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Research Report

The function of hypothalamus–pituitary–adrenal axis in children with ADHD

*Liang Ma, Yan-Hui Chen**, *Hui Chen, Yan-Yan Liu, Yan-Xia Wang*

Department of Pediatric, Union Hospital, Fujian Medical University, Fujian Fuzhou 350001, China

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ABSTRACT

Objective: To explore the relationship between hypothalamus–pituitary–adrenal (HPA) axis and Attention Deficit Hyperactivity Disorder (ADHD) in non-stress states. *Method:* 128 male children with ADHD aged between 6 and 14 years old were recruited, while 30 healthy male children were chosen as a control group. The diagnostic material was based on DSM-IV. The included ADHD children were further classified into the three sub-groups: ADHD-predominantly inattention type (ADHD-I) ($n=44$), ADHD-predominantly hyperactive impulsive type (ADHD-HI) ($n=32$), and ADHD-combined type (ADHD-C) ($n=52$). The levels of cortisol and adrenocorticotropin hormone (ACTH) were evaluated by the automatic particle enzyme immunoassay and electrochemiluminescence respectively per morning (8:00 am). Intelligence test was assessed by the Raven's Standard Progressive Matrices. *Results:* The children with ADHD had significantly lower intelligence quotient (IQ) (84.5±11.3) in contrast to the control group (98.6±12.4, $P<0.01$), although the lower level of IQ in ADHD-C group (79.2±10.7) was also found when compared with other two sub-groups [ADHD-I (85.6±10.4) and ADHD-HI (91.3±12.6)]. In addition, no significant difference between the ADHD-HI group and the control group regarding the level of IQ were revealed. The level of cortisol in the ADHD group (226.47±129.12 nmol/L) was significantly lower than that of the control group (384.53±141.43 nmol/L, $P<0.001$). The level of cortisol of the ADHD-HI group (154.36±71.62 nmol/L) was significantly lower than that of other two groups [ADHD-I group (219.42±117.66 nmol/L) ($P<0.01$) and ADHD-C group (258.30±136.39 nmol/L) ($P<0.01$)]. There were no significant differences in the ACTH level either between the ADHD and the control group ($P>0.05$), or between sub-groups in ADHD ($P>0.05$). *Conclusion:* In the non-stress states, the existence of dysfunction of the HPA axis (lower plasma cortisol) in children with ADHD might be due to the under-reactivity of the HPA axis ; the low plasma cortisol level might contribute less to the outcomes of cognitive behavior of ADHD children and instead more closely relate to the core domains of attention deficit, hyperactivity and impulsive behavior of ADHD patients.

1. Introduction

Attention deficit hyperactivity disorder (ADHD), characterized by inattention, span of attention grow downwards, hyperactivity, impulsivity and often accompanied by conduct problems and learning disorder, is a common developmental and behavioral disorder in childhood. Its prevalence has been estimated to be 3%–6% of the child population (Wang, 2007).

* *Corresponding author*. Fax: +386 2419 220.
E-mail address: yanhui_0655@126.com (Y.-H. Chen).

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The increasing researches indicate that ADHD not only severely affects children's study, physical and mental health, part of them even have possible chronic impact after the sufferers grow up. Moreover, the employment, family and social function would be seriously destroyed (Wang, 2007; Gadow and Weiss, 2001). The hypothalamus–pituitary–adrenal (HPA) axis plays an important role in regulating central nervous system neurotransmitters and behavior, such as attention, emotion, learning, memory and movement (Turner-Cobb, 2005; Marquez et al., 2006). The HPA axis is an elaborate system of checks and balances that, beyond the maintenance of a circadian rhythm, allows mammals to adapt to changes in their environment. Under stimulatory conditions neurons in the paraventricular nucleus of the hypothalamus (PVN) secrete corticotropin releasing hormone (CRH) into the hypophyseal portal circulation. In the anterior pituitary, CRH induces production of ACTH, which is released into the systemic circulation to stimulate formation and release of cortisol from the adrenal cortex. Elevated serum cortisol immediately begin to interact with corticoid receptors to inhibit the stress response via negative feedback (Talge et al., 2007). For the past few years, some researches found that the patient with ADHD had an under-reactivity of the HPA axis when exposed to stress (van West et al., 2009). Furthermore, we have found close correlations between the under-reactivity of the HPA axis and the performance of individuals with ADHD. Therefore, the aim of the current study is to investigate the role of the HPA axis in ADHD by the assay of the plasma cortisol and ACTH.

2. Results

2.1. Comparison of general information

There were no significant difference in the gender and age ($P>0.05$) between the ADHD group and the control group. No obvious abnormality in head CT/MRI was found between the ADHD and the control groups ($n=2$), although the rate of abnormality of electroencephalogram (EEG) in the ADHD group (35.94%, $n=46$) was higher than the control group (6.67%, $n=30$) ($\chi^2=9.85$, $P<0.01$). The abnormalities of EEG in ADHD children mainly was increase of slow wave, the minority assumes the quick wave ($n=8$, 17.93%) and few assumes sharp waves ($n=5$, 10.87%).

Raven's Standard Progressive Matrices results: Although the intelligence quotient (IQ) of all children in the case of ADHD was above 70, average was 84.5±11.3, it was still lower than the control group of normal children (98.6±12.4, $P<0.01$); the comparison of IQ among different subtypes of ADHD children, group ADHD-C was less than group ADHD-I and group ADHD-HI, and the degree of intellectual impairment of ADHD-HI children was least among these three subtypes. Compared with the control group, the IQ of ADHD-HI children showed no significant difference ($P>0.05$) (Table 1).

2.2. Comparison of plasma cortisol and ACTH levels

Table 2 showed that, compared with the control groups, ADHD groups and three sub-type groups had significant lower cortisol levels ($P<0.001$). The descending order of the plasma cortisol level is ADHD-HI, followed by ADHD-I and then ADHD-C, although no significant difference between the ADHD-I group and ADHD-C group was found ($P>0.05$). There was no significant difference in ACTH levels either between the ADHD and the control group, or among three ADHD sub-groups ($P>0.05$).

3. Discussion

Owing to the high incidence rate and great harm caused by ADHD, abundant ADHD studies were performed in numerous countries. Those studies tried to explore the etiology and pathogenesis either from heredity, neuro-biochemistry, neuro-pathology and neuro-psychology. The research suggests that developmental and metabolic disorders of catecholamine as a kind of neurotransmitter in central nervous system might play an important role in the development of ADHD. Recently, people began to pay close attention to the role of the HPA axis in the ADHD (Hanna et al., 1996; Hong et al., 2003; Blomqvist et al., 2007), which might play an important role in the neurotransmitters regulation of central nervous system in ADHD.

By far, the HPA axis is the most studied of the endocrine axes and many researches show that glucocorticoid plays an important role in the various domains, such as attention, learning and memory and so on. In the normal condition, increased hypothalamus CRH raise glucocorticoid concentration, which can cause a series of biological responses, such as alertness, attention and increased ability in learning and memory (Hanna et al., 1996). Blomqvist (Blomqvist et al., 2007) recruited 18 ADHD children (13/18 cases were ADHD-HI and 71 healthy children undergoing a clinical dental examination as a kind of stress. The result of their study showed that cortisol levels were lower in the ADHD-HI group compared with the control. A later study performed by Kaneko and others (Kaneko et al., 1993) also found similar results in 43.3%–46.7% of ADHD sufferers. These results suggest abnormalities in HPA axis function in some children with ADHD in the stress states, especially those exhibiting severe hyperactivity. The above studies suggest that children with ADHD in the stress states also suffer from HPA axis dysfunction. Furthermore, low cortisol levels are considered to significantly correlate with attention deficit, hyperactivity, impulsive behavior (Blomqvist et al., 2007).

To date, no study has been carried out to explore the relationship of base plasma cortisol levels and ADHD. Our

Table 1 – Raven's matrices results in ADHD patients and controls ($\bar{x}\pm s$).

	n	IQ
ADHD	128	84.5±11.3**
ADHD-I	44	85.6±10.4 **
ADHD-HI	32	91.3±12.6
ADHD-C	52	79.2±10.7 **
Control	30	94.6±12.4

Compared with the control group, **$P<0.01$.
Compared between the three ADHD sub-type .groups, $F=12.14$, $P<0.001$.

Table 2 – Plasma cortisol levels in ADHD patients and controls ($\bar{x}\pm s$).

	n	Cortisol levels (nmol/L)	ACTH levels (pg/mL)
ADHD	128	226.47±129.12**	23.93±7.49
ADHD-I	44	219.42±117.66**	23.96±8.82
ADHD-HI	32	154.36±71.62**	23.69±7.92
ADHD-C	52	258.30±136.39 **	24.29±7.62
Control	30	384.53±141.43	23.39±7.82

Compared with the control group, $^{**}P<0.001$.
Compared between the three ADHD subtype groups in cortisol, $F=7.85$, $P<0.01$.
Compared between the control group, ADHD and three ADHD subtypes groups in ACTH, $F=0.056$, $P>0.05$.

study showed that base plasma cortisol levels of ADHD children were lower than that of control (224.57±131.39 nmol/L vs 376.83±136.36 nmol /L, by the test of fully automated particle enzyme immunoassay) ($P<0.001$), suggesting the existence of the low plasma base cortisol in ADHD children. The sub-group comparison study showed that the plasma cortisol level in the ADHD-HI group was significantly lower ($P<0.01$) compared with the ADHD-I group and the ADHD-C group, which is similar to the outcomes of other studies from other countries (Kaneko et al., 1993), implying the dysfunction of the HPA axis regulation among different subtypes of ADHD. Kariyawasam also found that salivary cortisol was significantly lower in the ADHD/ODD group than in the controls (Kariyawasam et al., 2002), suggesting a dysfunction of control of the HPA axis in ADHD children. To further investigate the function of the HPA axis in children with ADHD, we conduct head CT or MRI as well as electrophysiological study (EEG) in part of recruited children, together with ACTH level testing of plasma. Imaging study showed no significant difference between groups and all included subjects had no organic lesion. In the EEG study, despite the abnormal rate of the ADHD groups (35.94%) were higher ($P<0.01$) than that of the control groups (6.67%), no specific focal abnormal EEG changes has been found as increased slow waves, a small number of fast EEG and sharp waves emerge. The levels of plasma ACTH in ADHD children did not show significant differences compared with controls, suggesting dysfunction of the HPA axis regulation in ADHD children. Normally, when blood cortisol decreased, through feedback regulation of the HPA axis by the release of CRH and ACTH in pituitary and hypothalamus, the HPA axis can keep the cortisol levels stable. In view of the feedback regulation of cortisol on ACTH in the HPA axis regulation, we assumed that low cortisol levels in ADHD patients under non-stress might be due to the lower reactivity of the HPA axis.

Raven's matrices shown that the IQ (84.5±11.3) of ADHD children is lower than the control group (98.6±12.4, $P < 0.01$). Although the ADHD-C group had the lowest IQ, followed by ADHD-I and ADHD-HI, respectively, there is no significant difference between the ADHD-HI children and the control group, suggesting that the low plasma cortisol level might contribute less to the outcomes of cognitive behavior of ADHD children and more closely relate to the core domains of attention deficit, hyperactivity and impulsive behavior of ADHD patients.

There are certain limitations to our study. The relationship between the HPA axis and ADHD in stress states should be explored in the future studies. It would be better if a dexamethasone suppression test be done to explore the cause of lower cortisol in ADHD children. We once tried to gather saliva sample, but the cortisol level in salivary has a large variability and it is hard to obtain the salivary sample because the patients cannot cooperate. Another limitation of this study is that it cannot avoid the invasive collection techniques required for plasma measurements.

4. Experimental procedures

4.1. *Subjects*

4.1.1. *ADHD group*

128 Chinese Han male children with ADHD, aged between 6 and 14 years (9.6±2.4), were recruited from June 2008 to March 2009 from the psychiatric outpatient's clinic at Union Hospital of Fujian Medical University. Two child psychiatrists made the diagnoses of ADHD based on the Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition (DSM-IV) (American Psychiatric Association, 1994). In all cases, there were 44 children with ADHD-predominantly inattention type (ADHD-I), 32 children with ADHD-predominantly hyperactive impulsive type (ADHD-HI) and 52 children with ADHD-combined type (ADHD-C). Exclusion criteria included childhood schizophrenia, mood disorder, autism, mental retardation, oppositional defiance disorder, conduct disorder, anxiety disorder, other parenchymal diseases and comorbids.

4.1.2. *Control group*

30 healthy boys, aged between 6 and 14 years (10.2±3.3), were chosen as the control group. The current study was approved by the Research Ethics Committee of Fujian Medical University, while the written consent forms were obtained from the parents.

4.2. *Methods*

4.2.1. *Experimental procedure*

All subjects reported to the laboratory on two occasions (test day one: measurements of the cortisol and ACTH level; test day two: intelligence test). The participants reported to the laboratory in the morning and were instructed to refrain from physical exercise and large meals at least 90 min before the onset of the assessment. Time of day was controlled to avoid baseline level or reactivity differences due to the circadian rhythm of HPA axis activity. In order to minimize the impact of the perceived novelty of the laboratory environment on HPA axis responses the Intelligence test was always performed on the second test day. The time interval between the intelligence test and the measurements of the cortisol and ACTH was at least 48 h.

4.2.2. *Blood preparation collection*

All blood samples for the measurements of the cortisol and ACTH level were collected at 8:00 am, centrifuged (3000 rpm/min) in 10 min at 4 °C and were stored in a −80 °C medical freezer. Then, 50 μl of clear samples have been pippetted into test-wells to evaluate the level of cortisol of and ACTH.

4.2.3. *Cortisol and ACTH determination in plasma*

The levels of cortisol and ACTH were evaluated by DxI 800 automatic particle enzyme immunoassay (Beckman Coulter, Inc.) and MODULAR ANALYTICS E170 electrochemiluminescence (F. Hoffmann-La Roche, Ltd.) separately. Specimen handing and determination were strictly carried out according to the protocol.

4.2.4. *Intelligence test*

Raven's Standard Progressive Matrices (SPM) (Zhang and Wang, 1985) computer software was used to test intelligence. After the instruction, the items are presented according to their difficulty level. Standard form of the SPM according to Raven is estimated to be completed in 45. The booklet comprises five sets (A to E) of 12 items each: A (To measure the ability of observation, comparison and imagination to graph); B (To measure the ability of analogy); C (To measure the recognition in graphic system Change); D (To measure the recognition in graphic alignment and system Change); E (To measure the ability of comprehensive analysis, abstraction). One point is given when a correct choice is made and total scores are 60 points. The raw-scores are converted to standard scores by computer, and then are converted to intelligence quotient (IQ).

4.2.5. *EEG acquisition*

EEG was recorded from 32 electrodes using the Geodesic Sensor Net (PL270, Dantec, Denmark). Impedances were <5 k ohm. EEG was recorded with reference to the vertex (Cz) electrode, amplified, and analog filtered (elliptical) between 0.1 and 35 Hz. Time scale is 30.0 mm/s and sensitivity is 10.0 μV/mm. Electrode application and experimental procedures were well tolerated by all subjects. Recordings were carried out in an electrically shielded, sound attenuated booth. Subjects were instructed to refrain from movement, were monitored for eye and head movements via video camera, and were reminded as necessary to remain still.

4.2.6. *Image acquisition*

CT scans were obtained using a standard CT scanner (Somatom 64; Siemens, Erlangen, Germany).We obtained contiguous transaxial images of the head at 10 mm intervals with a slice thickness of 10 mm. Subjects underwent a plain scanning. MR images were obtained using a 3.0-T superconducting MRI scanner (Magnetom Sonata Vision; Siemens). All MR studies included fast spin echo T1- and T2-weighted images. Transaxial, coronal, and sagittal images were obtained.

4.2.7. *Statistical analysis*

The statistical analysis was performed using the software SPSS, Version 15.0. Comparisons between the two groups were made using the Student's *t*-test in the cortisol levels and IQ result. Comparisons between multiple groups were made using ANOVA analysis in the cortisol levels, ACTH levels and IQ result. The chi-square test was used to analyze the distributions of variables between the two groups in the cortisol levels, ACTH levels and IQ result. The size of test $\alpha = 0.05$.

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