Relationship between Cortisol Concentrations and Tic Severity in Children with Tourette's Syndrome

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Tourette's syndrome is a movement disorder characterized by persistence of involuntary, sudden, rapid, recurrent, non-rhythmic, stereotyped motor movements, or vocalizations known as tics for a period of over a year. Often in individuals with Tourette's syndrome, the presentation of stressful situations or stimuli exacerbate the prevalence of the tics. Cortisol is a stress marker that is released by the adrenal glands in response to stress. We examined the potential relationship between resting serum cortisol concentrations and severity of tics in school-age children with severe, mild, and no tics. No significant differences were found in cortisol concentrations between the three subject groups. This negative finding suggests that basal cortisol does not contribute to tic severity but leaves open the possibility that hormonal responses to stressful stimuli may contribute.

INTRODUCTION

A tic as defined by the latest edition of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) is an involuntary, sudden, rapid, recurrent, non-rhythmic, stereotyped motor movement or vocalization. The best studied tic disorder is Tourette's syndrome, which is characterized by the presence of both multiple motor tics and one or more vocal tics throughout a period of more than one year, during which period there was never a tic-free period of more than three consecutive months (Chappell et al., 1994; Hoekstra et al., 2004).

An exacerbation of the tics in Tourette's patients is seen during times of emotional distress (Findley et al., 2003; Silva et al., 1995). Part of the body's response to stress is to release various endogenous compounds to alter the body's physiological state to deal with the demand. However, the question as to whether stress-related agents are part of the etiology of Tourette's syndrome or are exclusively intensifiers of Tourette's syndrome symptoms still remains. To analyze this relationship, we examined the relationship between severity of tics and basal serum cortisol levels in school-age children.

Cortisol is the principal form of glucocorticoid in humans. Glucocorticoids are hormones produced in the adrenal cortex that mobilize energy stores involved in maintenance of brain and muscle function in a stressful situation and modulate inflammation and other immune responses. They act by forming complexes with receptors that regulate genes involved in inflammatory and immune responses, blocking leukocyte access to sites of inflammation and functionally interfering with other molecules involved in immune response (for reviews see Boumpas et al., 1993; Carrasco, 2002). In response to a stressor, there is a chain of chemical signaling from the hypothalamus to the pituitary gland and terminating on the adrenal gland, which is called the hypothalamic-pituitary-adrenal (HPA) axis (Habib et al., 2001). Activation of the HPA axis leads to release of Adrenocorticotropic hormone (ACTH) by the pituitary gland, which in turn increases circulating levels of glucocorticoids. The glucocorticoids participate in maintenance of homeostasis and negative feedback of the HPA axis at the hypothalamic and pituitary levels (de Kloet, 1995).

METHODS

Subjects and Assessment of Tic Severity

We acquired serum samples of 34 school-age children from the laboratory of Dr. Tanya Murphy from Shands at the University of Florida. To assess the level of tics and choreiform movements, Dr. Murphy's group used two methods of evaluation. A choreiform screening was conducted during which the children had to wait in line in groups of five. While the children would wait for their turns, they were observed for three minutes at rest, with tics and behaviors noted. The tics noted consisted of six types: facial, shoulder, arms/hands/fingers, legs, vocal, and other. Additional behaviors were also counted, including fidgeting in line, twirling hair, excessive touching/tapping, picking skin/nose, cracking joints, hitting/shoving/pulling hair, balance/swaying, and grimacing (non-tic facial movements).

To assess choreiform movements, the rater instructed the children to hold their arms out with palms downward with fingers separated and then palms upward for 20 seconds each, with choreiform movements noted during each interval. The choreiform movements were scored on a scale from 0–3: 0 = no twitches, 1 = 2–5 isolated twitches, 2 = 6–10 twitches in bursts, or 3 = continuous twitches. Choreiform scores of 1 were disregarded to reduce

potential for false positives (Murphy 2007). Using the observations of tics and choreiform movements, the children were classified into groups of no tics, mild tics, and severe tics. Blood samples were then taken from the children, and serum was isolated, which was used in our experiment.

Serum Samples

Because of the natural cycle of cortisol levels throughout the day, the timing of the collection of the samples was critical. Fifteen subjects with severe tics had serum samples collected between 1610 and 1650 hours. Twelve subjects with mild tics had serum samples collected between 1610 and 1725 hours. Seven control subjects had serum samples collected between 1500 and 1800 hours. Samples were collected and placed in -80 ^{o}C freezer for storage within 15 minutes to prevent cortisol degradation.

Serum Cortisol Immunoassay

The serum samples were later thawed, and cortisol was assayed with an enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN). This assay was performed by an experimenter who was blind to the tic severity scores of the subjects.

Goat anti-mouse polyclonal antibody was provided in a 96 well polystyrene microplate in 12 strips of 8 wells. The cortisol conjugate (6 mL) was cortisol conjugated to horseradish peroxidase (HRP-cort) with red dye and preservatives. The cortisol standard was 100 ng of cortisol in buffer with preservatives, which was lyophilized prior to packaging. Mouse monoclonal antibody to cortisol (6 mL) was provided in buffer with blue dye and preservatives. Two 21 mL vials of buffered protein base with preservatives constituted the calibrator diluent RD5-43. 21 mL of 25-fold concentrated solution of buffered surfactant with preservatives served as wash buffer concentrate. Color reagent A consisted of 12.5 mL of stabilized hydrogen peroxide, and color reagent B was 12.5 mL of stabilized chromagen (tetramethylbenzidine). Stop solution was 6 mL of 2 N sulfuric acid (H_2SO_4). Four adhesive films were used as plate covers to prevent evaporation during incubation of the assay.

The cortisol standard was reconstituted with 1.0 mL of deionized/distilled water to produce 100 ng/mL stock solution of cortisol standard and gently shaken on a microplate shaker. The reconstituted cortisol standard was allowed to sit for 15 minutes to allow complete reconstitution. Serum samples were diluted 20-fold by adding 380 µL of calibrator diluent RD5-43 to 20 µL of each serum sample, and all tubes were vortexed. Seven test tubes were readied for dilution of cortisol standard. A 100 µL aliquot of cortisol standard was pipetted into the first test tube with 900 µL of calibrator diluent RD5-43 for an initial ten-fold dilution of the cortisol standard to a concentration of 10 ng/mL. For each pipetting, the pipette was filled up, discharged back into solution, and then refilled to ensure maximal precision and accuracy during pipetting measures. In the six subsequent tubes serial dilutions of 500 µL of cortisol standard were pipetted with 500 µL of calibrator diluent RD5-43 to form diluted standards of 5, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/mL). All test tubes were vortexed following addition of cortisol standard and Calibrator Diluent RD5-43 to maximize mixing. Calibrator diluent RD5-43 served as the zero standard (B_0). Calibrator diluent RD5-43 was added into NSB (150 µL) wells and B_0 (100 µL) wells. Each dilution of cortisol standard added to the appropriate wells (100 µL per well) to form the standard curve, and serum samples (100 µL per well) were added in duplicate to wells according to a pre-planned set-up of the microplate. Horseradish peroxidase conjugated to cortisol (HRP-cortisol) was added to each well (50 µL per well). Mouse anti-human antibody was then added to each well (50 µL) except the NSB well. The adhesive film was then placed over the top of the microplate. The microplate was then incubated on a horizontal orbital microplate shaker (0.12" orbit) for 2 hours at 500 rpm.

After the microplate finished shaking, the wells were washed using 400 µL of wash solution per well. After all the wells were filled, the microplate was flipped and blotted onto a paper towel. The plate was shaken and blotted quite vigorously to ensure all unbound solution had been removed. This process was repeated four times.

The color reagents A (Hydrogen peroxide) and B (chromagen) were mixed together to form a substrate solution shortly before the microplate finished shaking. The substrate solution was then added to each well in the microplate (200 µL per well). The microplate was wrapped in aluminum foil and incubated for 30 minutes to allow for the substrate solution to react with the contents of each well. After 30 minutes, 50 µL of Stop solution was added to each well to prevent any further development of the color reaction. The plate was gently tapped to ensure thorough mixing in each well. Optical densities were taken using a microplate reader set at 450 nm with correction set at 570 nm.

Data Analysis

Once optical density (OD) readings were obtained, the blind on the samples was broken. The OD value of the NSB well was subtracted from the OD of each standard and sample well to correct for non-specific binding of cortisol in each microplate well. The maximum possible binding of cortisol was obtained from the B_0 well.

A standard curve was generated using GraphPad Prism software, plotting logit vs. log[cortisol] and log[cortisol] vs. percent cortisol bound. An average OD value was calculated for each set of duplicate samples. The OD values were divided by B_0 OD value to determine the

percent of maximum binding of cortisol in the wells and to calculate the logit values for each averaged sample. The log [cortisol] values were then calculated by extrapolation from the linear regression of the plot of logit vs. log [cortisol] values of the standard curve. Finally, antilogs were calculated and the cortisol concentrations were corrected for the 20-fold dilution. Between groups (i.e., tic severity), differences in cortisol concentrations were assessed with a one-way ANOVA.

RESULTS

The linear regression of the standard curve revealed a very strong goodness of fit ($r^2 = 0.992$; $p < 0.001$), with a limit of detection less than 1.0 ng/ml. The NSB well revealed an extremely low non-specific binding (OD = 0.013; equivalent to 1.23% of the B_0).

There were no significant differences in cortisol concentrations ($F=0.3658$, $p>0.05$) between the group with severe tics, those with mild tics, and the controls (see Figure 1).

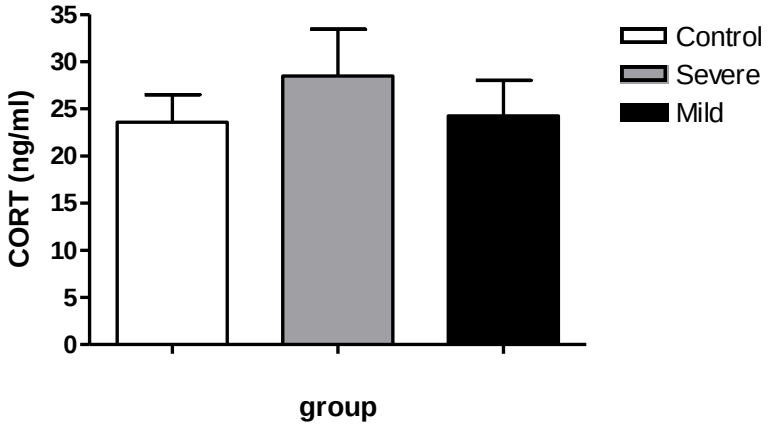

Figure 1: Cortisol concentrations for the groups with severe tics, mild tics, and no tics.

DISCUSSION

In this study, we investigated the relationship between cortisol and severity of tics in school-age children with Tourette's syndrome. Similar studies have been performed in the past on the role of the HPA axis in Tourette's syndrome, but none specifically looked at cortisol levels or at basal functioning of the HPA axis in Tourette's patients. Chappell et al. (1996) investigated stress-responsiveness of patients with Tourette's syndrome or obsessive-compulsive disorder (OCD) in comparison with normal controls by measuring cerebrospinal fluid (CSF) concentrations of corticotrophin releasing hormone (CRH) obtained through a lumbar puncture. They found significantly elevated levels of CRH in Tourette's patients as compared with the OCD patients and normal controls. Interestingly, no relationship was seen between the CRH concentrations and measures of depression, anxiety, OCD-like behaviors, and tics. Our results are consistent with these findings as no correlation was seen between severity of tic presentation and basal cortisol levels.

Considering the data from the Chappel et al. (1996) study, in combination with the current data, it seems that cortisol is a contributor to the exacerbation of symptoms during times of duress, but it is unrelated to the basal expression of tics and associated behaviors in Tourette's syndrome outside of stressful situations.

On the other hand, it is interesting to note the role of cortisol in suppressing the immune response because there appears to be a relationship between early streptococcus infections and the early manifestation of Tourette's symptoms in individuals identified as having Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus (PANDAS) (Murphy 2007). Thus, elevated cortisol could potentially play a role in etiology of Tourette's syndrome associated with PANDAS, but there was no evidence of ongoing differences in basal cortisol function in the current study.

Future studies should examine the potential that circulating cortisol concentrations may be abnormally elevated during stress exposure in patients with Tourette's

syndrome. This would determine if the downstream activation of the HPA axis concurs with the abnormal stress-responsive activation of CRH (Chappell et al., 1996) in these patients. It would also be interesting to know if basal CRH concentrations (obtained from post mortem samples) are abnormal in patients with Tourette's syndrome. In summary, stress has a clear role in the presentation of tics in individuals with Tourette's syndrome, and these patients have abnormal stress-responsive elevations in CSF CRH concentrations (Chappell et al., 1994); however, the basal HPA axis tone appears to be unrelated to the diagnosis or severity of Tourette's syndrome. The relationship of the HPA axis response to stress with Tourette's syndrome is still relatively unexplored.

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