the current study, testosterone and cortisol in relation to aggression were studied in a large general population sample of both boys and girls. As the most consistent relations from previous studies were found in specific samples in which aggression is most likely relatively severe, both linear and categorical analyses were performed.

Methods

Participants

Participants were 254 adolescents (143 boys and 111 girls), with a mean age of 17.26 years (SD=0.43). They were recruited from the RADAR (Research on Adolescent Development And Relationships) study. RADAR is a Dutch population based cohort study, with over-sampling (50%) of boys and girls with a borderline-clinical score on the externalizing scale of the Teacher's Report Form (TRF, Achenbach, 1991a) at age 11. The cohort was subsequently assessed yearly at the participants' home. This paper deals with the behavioral assessment performed at age 17 in February and March of 2010, and a lab session between January 2010 and January 2011 which included assessment of cortisol and testosterone levels.

All participants and their parents have provided written informed consent and received a reimbursement for their participation. The RADAR study has been approved by the responsible medical ethics committee, and was conducted in accordance with the Declaration of Helsinki.

The total cohort consisted of 497 adolescents, and 418 adolescents participated in 2010. Of the 418 adolescents, 303 participated in the lab session. The 303 participants did not differ from those not participating in the lab session on age, gender or level of aggressive behavior (all $p > .10$). For 263 adolescents, sufficient saliva was sampled to assay for testosterone and cortisol. Of these 263 adolescents, four participants did not provide information on alcohol or nicotine use, and five were excluded on the basis of outlier analyses (see statistical analyses), resulting in 254 adolescents were included in the final analyses (see Table 6.1).

Aggressive behavior

Aggressive behavior was assessed by means of the Youth Self Report (YSR, Achenbach, 1991b), administered to the adolescents. The YSR aggression scale consists of 17 items, which are scored on a three-point scale (0 = not true, 1 = somewhat true, 2 = very true or often true). Cut-off scores distinguish scores in the borderline-clinical range from those in the normal range. Good reliability and validity have been reported for the Dutch version (Verhulst et al., 1997).

Testosterone and cortisol

Testosterone and cortisol were assessed during resting conditions of the lab session. Testosterone and cortisol were assessed in one saliva sample assessed by passive drooling. The average time of collection was 3:58 p.m. (SD 26 min).

Saliva was stored uncentrifuged at -20°C until analysis. Testosterone was analyzed using an in-house competitive radio-immunoassay employing a polyclonal anti-testosterone-antibody (Dr. Pratt AZG 3290). [1,2,6,7-^{3}H]-Testosterone (NET370250UC, Perkin-Elmer) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 20 pmol/L. Inter-assay variation was 15.5 - 6.8% at 36 - 160 pmol/L respectively (n=20).

Cortisol was analyzed using electrochemiluminescence immunoassay (ECLIA). The lower detection limit was 0.5 nmol/l, with mean intra-assay and inter-assay coefficients of variation of 3.4% and 12.2%.

Control variables

Control variables were assessed at the lab session. During the lab session temperature and humidity of the test room were recorded, and the season of saliva sampling was taken into account. Intake of food, drinks, alcohol, nicotine and drugs, as well as physical exercise, within the last 24 hours, were assessed. Stressful situations, medication use, diseases, physical and dental conditions (including allergies and oral bleeding), body mass index (BMI), as well as menstrual phase and contra-conceptive (OC) use for females were assessed as well. Pubertal status was assessed a year prior to the other assessments, at age 16 by means of a modification of the Pubertal Development Scale (PDS, Petersen et al., 1988).

Statistical analyses

Outliers in testosterone and cortisol were defined as 3 SD above the mean and excluded (n = 5). As the distribution of cortisol values was positively skewed, a square root transformation was performed, after which values were normally distributed.

For the interaction between testosterone and cortisol, values were standardized by means of a Z-transformation, separately for boys and girls. For the testosterone to cortisol ratio, a T-transformation (mean 50; SD 10) was performed separately for boys and girls. Individual testosterone/cortisol ratio scores were calculated, as in Hermans, Ramsey & van Honk (2008).

Of the control variables, only alcohol and nicotine use were associated with both aggression, as well as testosterone and/or cortisol. These were therefore controlled for in the analyses. Four linear regression analyses were performed with aggression as the dependent variable. First, main effects of testosterone and cortisol respectively were examined in two separate analyses. Next, the interaction term between testosterone and cortisol was added to the main effects. In the fourth model, the ratio of testosterone to cortisol was entered individually as a predictor. Gender by joint testosterone-cortisol effects were tested in each model. If interaction effects with gender were not found, gender was controlled for in the analyses which included both boys and girls.

Post hoc, categorical analyses on significant relations of testosterone and cortisol with aggression were further examined with logistic regression analyses, to examine whether borderline-clinical aggressive behavior can be distinguished from aggressive behavior in the normal range.

Results

In Table 6.1 descriptive statistics are shown. It can be seen that boys showed higher levels of cortisol, testosterone, and more often reported to have used alcohol in the 24 hours before sampling.

Table 6.1. Descriptive statistics

	All participants		**Boys**		**Girls**			
	Mean/n	**SD/%**	**Mean/n**	**SD/%**	**Mean/n**	**SD/ %**	**t-test/ χ^2**	**p**
N	254		143	56.3 %	111	44.7 %		
Age	17.25	0.39	17.27	0.40	17.22	0.38	1.092	.276
Aggressive behavior	6.31	5.16	6.57	5.63	5.97	4.50	0.906	.336
Cortisol	6.24	3.27	6.86	3.37	5.46	2.96	3.449	.001
Testosterone	182.12	113.81	265.91	78.50	74.18	30.87	24.305	<.001
Alcohol use	44.00	17.3 %	33.00	23.1 %	11.00	9.9 %	7.565	.007
Nicotine use	62.00	24.4 %	34.00	23.8 %	28.00	25.2 %	0.071	.883

Note. Untransformed levels of testosterone and cortisol are presented, whereas in the analyses these were standardized for boys and girls separately. Alcohol and nicotine used in the 24 hours before sampling.

In Table 6.2 results of linear regression analyses are shown. A trend appeared for a negative association of cortisol with aggressive behavior for all participants, indicating that those with lower cortisol levels reported higher levels of aggression. Analyses for boys and girls separately showed that this was not significant for girls and only marginally significant for boys. No main effect of testosterone was found in association with aggressive behavior. The interaction variable testosterone x cortisol was also not significantly relation to aggressive behavior. However, a positive ratio of testosterone to cortisol was found in association with aggressive behavior for all participants, although only marginally significant.

Gender differences were examined by adding a gender x testosterone x cortisol interaction, and a gender x testosterone/cortisol ratio. These interaction terms were not significant for the gender x testosterone x cortisol interaction (B = -0.326, p = .631) nor for the gender x testosterone/cortisol ratio (B = -0.000, p = .643) in association with aggressive behavior.

Table 6.2. Results of the four linear regression analyses predicting aggression.

	All participants			Boys			Girls		
	B	Adj. R^2	p	B	Adj. R^2	p	B	Adj. R^2	p
Cortisol	-.612	.064	.060	-.892	.092	.052	-.212	.026	.635
Testosterone	.184	.052	.572	.068	.067	.885	.502	.036	.260
Testosterone*Cortisol Interaction	.142	.060	.666	.278	.082	.544	.046	.021	.923
Testosterone/Cortisol Ratio	2.267	.065	.051	2.653	.083	.114	2.184	.042	.164

Note. Results are controlled for gender (for analyses including all participants), alcohol and nicotine use in the last 24 hours. B's for main effects and interaction are based on Z-scores, B's for the ratio are based on T-scores.

Post hoc, it was examined whether the ratio of testosterone to cortisol was associated with aggression in the borderline clinical range (n=24, of whom 5 girls) versus aggression in the normal range. A logistic regression analysis controlling for gender, nicotine and alcohol use confirmed the dimensional analysis, and showed that an increased testosterone to cortisol ratio is associated with aggression in the borderline-clinical range (*OR* = 5.341, *Nagelkerke* R^2 = .115, p = .027).

Discussion

Aggression in adults and adolescents has been posed to be related to high levels of testosterone in combination with low levels of cortisol. This hormonal interrelation may aid in understanding individual differences in the occurrence of aggressive behavior. Until now, research mainly focused on specific (i.e. delinquent adolescents or clinic-referred disruptive children), small and/or male samples, and results are inconclusive. The present study aimed to examine the generalizability of the relation of high testosterone and low cortisol with aggression, to general population adolescents and female samples. The current findings indicate that this relation is indeed present in general population adolescents as well. Although only marginally significant, the ratio of testosterone to cortisol explained 6.5% of the variance in aggressive behavior. This finding was further substantiated as it was found that the testosterone/cortisol ratio was significantly elevated in general population adolescents with high levels versus normative levels of aggression (OR = 5.3). Gender differences were not found in the relation of testosterone and cortisol with aggression.

The current results corroborate previous findings (Dabbs et al., 1991; Glenn et al., 2011; Popma et al., 2007a), and extend them to a large sample of general population adolescents. Specifically, when the ratio of testosterone to cortisol was high, that is, intra-individually one has higher levels of testosterone relative to cortisol, aggression was higher. These results suggest that also normative, non-pathological levels of aggression in general population adolescents, may be related to the same neurobiological risk

factors as e.g. violent aggression in incarcerated youths (Dabbs et al., 1991), aggression in delinquent boys (Popma et al., 2007a), and psychopathic traits in adults (Glenn et al., 2011). Although this relation is more distinct (and significant) when comparing severe to normative levels of aggression, it would be too strong to interpret this as the relation between the testosterone-cortisol ratio being stronger for severe aggression. This possibility requires further investigation.

Although generally large gender differences exist in the levels of aggressive behavior, and testosterone levels are not only much lower in girls as compared to boys, but also differently produced, gender interactions were not found in the current study. Boys and girls have not previously been directly compared on this relation, and studies in female samples were few and inconsistent (Denson et al., 2012; Pajer et al., 2006). The current findings suggest that the testosterone/cortisol ratio in relation to aggression would be in the same direction for boys and girls. Yet splitting the sample in boys and girls did result in too small a sample for both genders, which increased the uncertainty in the estimates, and a relation could no longer be detected. To confirm that relation between the ratio of testosterone to cortisol and aggression is the same across genders, larger samples of (more severely aggressive) boys and girls should be included in future studies.

The ratio of testosterone to cortisol could be interpreted as an index of the imbalance between the HPA and HPG axes within a specific individual. On receptor level, both testosterone and cortisol bind to steroid-responsive centers in the amygdala (Wood, 1996). The amygdala has a key role in emotional processing (LeDoux, 2000), and where increased testosterone facilitates fight/approach reactions, increased cortisol has an opposing effect by facilitating flight/fright reactions (Schulkin, 2003). On the behavioral level, a disturbed balance towards higher testosterone over lower cortisol is thus indicative of increased approach and reduced fear, increasing the likelihood of an aggressive outcome. Recent theories on the neurobiological modulation of aggression, implicate serotonin as a third important player, which may distinguish reactive aggression from proactive aggression (Montoya et al., 2012; Terburg et al., 2009). With increasing evidence for a high testosterone to cortisol ratio as an important factor for aggression, future research including serotonin and subtypes of aggression is necessary to further elucidate the neurobiological mechanisms for aggression.

The results of the present study should be interpreted in the context of some limitations. First, testosterone and cortisol were analyzed from a single saliva sample, not allowing to take the mean of several samples, which could have minimized the effect of pulsatility. Second, sample sizes for boys and girls separately appeared to be too small to directly examine gender differences in the testosterone/cortisol ratio in relation to aggression. Although the current findings do point towards similar associations between the ratio of testosterone and cortisol in relation to aggression across both genders, more research in even larger samples is necessary. Third, aggression was assessed with the Youth Self Report, this is a very broad measure of aggressive behavior, and does for instance not distinguish between reactive aggression and proactive aggression. The advantage of the YSR, on the other hand, is that this is a highly valid, reliable and often used measure.

In conclusion, current findings extends previous research by showing that high testosterone and concurrent low cortisol in relation to aggression appears to represent variation along a spectrum that includes normative levels of aggression. The ratio of testosterone to cortisol could serve as a useful basis for further theories and studies to increase our understanding of aggression.