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## Slower cortisol response during ACTH stimulation test in autistic children

Accepted: 16 April 2007  
Published online: 14 September 2007

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### Introduction

Autism belongs to the group of pervasive developmental disorders that are characterized by a lack of social communication skills, improper language development, limited interests and stereotypical behavior [1, 15]. Despite the fact that it was described 60 years ago, its etiology remains a mystery, which is making autism very challenging from etiological, as well as therapeutical perspective [4, 25, 32, 33]. Many

■ **Abstract** Autism is a hereditary, pervasive neurodevelopmental disorder that starts early in life. The main characteristics of the autism are impairment in social interactions, difficulties in adapting to novel environmental situations and improper reaction to stress. Since the Hypothalamic-Pituitary-Adrenocortical (HPA) axis plays a key role in the response to stress and because the previous research found abnormalities in HPA system, we conducted a study to test several elements of the HPA axis. Because autism is a heritable disorder, autistic subjects were studied as well as their parents. Cortisol circadian rhythm, cortisol daily secretion and its suppression response to dexamethason had been measured from saliva or urine samples of the autistic children and their parents. Cortisol secretion response after ACTH stimu-

lation was done with the autistic children only. The cortisol elevation after ACTH stimulation among the autistic individuals was slower ( $P = 0.017$ ) than in healthy controls. No differences were found in salivary cortisol circadian rhythm or suppression response, as well as in cortisol daily excretion. These data indicate that, compared to healthy subjects, autistic individuals have fine differences in cortisol response to ACTH stimulation or possibly to other types of stress.

■ **Key words** autism – ACTH – cortisol – saliva

genetic studies [24, 30, 31], hormone and neurotransmitter analysis [10, 26, 28] and studies aiming to find the cause of autism in neurological abnormalities and lesions [2, 12, 19, 22], did not find reasonable explanation for the mechanisms underlining development of autism. The hypothalamic-pituitary-adrenocortical (HPA) axis, as a part of limbic system, deserves special attention, since it is the basis for emotions and social interactions, functions that are affected in autism. There is a plenty of evidence that

the limbic system function, together with the HPA axis, has been impaired in autistic children (lack of feelings, miscomprehension of hearing, visual and tactile stimuli, abnormal reaction to almost all stress causing factors) [2, 7, 19, 22]. Furthermore, the HPA axis is mediating response to stress, which is largely modified among autistic individuals [6, 32]. One of the approaches to test the HPA axis is a measurement of its hormones and their subsequent targets, which was used in many studies [8, 29]. Although many studies were indicating about abnormalities in the HPA axis in autistic patients, their findings were not consistent; moreover, they were sometimes contradictory. Some studies found abnormal cortisol or ACTH levels among autistic individuals [3, 14, 21], while others did not find a difference [9, 18, 23, 27, 29], which indicates that more complex studies need to be done.

In our previous study, we found that the cortisol level in plasma was lower, while ACTH was elevated among autistic individuals [14]. Our results hold high statistical significance for observed cortisol difference; therefore, we conducted more profound research of cortisol functioning. In this study we tested: daily rhythm of cortisol secretion, total daily cortisol secretion, cortisol suppression and cortisol stimulation response. Since autism is a heritable disorder, some changes found in autistic individuals should be present in their parents. In order to test this hypothesis, we included parents of autistic individuals in the study.

## Methods

Autistic individuals included in the study, were diagnosed according to the DSM-IV and ICD-10 criteria; diagnosis of autism was determined by child psychiatrist. They were examined by child psychologist and tested by Childhood autism rating scale, Stanford-Binet scale and Autistic Diagnostic Interview. Autistic individuals were recruited at the Center for Autism in Zagreb and Split, and they were all males. This study was approved by the Ethical Committee of the Split University Hospital. All the participants (parents for themselves and for children) provided a written informed consent after they received comprehensive information about the nature of the study.

Control group included healthy boys, without psychiatric and endocrinology disorders, matching autistic children regarding their age. Age of autistic individuals was  $11.9 \pm 2.97$  and of control individuals  $11.6 \pm 4.27$ ; we had nine autistic and seven control individuals. Parents of the control individuals were included in the study and were without psychiatric or endocrine abnormalities, as they reported during an interview. Parents of the control individual were examined by a psychiatrist and were tested by the

GHQ-60 test. Parents of the autistic individuals were included into study because they have autistic child. The control individuals for the ACTH test were healthy adult males, since it was difficult to obtain appropriate children for controls.

The salivary cortisol samples for a daily rhythm analysis were collected from nine autistic families (mother, father and autistic son) and seven control families (mother, father and son). The salivary samples were collected 6 times during the day: immediately after awakening, 30 min after awakening, just before lunch, 60 min after lunch, at 6 p.m. and just before going to sleep (around 10 p.m.). The samples were collected and kept until the analysis, as it was described previously [11]. Free cortisol in saliva was determined by saliva cortisol kit (DSL, Texas).

Dexamethason suppression test was performed among the autistic and the control family members, as previously described [5, 13, 20]. Briefly, 1 mg of dexamethason was given "per os", around 10 p.m. one salivary sample was taken prior to dexamethason treatment and another sample for the cortisol measurement was taken the following morning, just after awakening.

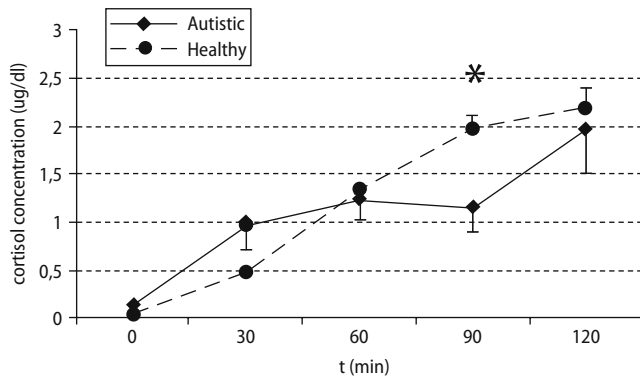
Daily secretion of cortisol was calculated from the cortisol concentration in urine and urine volume, after collecting urine during 24 h period, from the autistic and the control family members. Urinary cortisol concentration was measured using a standard RIA test, (Immuntech, France).

Quick (rapid) ACTH stimulation test was performed on seven autistic individuals and on five control individuals, following the usual procedure [5, 13, 20]. Five salivary samples were collected, one sample prior to the test and at 30, 60, 90, and 120 min after the ACTH application.

For statistical analysis of repeated measures of saliva cortisol, between healthy and autistic children, and also within groups of their mothers and fathers, the Mann-Whitney *U* test was used. Also, when autistic children were grouped with their parents and compared with control families, similar analysis was performed. For comparison of ACTH stimulation responses, between autistic boys and healthy males, the Mann-Whitney *U* test was used. Urinary cortisols were compared using *T*-test for independent variables. For comparison of absolute differences in cortisol levels between different measuring points of saliva cortisol measurement, analysis of variance (ANOVA) test was used.

## Results

Important finding of this study is a statistically significant difference in a salivary cortisol levels during



**Fig. 1** Cortisol response to rapid ACTH test. Autistic and control subjects were given 250 µg of Tetracosactrine (Synacten®) in intramuscular injection; salivary samples were collected before (time 0), and 30, 60, 90 and 120 min after injection of ACTH. Salivary cortisol concentrations were measured with ELISA assay. Data represents mean and unilateral standard error (bars). \* $P = 0.017$

the ACTH stimulation test between autistic and normal individuals. The difference is evident at 90 min time point ( $P = 0.017$ ), but the difference in trend can be observed in all measured time points (Fig. 1).

When the degree of a cortisol increase was measured between 30 and 90 min time points, the difference was even more obvious ( $P < 0.01$ ), indicating a slower increase of plasma cortisol level among autistic individuals.

There was no significant difference in measured values of cortisol circadian rhythm among all study subjects (Fig. 2) as well as in the cortisol values after dexamethason suppression test (results not shown).

Also, daily cortisol secretion measured via 24 h urine did not differ between autistic individuals and their parents when compared with healthy controls

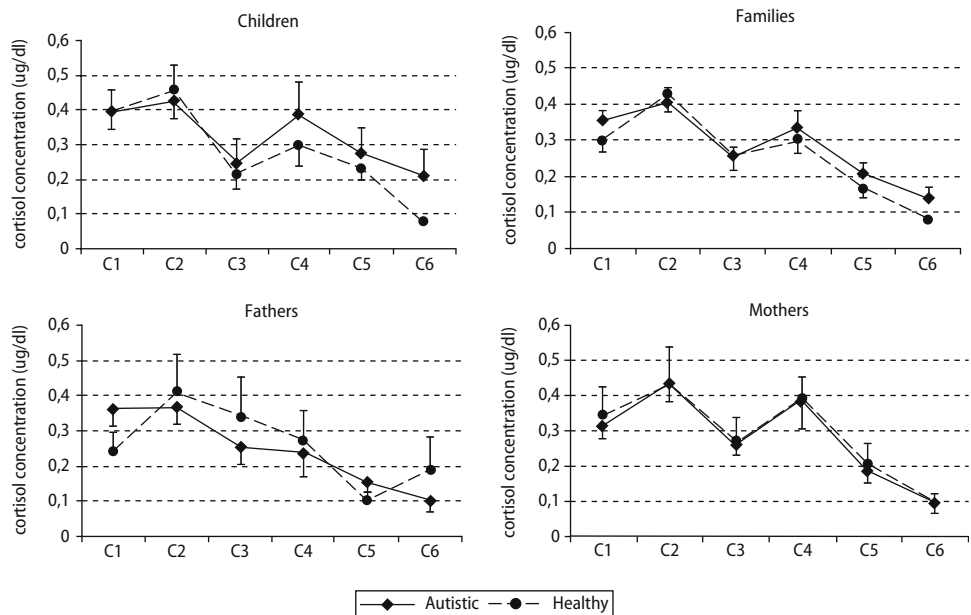
( $110 \pm 77$  nmol/l vs.  $70 \pm 58$  nmol/l;  $P = 0.26$ ,  $T$ -test).

## Discussion

In this study, the hypothalamic-pituitary-adrenocortical axis functioning in autistic individuals, their parents and healthy controls was analyzed. Namely, cortisol circadian rhythm, cortisol suppression response (via dexamethason test), cortisol stimulation response (using rapid ACTH test) and daily cortisol secretion (in 24 h urine) were tested.

Our results show that cortisol release following ACTH administration was slower in autistic individuals, when compared with healthy, sex matched individuals. Slow cortisol response to ACTH stimulation among autistic individuals could explain our previous finding of lower cortisol, which was found after venepuncture performed to obtain serum sample [14]. This, slower response of main anti-stress hormone—cortisol, could help explain higher susceptibility of autistic individuals to various stressors [9, 10, 16, 17]. One recent study [3] shows that autistic children responded to social stress with more cortisol release, which is in contrast to our data. Possible explanation for this discrepancy could be that mechanisms involving the interpretation of an emotional response to a social stressor, plays an important role in cortisol release, which is completely lacking in ACTH stimulation test [14, 27]. Although we see slower cortisol release in autistic subjects, finally, after 2 h they reach the same cortisol level as healthy controls. It needs to be noted

**Fig. 2** Daily cortisol secretion curves. Salivary cortisol concentrations were measured and compared between autistic and control children, mothers or fathers of autistic and control children. Also, cortisol concentrations of families with autistic children were compared with control families (mother, father, and child). Cortisol salivary samples: C1- immediately after awakening; C2- 30 min after awakening; C3- just before lunch; C4- 60 min after lunch; C5- 6 p.m.; C6- just before going to sleep (around 10 p.m.). Data represents mean and unilateral standard error (bars)



that parents of autistic children did not undergo the ACTH stimulation test.

In the other part of the study, autistic children and their parents as well as healthy children and their parents were analyzed. In contrast to ACTH stimulation test, by analyzing cortisol circadian rhythm, cortisol suppression response and total daily cortisol secretion, we did not observe any difference. No difference was detected when parents of autistic and non-autistic children were compared. Also, comparison of autistic and healthy children did not show any difference, as well as comparison of autism-affected families with non-affected families. Not seeing a difference in cortisol circadian rhythm, cortisol suppression response and total daily cortisol secretion, could be due to the fact that the difference between

parents is not big enough in order to be detected by the methods used in this study. Thus it could be more appropriate to do more sensitive, blood tests for cortisol measurements for comparing parents of autistic and healthy children. However, not seeing difference in cortisol circadian rhythm is in parallel with the same amount of cortisol daily secreted in urine.

In conclusion, we found that autistic individuals have slower cortisol secretion in saliva after ACTH stimulation. That finding should direct some future studies of autism.

■ **Acknowledgement** We thank to all the children with autism and parents that participated in the study. Also, we are very thankful to the personnel of the «Center for Autism» in Split and Zagreb.

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