Enrique F. Maldonado, Mª Victoria Trianes, Antonio Cortés, Encarnación Moreno, Milagros Escobar

Salivary Cortisol Response to a Psychosocial Stressor on Children Diagnosed with Attention-Deficit/Hyperactivity Disorder: Differences Between Diagnostic Subtypes

Universidad Complutense de Madrid
España

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Keywords: attention-deficit/hyperactivity disorder (ADHD), cortisol, Trier social stress test for children (TSST-C), children.

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Universidad de Málaga (Spain)

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Keywords: attention-deficit/hyperactivity disorder (ADHD), cortisol, Trier social stress test for children (TSST-C), children.

El objetivo del presente estudio fue comparar la reactividad del eje HPA de niños diagnosticados con distintos subtipos de TDAH en comparación con un grupo de control sano. En el estudio fueron incluidos 66 niños: 33 con TDAH (10 con predominio inatento, 9 con predominio hiperactivo-impulsivo, y 14 con sintomatología combinada) y 33 controles sanos. Como estresor se utilizó el Test de Estrés Social de Trier versión infantil (TSST-C). Este test incluye dos estresores: primero, tras una fase de preparación, los niños deben concluir la narración de un cuento previamente iniciado por uno de los dos entrevistadores; y en segundo lugar, tienen que realizar una tarea cognitiva bajo presión de tiempo ante los entrevistadores. Las muestras de saliva fueron obtenidas a -1 y a +1, +10, +20 y +30 minutos después de la inducción de estrés. El ANOVA de medidas repetidas arrojó un efecto tiempo estadísticamente significativo aunque no se observó la respuesta de cortisol esperada en ninguno de los grupos. Se observó una diferencia en la respuesta del grupo de niños con subtipo hiperactivo-impulsivo que también se constató cuando se comparó su AUCG con la del resto de los grupos. El grupo TDAH con predominio hiperactivo-impulsivo mostró niveles significativamente más bajos de cortisol que el grupo control y el resto de los grupos experimentales al enfrentarse al TSST-C.

Palabras clave: trastorno por déficit de atención/hiperactividad, cortisol, test de estrés social de Trier versión infantil, niños.

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Attention Deficit Hyperactivity Disorder (ADHD) is a developmental disorder that accompanies the individual along his/her lifespan. It is characterized by poor attention capacity from a developmental point of view (Barkley, 1998, 2006; Barkley, Murphy, & Bauermeister, 1998). In addition, it is considered a neurological disorder with a strong genetic component (Biederman & Faraone, 2005; Faraone et al., 2005). The American Psychiatric Association (2000) establishes the following diagnostic subtypes in the DSM-IV-TR: Predominantly inattentive type of ADHD (ADHD-IN) when it only meets the conditions for attention deficit; predominantly hyperactive/impulsive type of ADHD (ADHD-HY) when it meets the conditions for hyperactivity and impulsivity; and finally the combined type (ADHD-CO) when it meets all conditions for attention deficit, impulsivity and hyperactivity. However, when none of the above diagnostic conditions are met, a non-specific type of ADHD can also be diagnosed (Coghills, Rohde, & Banaschewski, 2008). From an epidemiological point of view, the prevalence of ADHD in children is estimated in 6.48% (Polanzeug, Lima, Horta, Biederman, & Rohde, 2007) and is higher among boys than girls (boy/girl ratio of 2.45:1). In one of the very few research studies on the prevalence of this disorder carried out in Spain, Cardo, Servera, and Llobera (2007) have reported a total prevalence of 4.6% and a prevalence of 1.06% for the hyperactive subtype, 1.06% for the inattentive subtype and 2.25% for the combined subtype.

Although there are plenty of research studies on the endocrinological correlates of this disorder in the literature review (Lurie & O’Quinn, 1991), the raised interest by investigators to regulate precise evaluation of the Hypothalamic-Pituitary-Adrenal axis (HPA) in children diagnosed with ADHD has been much more recent and limited. The HPA axis regulates the basal level and the stress response of the cortisol hormone – which is the main glucocorticoid in the human body – and it constitutes one of the two main subsystems where the physiological system of human stress is composed of (Chrousos & Gold, 1992). Among the few research studies that have evaluated basal cortisol levels in children diagnosed with ADHD, either no statistically significant differences were shown (Schultz, Halperin, Newcorn, Sharma, & Gabriel, 1997; Valdizán, 2004) or high basal levels of cortisol were rarely observed (White & Mulligan, 2005). Further research has shown that the most severe forms of this disorder are characterised by a well-distinguished hyporeactivity of the HPA axis to mental or cognitive stressors (Hong, Shin, Lee, Oh, & Noh, 2003; King, Barkley, & Barret, 1998; Shin & Lee, 2007). In this respect, it is worth stressing that the presence of a comorbid aggressive symptomatology, - including that associated to diagnoses of conduct disorder and/or oppositional defiant disorder (Kariyawasam, Zaw, & Handleby, 2002; Snoek, van Goozen, Matthys, Buitelaar, & van Engeland, 2004; Yang, Shin, Noh, & Stein, 2007) – as well as impulsive hyperactive symptomatology (Blomqvist et al., 2007; Hong et al., 2003; Kaneko, Hoshino, Hashimoto, Okano, & Kumashiro, 1993; Shin & Lee, 2007; Yang et al., 2007) have constituted two independent factors to be associated to these alterations in the basal-circadian activity and/or reduced reactivity of the HPA axis. However, it has recently been observed that the inattentive subtype with severe symptomatology – six or more inattentive symptoms – can also present hyporeactivity in the cortisol response to a standard laboratory psychosocial stressor (Randazzo, Dockray, & Susman, 2008). Overall, these results may be interpreted from Raine’s theory (1996), which views the cause of ADHD to stem from lack of fear and lack of physiological arousal experienced by these children when facing a challenge. Likewise, these results could also be interpreted from the viewpoint of those research studies that suggest that a low level of physiological arousal could drive the child to search for sensations that could eventually trigger a decrease in the hormonal response to stress after repeated activation (Alink et al., 2008).

In line with our literature review, there has not yet been any child research with a control group to compare simultaneously the reactivity of the HPA axis to a laboratory psychosocial stressor like the TSST-C among the three ADHD diagnostic subtypes. In this sense, most reviewed studies have included patients on treatment and/or diagnosed with an only diagnostic subtype according to the DSM-IV-TR criteria. Due to this gap in the literature, we suggest the following hypothesis regarding the purpose of this study: according to the literature review, when comparing the cortisol response of the three ADHD diagnostic subtypes without treatment against a control group only experimental subjects belonging to the ADHD-HY group will show less cortisol reactivity in relation to the rest of the groups (experimental and control).

Materials and Method

Participants

This research study has selected a communitarian sample of 66 boys and girls from seven nurseries and Elementary Education schools in the metropolitan town of Fuengirola in Málaga, Spain. This selection was based on the initial evaluation of an original sample of 401 boys and girls ranging in age from 5 to 8 years old. In order to diagnose Attention Deficit Hyperactive Disorder (ADHD) and its various subtypes according to the DSM-IV-TR criteria, a multi-dimensional and multi-informant procedure (Cortés, 2006) was applied: firstly, the scores from the Teacher Report Form (Achenbach & Rescorla, 2000, 2001) were filled and completed by each child’s class tutor, and secondly the diagnostic criteria were compared to during the parents’ interview, in which an adapted version of the review of
children disorders by Barkley (1998) was used. Following this procedure, 15 boys and 18 girls diagnosed with ADHD were selected for the experimental group, from which 10 children suffered from the prevalent inattentive ADHD type, 9 children from the prevalent ADHD hyperactive-impulsive type and 14 children from a combination of both ADHD symptomatology. The control group was composed of 33 healthy children (19 boys and 14 girls) who did not report any ADHD symptomatology and were randomly chosen among the participants in the study (see Table 1). Given that psycho-stimulant treatments are known to alter basal levels together with the cortisol response of this type of samples (Lurie & O’Quinn, 1991), according to the information provided by their families none of the children included in the ADHD group were on any medical treatment. A further inclusive criterion was for ADHD participants not to have history of neither conduct nor oppositional defiant disorder. These disorders have been associated to alterations in basal levels and in the HPA axis reactivity to stress (Alink et al., 2008; Kariyawasan et al., 2002) and show a high comorbidity with ADHD (Coghill et al., 2008).

The parents or legal guardians of every child participating in this research study had signed an informed consent form prior to data collection. The study protocol counted on the approval from the Department of Education in the Regional Government of Malaga, which is dependant on the Ministry of Education in the Andalusian Regional Government (Junta de Andalucía), as well as from the relevant boards of directors and school boards. The epidemiological results of this project are shown in Cortés (2009).

**Adapted Trier Social Stress Test Version for Children (TSST-C)**

An adapted children’s version of the Trier Social Stress Test (TSST-C) was used in this research as a standard laboratory psychosocial stressor (Kirschbaum, Pirke, & Hellhammer, 1993). The TSST-C incorporates both a psychosocial stressor and a cognitive stressor in the format of an interview applicable to children. The test is divided into three subsequent steps: anticipation, public speaking, and mental stress. After 30 minutes of rest period before the start of the interview, the first step involves inviting the child to enter a room where two research assistants are awaiting him/her, introducing themselves as interviewers responsible for assessing his/her performance. The researcher kindly explains instructions to the child. The child is instructed that he or she has four minutes to improvise a story from a starting paragraph read by the researcher. After this anticipation period, the second step involves asking the child to stand in front of a microphone and a video-camera to continue the narration of the story that the researcher has begun and that the child needs to finish with his or her own words. This step is required to last for at least five minutes while it is being recorded. Should the child interrupt his or her narration, he or she is encouraged by the researcher to continue by answering a script of predetermined questions. Once this step has concluded, the third step involves asking the child to sit through a cognitively stressor for yet another five minutes. Due to the complexity of the original task of numeric extraction (thought for this step and suggested for 7-10 years old), which difficulty has been corroborated by teachers from some of the participating schools, this task was substituted by the Matching Familiar Figures Test-20 (MFFT-20) under time restraint. After the MFFT-20, each child’s performance was positively reinforced by telling him or her that he or she did very well on the test. All tests needed to be performed in the morning (due to school schedule conflicts) where data were collected between 10:30 AM and 12:30 PM in all cases in order to minimize the interference of the circadian rhythm of cortisol release. Saliva samples were taken one minute before the child entered the interview room (pretest values of free cortisol in saliva) and then gradually one (+1), ten (+10), twenty (+20) and thirty (+30) minutes after having concluded the stress-induction situation in the waiting room.

**Determination of Free Cortisol in Saliva**

Salivette® saliva collection kits were used to obtain saliva samples in children. Immediately after the samples were obtained, they were frozen at -20°C for a month before being tested and assayed. In preparation for their assay, the samples were centrifuged at 3,500 rpm for 10 minutes in order to get a clear and watery supernatant of low viscosity. In order to detect free cortisol, a fluorescence immunoassay (DELFIA) was employed. The DELFIA is described in detail in Dressendorfer, Kirschbaum, Rohde, Stahl, and Strasburger (1992). The lower limit of detection of this analysis is 0.43 nmol/l. The inter and intra-assay coefficient of variation was below 10% along the range that was expected from the cortisol levels. The analyses were conducted in the laboratory of the Department of Biological Psychology in Dresden Technical University (Germany).

**Procedure**

Before data collection, the parents of the selected children were invited to attend an information session where they were given a general explanation (both spoken and written) on the research objectives and their participation in the project. In order to reduce the anxiety caused by the anticipation of the test, children were told by their parents that they were going to participate in a school contest. Furthermore, children were not told in advance on which date they were going to take the test or they knew the details involved in the study (e.g., interviewers, video-recording and/or type of test). The day of the test, the TSST-C was...
individually administered to each participant from 10:30 AM to 12:30 PM. Each school used three different rooms: a waiting room, an interview room and a third room to collect the saliva samples after the stressful situation took place.

Statistical Analysis

To begin with, the normality of the values of cortisol data was assessed in each measure by using the Kolmogorov-Smirnov test. The area under the curve with respect to ground (AUC_G) and with respect to increase (AUC_I) were estimated using the formula suggested by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). AUC_G and AUC_I are two different approaches to estimate the area under the curve of the cortisol response to the TSST. They were suggested by Pruessner et al. (2003) and widely employed in the literature of the field. While the formula used for the estimation of the AUC_G is especially sensitive to the total response when comparing the response from the two groups, the formula used for the estimation of the AUC_I is especially sensitive to increase changes. In order to compare the distribution of the gender variable in the different experimental groups, a chi-square test was conducted ($\chi^2$). In order to compare the distribution of the age variable and the time of the test variable among the different experimental groups, a T-Student test was conducted ($t$). In order to compare the cortisol values among the baseline measurement, the AUC_G and the AUC_I, the T-Student test was also used. In order to conduct the statistical analysis of the endocrine response of cortisol to a psychosocial stressor, two repeated measures ANOVAs were conducted on two factors: a time factor with five repeated measures (free cortisol in saliva) and the diagnostic group independent factor (control vs. ADHD and control vs. ADHD subtypes).

Time of the test, gender and age were used as covariables. A value of $p < .05$ was adopted as the indicator of statistical significance in the results. All the results shown are mean $\pm$ SEM (except otherwise indicated). All the statistical analyses were performed with the SPSS 12 statistical program.

Results

In a preliminary data analysis, the cortisol values in saliva were observed to be distributed normally (therefore non-transformed direct values obtained are presented) and the gender variable was not homogeneously distributed in each of the experimental groups ($\eta^2 = 11.028, p = 0.012$) and therefore it was included as a covariable in a further analysis to control its effect (see Table 1). No statistically significant differences were observed in the distribution of the age and time of the test variables $F(3,65) = 2.665, p = 0.056$ and $F(3,65) = 1.379, p = 0.257$, respectively.

With the purpose of comparing the HPA axis response to TSST-C of children diagnosed with ADHD with that of controls, a first repeated measures ANOVA was conducted. While this ANOVA threw statistically significant differences in the time intra-subject factor ($F(4,256) = 6.129, p = 0.005, \eta^2 = 0.087$), the typical pattern of cortisol response—powerful and with several minutes of latency - to this laboratory psychosocial stressor was not observed (see Fig. 1). Paired contrasts of data extracted from the total sample only revealed decreased differences between the +20 and +30 minute measurements in the total sample. A statistically significant difference was also observed in the inter-subject group factor (control group vs. ADHD; $F(1,64) = 7.497; p = 0.008, \eta^2 = 0.105$) and the experimental group showed more reduced cortisol levels in comparison with the control group. In relation to the latter result, a statistically significant difference in both the pre-test cortisol value ($t = 2.050, p = 0.045, d = 0.502$) and the AUC_G value between the control group and the ADHD group ($t = 2.759$, $p = 0.008, d = 0.679$) was also observed (see Table 2), unlike in the AUC_I parameter ($t = 1.373, p = 0.175$). Therefore, the ADHD group showed more reduced cortisol levels than the control group during the entire test (see Figure 1). No statistically significant interaction was observed between the time and group factors ($F(4,256) = 0.946; p = 0.438$). In this first ANOVA, the following covariables were included: gender, age and time of the test. As none of these covariables showed a statistically significant effect, they were excluded from the final statistical analyses.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>ADHD Group</th>
<th>ADHD-IN Subgroup</th>
<th>ADHD-HY Subgroup</th>
<th>ADHD-CO Subgroup</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>33</td>
<td>33</td>
<td>10</td>
<td>9</td>
<td>14</td>
<td>66</td>
</tr>
<tr>
<td>Age (Range)</td>
<td>6.21±0.13</td>
<td>6.45±0.15</td>
<td>6.60±0.22</td>
<td>5.77±0.36</td>
<td>6.78±0.18</td>
<td>6.33±0.14</td>
</tr>
<tr>
<td></td>
<td>(5-8)</td>
<td>(5-8)</td>
<td>(6-8)</td>
<td>(5-8)</td>
<td>(6-8)</td>
<td>(5-8)</td>
</tr>
<tr>
<td>Ratio Boy/girl</td>
<td>19/14</td>
<td>15/18</td>
<td>6/4</td>
<td>7/2</td>
<td>2/12</td>
<td>34/32</td>
</tr>
<tr>
<td>Time of the test</td>
<td>11:28±0.005</td>
<td>11:34±0.05</td>
<td>11:30±0.09</td>
<td>11:21±0.08</td>
<td>11:46±0.08</td>
<td>11:31±0.03</td>
</tr>
</tbody>
</table>
With the purpose of dealing in depth with the analysis of the differences found between the control group and the rest of the groups of ADHD included in the DSM-IV-TR and without the comorbid presence of neither a conduct nor an oppositional defiant disorder. The result that first captured our attention was the absence of a response to a standard psychosocial stressor from all experimental groups as well as the control group. Secondly, it was observed that the ADHD group, in particular the ADHD-HY subtype showed significantly more reduced cortisol levels than the control group and the rest of the experimental participants during the entire stressful situation.

With regards to our first finding, i.e. the absence of a significant response from the free cortisol levels with a latency of about 15-20 minutes in relation to the starting moment of the stressful period (Buske-Kirschbaum et al., 1997; Buske-Kirschbaum et al., 2003) could be interpreted as a result of the so-called “stress hypersensitiveness period” of the HPA axis that some researchers have observed among the same childhood ages as a response to similar situations of laboratory stress (Gunnar & Donzella, 2002; Gunnar & Fisher, 2006). According to these researchers, this period goes from the first year of life (Gunnar & Donzella, 2002) up to childhood and approximately the beginning of adolescence (Gunnar & Fisher, 2006; Gunnar & Quevedo, 2007). An increase in the negative regulation of the HPA axis and a reduction in the adrenal cortex sensitivity to the ACTH have been suggested as the main causes for a reduction in the response of this hormonal system (Lashansky et al., 1991). In relation to this, and as far as we are concerned, this is the first research study to apply the TSST-C in a sample with an average age of six without observing any response from the HPA axis. However, this result must be taken carefully as there is a possibility that the resting period that was included in the original design – together with the fact that data was collected in the morning – might have altered or hindered the observation of a weak response from the HPA axis among participants.

Our second discovery seems to be of particular relevance given that it was obtained from an experimental sample.
composed of non-treated children diagnosed with ADHD – according to the international criteria to diagnose the three main subtypes of this disorder mentioned in the DSM-IV-TR –, ruling out the possible confusion that the presence of further comorbid psychopathological disorders of an externalising type would have represented. While the formal hypothesis previously introduced as an objective cannot be answered given the absence of reactivity among all participant groups, our results do allow us to state that the ADHD group – in particular children diagnosed with ADHD-HY – presented a significantly reduced cortisol response during the course of a stressful situation (such as the one triggered by the TSST-C) in comparison with children who do not suffer from this disorder or children diagnosed with a different ADHD subtype. These reduced cortisol levels were a constant during the entire test albeit the variables gender, age and time of the test being statistically controlled. In relation to the latter, it is worth stressing the differences observed between the control group and the ADHD-HY group with regards to the AUCİ but not to the AUCİ. According to Puressner et al. (2003), the AUCİ is a particularly close approximation to the level of total production of the hormone under study as the AUCİ parameter is to increases during the observation time. To sum up, the above results support and cover the isolated observations made by some researchers of this ADHD-HY subtype (Blomqvist et al., 2007; Hong et al., 2003; Kaneko et al., 1993; Shin & Lee, 2007; Yang et al., 2007). Such results can be compatible with a variety of hypotheses that explain the origin of these differences (Alink et al., 2008).

However, this research study presents some limitations that prevent the results from being totally generalised. In the first place, the limited number of children composing each experimental group, and in particular, each ADHD subgroup. Nevertheless, it is important to stress again that none of the children involved in this research had ever taken typical medication for their disorder (psycho-stimulant medicines), and that participants with a comorbide diagnosis of either conduct disorder or oppositional defiant disorder were excluded from the research, which made it very difficult to make up the sample. Moreover, the effect size of all significant contrasts obtained was either medium or high according to the criterion established by Cohen (1988). In the second place, the administration of the TSST-C in the morning could have also been affected by the well-known diurnal rhythm of the cortisol levels, with a peak 30 min. after waking up, moderated levels for the first hours in the morning and eventually more reduced values during the day until reaching the nadir in the first hours of sleep. However, as it has been observed, the time of the test was statistically controlled in all contrasts and tests were taken in the short period of two hours in the morning, so that this limitation could only affect the detection of slight changes in the response. In the third place, the fact of taking students from the class to the waiting room could have also altered or hindered the observation of the cortisol response to the TSST-C, as it prompted anticipated responses among children. Finally, the gender variable had to be controlled statistically due to the non-homogeneous distribution of boys and girls in the experimental groups.

To conclude, this research supports the existence of a reduced response of the HPA axis in the ADHD to an experimental stressful situation. This reduced response is especially remarkable in the subgroup of children diagnosed with ADHD-HY, i.e. children with predominance of impulsivity and hyperactivity symptoms. This result suggests that further research on the cortisol awakening response as well as on the diurnal rhythm of cortisol release from children diagnosed with ADHD could bear a heuristic interest. However, and in line with discoveries made by expert researchers of this field (Gunnar & Fisher, 2006), there is evidence to suggest that our modified version of the TSST-C (as well as some other similar laboratory paradigms) might not be the optimal way to study the reactivity of the HPA axis response on boys and girls in such a low age rank (as there was in our research). In relation to this, it seems necessary to keep searching for situations with ecological validity to allow us to analyse the HPA axis response to psychological stress in children in a non-invasive way.

References


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