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Salivary cortisol and alpha-amylase diurnal profiles and stress reactivity in children with Attention Deficit Hyperactivity Disorder

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ABSTRACT

There is growing evidence for dysregulation of the stress system in individuals with Attention Deficit Hyperactivity Disorder (ADHD). The stress system includes neuroanatomical and functional components that function in concert to maintain homeostasis and its main effectors are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic/adrenomedullary nervous system (SNS). As stress system activity demonstrates a distinct circadian variation, we aimed to describe simultaneously, diurnal rhythms of both the HPA axis and the SNS in children with ADHD and a comparison group. Moreover, we attempted to investigate stress responses to a physical stressor, venipuncture, in both groups. Sixty-two prepubertal children with ADHD combined (ADHD-C) or inattentive (ADHD-I) type and 40 typically developing children provided saliva samples at six specific time points during a day, as well as before and 10 min after a scheduled morning venipuncture. Salivary cortisol and α- amylase were selected as reliable noninvasive biomarkers for HPA axis and SNS function and were measured in the samples obtained. Results revealed that children with ADHD-C had lower mean cortisol values both 30 min after awakening and at 18:00 h than controls ($p = 0.002$ and $p = 0.018$ respectively), as well as lower mean Cortisol Awakening Response (CAR) and Area Under the Curve for "wake to bed" period (AUC_i) values of cortisol ($p = 0.004$ and $p = 0.001$, respectively). Also, mean CAR and cortisol AUC_i were lower in children with ADHD-I than the control group ($p = 0.034$ and $p = 0.038$ respectively). Alpha-amylase measurements showed an increase over time ($p < 0.001$), which was similar in all three groups. Interestingly, α-amylase changes over time were correlated with the corresponding cortisol changes ($p < 0.001$). Venipuncture, elicited a significant increase only in α-amylase levels and more so in the control group ($p = 0.003$). These findings suggest a partial hypofunction of the stress system in children with ADHD.

1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD), the most common neurodevelopmental disorder of childhood with an estimated overall worldwide pooled prevalence of 7.2% (Thomas et al., 2015), is clinically presented with developmentally inappropriate levels of inattention, motor hyperactivity and impulsivity, with subsequent significant impact on behavioral, emotional, cognitive, academic, and social functioning (Thapar and Cooper, 2016).

As a neurodevelopmental disorder, ADHD is heterogenous not only in terms of clinical presentation and comorbidity but in terms of aetiopathogenesis, as well (Thapar and Cooper, 2016). From a neuropsychological aspect, poor response inhibition (i.e. a function enabling an individual to delay a determined response to an immediate environmental event) seems to be the central feature of ADHD (Barkley, 1997). In addition, response inhibition is thought to induce the stress response (Cloninger, 1988). Thereafter, King et al. (1998) first hypothesized that poor response inhibition should be linked to stress system dysfunction in ADHD.

The stress system includes neuroanatomical and functional

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structures that work in concert to maintain homeostasis through behavioral, physiological and biochemical changes (Chrousos, 2009; Johnson et al., 1992). The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic/adrenomedullary nervous system (SNS) are the effectors of the stress system as they regulate the secretion of glucocorticoids and catecholamines, respectively (Chrousos and Pervanidou, 2014).

Indeed, there is growing evidence that there is stress system dysfunction in patients with ADHD King et al. (1998) provided evidence of underactivity of the stress system in children with ADHD, supporting the hypothesis of poor response inhibition in ADHD. Moreover she suggested that HPA axis hypoactivity might be linked to the persistence of the disorder. Kaneko et al. (1993) and Blomqvist et al. (2007) suggested a connection between HPA axis hypofunction and symptoms of hyperactivity while Freitag et al. (2009) reported blunted HPA axis reactivity confined in the case of ADHD comorbidity with Oppositional-Defiant Disorder. Several other studies have also suggested that ADHD is associated to HPA axis hypoactivity (Isaksson et al., 2012; Ma et al., 2011; McCarthy et al., 2011; Van West et al., 2003). In contrast, Snoek et al. (2004) found normal, while Palma et al. (2012) noted increased HPA axis reactivity in children with ADHD.

Regarding the activity of the SNS, Konrad et al. (2003) reported a higher tonic secretion of epinephrine, while Wigal et al. (2003) demonstrated blunted epinephrine and norepinephrine responses in children with ADHD.

To the best of our knowledge, diurnal variations and stress responses of both the HPA axis and the SNS have not been studied in parallel in children with ADHD. Our study's main objective is to record simultaneously diurnal rhythms and stress responses of both the HPA axis and the SNS, in children with ADHD, via consecutive measurements of specific, extensively used, salivary biomarkers (cortisol for the HPA axis and α- amylase for the SNS) (Granger et al., 2007; Nater and Rohleder, 2009). Salivary measures of these systems provide the opportunity to study these systems together in an ecologically valid way with saliva, which is easily collected and non-invasive to participants (Katz and Peckins, 2017). This is of great importance in the case that participants are children, as in our study.

2. Material and methods

2.1. Participants

A total of 102 children (32.4% girls; mean age ± SD = 8.4 years ± 1.8) participated in this study. Sixty-two children (29% girls, mean age ± SD = 8.63 years ± 1.8) were clinically diagnosed with ADHD and 40 (37.5% girls, mean age ± SD = 8.05 years ± 1.74) typically developing children served as the comparison group. The ADHD group was derived from the "Unit of Developmental and Behavioral Pediatrics", First Department of Pediatrics, National and Kapodistrian University of Athens, "Aghia Sophia" Pediatric Hospital. The comparison group consisted of prepubertal children of typical development, without any developmental or neurological disorder who were recruited from neighboring schools. The study was conducted in the context of a collaborative research project involving the Unit of Developmental and Behavioral Pediatrics and the Department of Child Psychiatry of the National and Kapodistrian University of Athens, investigating the neuroendocrine profiles of children with neurodevelopmental disorders.

The ADHD group consisted of prepubertal boys and girls, clinically diagnosed with ADHD (combined or inattentive subtype). Exclusion criteria included: 1) clinical diagnosis of developmental delay/intellectual disability; 2) clinical diagnosis of psychiatric comorbidity; 3) underlying chronic neurological, genetic or chromosomal disorder; 4) history of prematurity and/or low birth weight; 5) pharmacological treatment for ADHD and 6) current corticosteroid treatment. Children of the comparison group were matched for sex, age, Body Mass Index (BMI) and Socioeconomic Status (SES) with the ADHD group. Children of the control group were assessed at the Outpatients Clinic of Pediatrics to exclude the presence of physical and mental health problems.

The project has been carried out in concordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). It was also approved by the Ethics Committee of the "Aghia Sophia" Children's Hospital. Informed consent was obtained from the parents and legal guardians of all participants.

2.2. Diagnostic instruments

A full medical history for each participant was obtained from the parents and all children underwent a pediatric physical examination to assess their physical health and estimate the stage of puberty, according to the Tanner scale (Tanner and Whitehouse, 1976). A developmental history was obtained by a developmental pediatrician to assess any delays in speech/language and motor function or behavioral and learning difficulties.

All children were interviewed by a developmental pediatrician and a child psychiatrist with Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL) interview for school-aged children. K-SADS-PL is a semi-structured clinical and diagnostic interview that evaluates the severity of symptomatology for 32 present and lifetime child and adolescent psychiatric disorders according to DSM-IV (Kaufman et al., 1997). A diagnosis of ADHD (combined, inattentive or hyperactive subtype) and exclusion of other disorders was made according to DSM-IV criteria (American Psychiatric Association, 1994), as the study was designed and started in 2009, four years before DSM-5 publication.

Raven's Colored Progressive Matrices (CPM) test was administered to screen for borderline or mild intellectual disability. CPM test is a non-verbal measure of distinction between various levels of maturity, through the quantification of a child's ability to evaluate differences and reason by analogy. It consists of 36 items classified in three sets, designed in gradual difficulty (A, Ab, and B), with 12 items for each set. The data collection in Raven's CPM is accomplished by extracting the raw scores and converting them to percentiles based on normative data of various groups (Raven and Court, 1998).

2.3. Study groups

Based on the symptomatology and the clinical criteria provided by the diagnostic evaluation, three study groups were formed, as follows: 1. ADHD combined type (ADHD-C) group (N = 42; 21.4% girls); 2. ADHD inattentive type (ADHD- I) group (N = 20; 45% girls) and 3. Control (Comparison) group (N = 40; 37.5% girls). None of the ADHD children was diagnosed with the predominately hyperactive- impulsive subtype and consequently no such study group was formed.

2.4. Neuroendocrine evaluation of diurnal variations and stress response

Salivary cortisol and α-amylase were selected as reliable non-invasive biomarkers for HPA axis and SNS function, respectively (Nater and Rohleder, 2009). The following neuroendocrine parameters were assessed in all participants: 1) Diurnal variation of salivary cortisol, 2) salivary cortisol area under the curve with respect to ground (AUCg) and with respect to increase (AUCi), for "wake to bed" period, as a measure of total cortisol output, 3) salivary cortisol awakening response (CAR) as shown by the increase in cortisol from waking to 30 min later, 4) diurnal variation of salivary α-amylase and 5) salivary α-amylase AUCg and AUCi, for "wake to bed" period, as a measure of total α-amylase output. Moreover, on another day, we recorded the levels of salivary cortisol and α-amylase before and after exposure to a physical stressor, venipuncture.

To assess diurnal variation of cortisol and α-amylase, cortisol and α-