Relationship between Levels of Testosterone and Cortisol in Saliva and Aggressive Behaviors of Adolescents[1]

YI-ZHEN YU[#,2] AND JUN-XIA SHI[*]

[#]*Department of Child and Adolescent Health and Maternal Care, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China;*
[*]*Xiamen Maternity and Child Hospital, Xiamen 361003, Fujian, China*

Objective To explore the endocrinal factors which influence the aggressive behavior of adolescents. **Methods** The levels of cortisol (CORT), testosterone (T), prolactin (PRL), and growth hormone (GH) in saliva from 20 aggressive students and 20 non-aggressive control students were measured by radioimmunoassay (RIA). The students were matched for their gender, age, grade, stage of pubertal development, and economic status of their families. **Results** The salivary T levels were 22.20±14.50 pg/mL and 19.54±12.52 pg/mL in aggressive male and female students, 13.20±6.85 pg/mL and 5.24±3.03 pg/mL in non-aggressive male and female students ($P<0.05$). The male aggressive students had a lower level of CORT in saliva than non-aggressive male students ($P<0.05$). There were no significant differences in the salivary levels of PRL or GH between the aggressive and non-aggressive groups. Correlation analysis revealed a negative relationship in male students between the salivary CORT levels and the aggression factor scores of the child behavior checklist (CBCL). In addition, the data also showed a positive relationship between the salivary T levels and the aggression factor scores of CBCL in female students. Multiple linear regression analysis showed that the salivary CORT level was an independent predictive factor for aggressive behaviors in adolescent boys. The higher the CORT level, the less aggressive the boys were. **Conclusion** CORT and T levels may play a certain role in adolescent aggressive behaviors.

Key words: Adolescents; Aggressive behavior; Testosterone (T); Cortisol (CORT)

INTRODUCTION

The manifestation of aggression is much more influenced by the learning processes during socialization in humans than in animals. In addition to the inter-individual differences, types of aggressive behaviors can also be combined at one time and changed in their occurrence during the life span intra-individually. In children and adolescents who are running through the different stages of social and biological development within a relative short period of life time, their aggressive behaviors can vary considerably. It has been shown that early aggressive behaviors can predict later violent crime and severe aggression, and seem to be an essential feature of later-onset violent crimes[1-2].

Endocrine factors play an important role in aggressive behaviors. In animal studies, androgens have been shown to be involved not only in organization, but also in activation of the aggressive behavior[3-5]. Over the years, a large variety of animal behaviors, paradigms, and species have been studied with respect to aggression[6-7]. Similar results have been reported from the studies performed on human subjects. Previous studies demonstrate a positive correlation between aggressiveness and plasma levels of norepinephrine (NE) and testosterone (T). The 'irritability' and 'resentment' are positively correlated with plasma levels of NE, T and cortisol (CORT)[8-9]. Gerra *et al.*[10] found that plasma levels of NE, CORT, and growth hormone (GH) in aggressive males are significantly increased during experimentally induced aggressiveness. Mattsson *et al.*[11] found that the plasma levels of T are higher in male than in female violent offenders at their early life. The testosterone levels are higher in cerebrospinal fluid from persons with antisocial personality disorders than from normal controls. The plasma levels of testosterone are also associated with lifetime aggressive behaviors and impulsive aggressiveness. These data suggest

[1]This research was supported by Health Department of Hubei Province (No. JX1B161).
[2]Correspondence should be addressed to Yi-Zhen YU. Tel: 86-27-83692709. Fax: 86-27-83692709. E-mail:yuyizhen650@163.com
Biographical note of the first author: Yi-Zhen YU, male, born in 1965, associate professor, majoring in health care of children and adolescents.

0895-3988/2009
CN 11-2816/Q

that androgen plays an important role in aggressive behaviors.

The role of endocrine factors in aggressive children and adolescents has not been extensively studied. This study was to explore the role of hormone levels in saliva, including cortisol (CORT), testosterone (T), prolactin (PRL) and growth hormone (GH), in formation of aggressive behaviors among 20 aggressive students and 20 non-aggressive control students, and to identify the possible biological profiles that might be responsible for adolescent aggressive behaviors.

MATERIAL AND METHODS

Subjects

Twenty aggressive students (10 males and 10 females) and 20 non-aggressive students were selected from 1051 students aged 11-16 years from two middle schools in Wuhan city, Hubei province of China. The average age of the aggressive and non-aggressive students was 12.8±1.6 years and 12.6±1.8 years, respectively (P<0.05). The aggressive students and non-aggressive controls were matched for gender, age, grade, Tanner stage, and family economic status. The students who were affected currently, or had a past history of endocrine or metabolic disorders, physical organic diseases, immune diseases, cerebral trauma, obesity or recent weight loss, drug, alcohol or tobacco addiction, psychiatric and personality disorders, were excluded from this study. Informed consent was obtained from the participants or their parents.

Evaluation of Aggressive Behaviors

The aggressive behaviors were measured using the child behavior checklist[12] (CBCL, parent questionnaire). CBCL is a standardized evaluation of behavioral problems and social competencies of children aged 4–16 years, based on the reports of their parents or others who know the children well[13] (Achenbach, 1991). The CBCL checks a broad range of emotional and behavior problems in children and identifies two major groups of problems: 'internalizing' (fearful, inhibited and over-controlled behavior) and 'externalizing' (aggressiveness, antisocial and under-controlled behavior). The complete CBCL "Parents' forms" were used to screen the aggressive behaviors and to calculate the aggressive scores. An aggressive student was defined as a student whose aggressive score of CBCL was equal to or above China's norm[12] (the score of aggressive factor ⩾18 for male adolescents and ⩾ 17 for female adolescents, respectively, Wang Xiangdong, 1999).

Collection of Saliva Samples

Saliva samples were collected as previously described[14] and stored at -20 ℃ until test[14]. The samples were free from contamination with blood, sputum and water. A 5.0 mL saliva sample was collected from each individual at the same time point during school day (1:30-2:30 pm). Saliva specimens were collected from all female students during their follicular phase of menstrual cycles.

Hormone Assays

The levels of salivary CORT, T, GH, and PRL were measured by radioimmunoassay using DFM-96 type ten-tube radioimmunity and γ counting apparatus. Radioimmunoassay kits were purchased from CHEMCLIN of Beijing[15-16]. All samples were assayed in duplicate. Intra-assay coefficient of variation (CV) averaged across all 40 participants was 4.7%, 3.1%, 3.8%, 4.6% for CORT, T, GH, and PRL, respectively. Inter-assay CV assayed in our laboratory was 8.7%, 7.9%, 3.5%, 4.6% for CORT, T, GH, and PRL, respectively.

Data Analysis

Student's t test was used to compare the mean salivary level of CORT, T, GH, and PRL between the case and control groups. Multiple linear regression analysis was used to analyze the relationship between scores of aggressive factor of CBCL and salivary levels of CORT, T, GH, and PRL, and to determine whether the salivary concentrations of CORT, T, PRL, and GH might be responsible for adolescent aggressive behavior. Statistical analysis was performed using SAS version 8.0 for windows[17] (SAS Statistics Software Package, SAS Institute Inc. Cary, NC 27513, USA) .

RESULTS

Salivary Levels of CORT, T, PRL, and GH

The mean levels of CORT, T, PRL, and GH in saliva in aggressive and non-aggressive students are listed in Tables 1 and 2. Compared with the non-aggressive students, the aggressive students had a higher T level and a lower CORT level in their saliva (P<0.05), but no difference was found in the salivary levels of PRL and GH. The same results were obtained from either the male or female students (Table 2). The salivary CORT and T level was 16.69 pg/mL and 22.20 pg/mL respectively in the aggressive male students, and 22.53 pg/mL and 13.20 pg/mL respectively in the non-aggressive male students ($P<0.05$). Similarly, the aggressive female students also had a lower salivary CORT level and a higher salivary T level than the non-aggressive female students ($P<0.05$).

TABLE 1

Salivary levels of CORT, T, PRL, and GH in aggressive and non-aggressive students ($\bar{x} \pm s$, pg/mL)

	Aggressive Students (*n*=20)	Non-aggressive Students (*n*=20)	*t*	*P*
CORT	15.54±6.85	21.04±5.16	4.37	<0.05
T	20.87±13.27	9.22±6.57	4.57	<0.05
PRL	6.58±5.07	6.00±2.23	0.43	>0.05
GH	1.78±0.77	1.97±0.73	0.85	>0.05

TABLE 2

Salivary Levels of CORT, T, PRL, and GH in Aggressive and Non-aggressive Students ($\bar{x} \pm s$, pg/mL)

	Male				Female			
	Aggressive Students (*n*=10)	Non-aggressive Students (*n*=10)	*t*	*P*	Aggressive Students (*n*=10)	Non-aggressive Students (*n*=10)	*t*	*P*
CORT	16.69±4.61	22.53±3.34	3.40	<0.05	11.90±8.48	17.51±7.40	2.50	<0.05
T	22.20±14.53	13.20±6.85	2.74	<0.05	19.54±12.52	5.24±3.03	3.68	<0.05
PRL	5.42±1.38	5.42±2.45	0.86	>0.05	7.73±7.03	5.64±2.05	0.88	>0.05
GH	1.61±0.55	1.83±0.65	0.80	>0.05	1.94±0.94	2.11±0.81	0.44	>0.05

Correlations between Aggressive Factor Scores of CBCL and Salivary Levels of CORT, T, PRL, GH

Spearman's correlation analysis for independent variables showed a negative correlation between the salivary level of CORT and the aggression factor scores of CBCL in male students (Table 3). The aggressive factor scores of CBCL were positively correlated with salivary T levels in female students. There was no significant correlation between the aggressive factor scores of CBCL and PRL, GH (Table 3).

TABLE 3

Correlation between Aggressive Factor scores of CBCL and Salivary Levels of CORT, T, PRL, GH

	CORT	T	PRL	GH
Aggressive Factor Scores of Male Students	-0.63**	0.28	-0.24	-0.08
Aggressive Factor Scores of Female Students	-0.41	0.53*	0.24	-0.07

Note. *$P<0.05$ *vs* male students, **$P<0.01$ *vs* female students.

Multiple Linear Regression

To determine whether the salivary concentrations of CORT, T, PRL, and GH are the independent factors for adolescent aggressive behaviors, we performed a multiple linear regression analysis using the aggressive factor scores as a dependent variable and the salivary concentration of CORT, T, PRL, and GH as an independent variable at SLE=0.05 and SLS=0.10. The results showed that the salivary concentration of CORT entered the male aggressive behavior regression equation (aggressive factor score =34.28-1.11 × CORT). However, the salivary hormone levels showed no significant correlation with the aggressive CBCL score in the female students (Table 4).

TABLE 4

Multiple Linear Regression Analysis of Adolescent Aggressive Behaviors

Variable of Entry Regression	Regression Coefficient	Standard Regression Coefficient	Determinant Coefficient	Multiple Determinant Coefficient	*P*
			0.40	0.37	
Constant	34.28				
CORT	-1.11	-0.63			<0.01

DISCUSSION

To examine the role of endocrine factors in adolescents' aggressive behaviors, we measured the levels of CORT, T, PRL, GH in saliva from 20 aggressive and 20 non-aggressive students. The salivary T levels in both male and female students with aggressive behaviors were significantly higher than those in non-aggressive male and female students. In contrast, the CORT levels in saliva were lower in the aggressive students than in the non-aggressive students. In female students, the salivary T levels were positively correlated with aggressive scores of CBCL. Furthermore, multiple linear regression analysis revealed that the salivary CORT levels were negatively related with the aggressiveness of male students. These results indicate that the salivary CORT and T levels might play a certain role in aggressive behaviors of adolescents.

It was reported that serum hormone levels correlate with hormone levels in matched saliva samples in a linear fashion[14]. Salivary samples are easily collectable, non-invasive, and pose no risk to the health of people. Therefore, salivary samples can be easily applied in investigation of the health and development of children. Due to the non-invasiveness and easiness of sample collection, salivary samples may help research the development of children. We examined the association between salivary hormone levels and aggressive behavior in adolescents by assaying salivary hormones.

Although there is evidence that both T and CORT have organizational and active effects on aggressive behavior[3,18-19], the available findings in humans are inconclusive[20]. Testosterone, an important factor in cerebral chemistry, is thought to be involved in the modulation of behaviors by interacting with growth factors, neurotransmitters, neruopeptide, neuroactive steroids and neuron second messengers. Animal studies demonstrated that testosterone affects aggression through neurotransmitters, 5-HT_{1A} and 5-HT_{1B}[21-22]. However, the molecular mechanism by which testosterone regulates human aggressive behaviors is not clear. A previous study reported that violent aggressive behaviors of males seem to correlate with the impairment of noradrenergic function, which in turn is probably regulated by gonadal hormones[21]. Consistent with this notion, our results show that the salivary T levels are significantly higher in the aggressive than in the non-aggressive male and female students and are positively correlated with the aggressive CBCL scores in female students. Our findings are consistent with the reported data, which demonstrate that plasma T level is higher in male than in female violent offenders at their early life and is associated with lifetime aggressive behaviors and impulsive aggressiveness[23-25].

Few studies are available on the association between CORT levels and human aggressive behavior. Kathleen Brewer-Smyth *et al.*[26] reported that CORT is lower in female prisoner inmates with violent criminal behavior. Low CORT levels are associated with aggressive behaviors[27-28]. In our study, salivary CORT negatively correlated with aggressve CBCL scores and was found to be an independent factor for the aggressive behaviors. CORT is a stress hormone secreted in response to stressful or threatening situations. It is obvious that the stress system may be involved in human aggressive behaviors[29]. People who are less sensitive to stress might have aggressive behaviors more quickly or easily because they are less concerned about the consequence of their actions. The potential mechanism underlying the association between aggression and CORT depends on the relationship between CORT and personality. Kagan *et al.*[30] reported that resting CORT levels correlate positively with the fearful attachment to the mother and reluctance to approach to unfamiliar individuals or events. Tarter *et al.*[31] found that CORT levels are negatively related to self-reported irritability. Dabbs[32] and Hopper[33] examined college students and found that CORT levels appear to be positively related to anxiety. The above findings suggest that people with a high resting CORT level are more likely to be cautious, sensitive to punishment and perspectives of others. On the other hand, people with a low resting CORT level have a low alertness to circumstance and dangerous behaviors. In addition, there is also evidence that cortisol is related to negative emotionality, which has been defined as a continuum of predisposition to distress, fear, anger, and hostility, indicating that CORT levels related to both personality traits and aggressive behavior are especially intriguing. This has raised the possibility that personality traits might have a link between low CORT level and increased aggressive behavior because individuals who are engaged in most aggressive behavior are often characterized by particular personality characteristics. The results of our study show that male adolescents with a lower cortisol level might have a lower self-control ability during stress, thus possibly leading to aggressive behaviors.

Very few studies are available on the relationship between aggression and PRL or GH[34-35]. In this study, no correlation was found between aggression and salivary PRL and GH levels. Whether the levels of PRL and GH are linked to aggression should be studied further.

The mechanism underlying the occurrence and development of aggression is still unclear. Besides the biological factors, psychological and social factors influencing aggressive behaviors have been reported[36-38]. For example, the prevalence of violence in television and video games greatly affects aggressive behaviors[39]. In contrast, during young adulthood, good behavior games (GBG) significantly reduce the rate of antisocial personality disorder as well as violent and criminal behavior among highly aggressive males. In addition, personality characters of adolescents play a certain role in their aggressive behaviors[40].

There are two major limitations in our study. One is the potential confounding factor which cannot be well controlled due to the complicated role of psychological and social factors in the formation of behavior. The other is the small sample size, thus, further study with a large sample size is needed to verify our results.

In conclusion, salivary levels of CORT and T are significantly associated with adolescent aggressive behaviors. Endocrine factors play a certain role in aggressive behaviors of adolescents.

ACKNOWLEDENTS

The authors thank all families which participated in the study, and also thank Professor Hua-Ming WANG, Anderson Cancer Center of Houston (USA), for his revision of our manuscript .

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(*Received April* 11, 2008 *Accepted August* 1, 2008)