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Interaction of Childhood Maltreatment with the Corticotropin-Releasing Hormone Receptor Gene: Effects on HPA Axis Reactivity

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Abstract

Background—Variation in the corticotropin-releasing hormone receptor (*CRHR1*) gene has been shown to interact with early-life stress to predict adult depression. This study was conducted to determine whether *CRHR1* polymorphisms interact with childhood maltreatment to predict HPA axis reactivity, which has been linked to both depression and early-life stress.

Methods—One-hundred twenty-nine White non-Hispanic adults completed the Childhood Trauma Questionnaire, the dexamethasone/corticotropin-releasing hormone test, and provided blood samples for genotyping of two *CRHR1* polymorphisms.

Results—Both rs110402 and rs242924 (which were in tight linkage disequilibrium, $D' = 0.98$) showed a significant interaction with maltreatment in the prediction of cortisol response to the Dex/CRH test ($p < .05$). For subjects with maltreatment, the GG genotype of each SNP was associated with elevated cortisol responses to the test.

Conclusions—Variation in the *CRHR1* moderates the effect of childhood maltreatment on cortisol responses to the Dex/CRH test. Excessive HPA axis activation could represent a mechanism of interactions of risk genes with stress in the development of mood and anxiety disorders.

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Keywords

Cortisol; Dex/CRH test; HPA axis; genetics; CRHR1 gene; gene-environment interaction

INTRODUCTION

Stressful life experiences increase risk for psychiatric disorders such as major depression and anxiety disorders. Genes that moderate the influence of adversity on depressive and anxiety disorders have recently been identified [1-3]. One likely mechanism of gene-environment interactions is that risk genes may confer sensitivity to stress, possibly through altered functioning of corticotropin-releasing hormone (CRH) and the hypothalamic-pituitary-adrenal (HPA) axis. Studies of rodents and non-human primates with early exposure to stress show enduring alterations of behavior as well as activity of CRH and the HPA axis, changes that parallel some of the abnormalities frequently seen in major depressive disorder (MDD) and post-traumatic stress disorder (PTSD) [4]. Recent studies of humans have also found associations between childhood adversity and enhanced or attenuated CRH and HPA axis function [5-8]. Preclinical work demonstrates that prolonged or excessive exposure to stress and glucocorticoids results in neurostructural changes in limbic brain regions that may contribute to the pathogenesis of stress-related disorders [9,10].

Genes that regulate activity of CRH and the HPA axis are likely determinants of the effects of stress and adversity on risk for depressive and anxiety disorders. Recent studies have examined variation in the gene coding for the CRH type I receptor, which mediates the hormonal and behavioral effects of CRH in response to stress [11]. CRH receptors have been found to occupy widespread areas of the primate brain, including the pituitary, amygdala, hippocampal formation, and throughout the neocortex; the CRH type 1 receptor is also expressed in cerebellar cortex, locus coeruleus, thalamus, nucleus of the solitary tract and striatum [12-14].

Variation in the *CRHR1* gene has been linked to major depression [2,15,16]. Bradley and colleagues recently found that several single nucleotide polymorphisms (SNPs) in this gene interacted with childhood maltreatment to predict depressive symptoms and in two separate samples found that a haplotype of the three most significant SNPs was protective against depressive symptoms among maltreated subjects as measured by the Childhood Trauma Questionnaire (CTQ) (2). Polanczyk and colleagues recently replicated the interaction of this haplotype and maltreatment according to the CTQ on the prediction of major depressive disorder in a representative community sample, but not in another community cohort using a different measure of maltreatment [16]. A gene by stress interaction influencing excessive alcohol use among adolescents has also been demonstrated [17].

Recent reports have identified HPA axis effects of genes linked to depression and anxiety [e.g., 18-20]. A preliminary study showed altered neuroendocrine function in relation to variation in the *CRHR1* gene in a sample of adolescents [20]. These studies have not examined influences of early adversity. In the current study, we tested the hypothesis that two of the *CRHR1* polymorphisms studied by Bradley and colleagues [2] and Polanczyk and colleagues [16] would interact with reported childhood maltreatment to predict altered cortisol responses to the Dex/CRH test.

METHODS

Subjects

Participants were 78 female and 51 male adults. Subjects were recruited from the community via flyers as well as through Internet and newspaper advertisements for “healthy adults” and “healthy adults with a history of early-life stress.” Only individuals who reported their race as White, non-Hispanic (not Black, Asian, Pacific Islander, Native American, or “Other”) were included in the present study, in order to reduce the possibility of population stratification. Individuals with lifetime psychotic disorders and bipolar disorder were excluded from participation, and the current study also excluded those with current alcohol or substance abuse or dependence, current MDD and current PTSD, due to possible neuroendocrine effects of these disorders. Participants completed a medical history, physical examination, electrocardiogram, and standard laboratory studies to rule out acute or unstable medical illness, endocrine disease, or ongoing treatment with drugs that might influence HPA axis function, including psychotropics, beta blockers, angiotensin-converting enzyme inhibitors, ketoconazole, metyrapone, and corticosteroids. Oral contraceptives were permitted. Subjects gave voluntary written informed consent to participate in this study, which was approved by the Butler Hospital Institutional Review Board.

Measures

The Structured Clinical Interview for DSM-IV (SCID; [21])—Current and lifetime history of Axis I psychiatric diagnoses were assessed using the SCID.

The Childhood Trauma Questionnaire (CTQ)—The 28-item version of the CTQ [22] was used to assess childhood maltreatment. The five CTQ subscales (physical abuse, sexual abuse, emotional abuse, physical neglect, emotional neglect) were significantly inter-correlated in this sample with r values ranging from .68-.70 for most pairs of subscales. Correlations of sexual abuse and physical abuse with the other subscales were weaker and r values ranged from .31-.67. Childhood maltreatment was defined as a “moderate to severe” score on any of five subscales. The remaining participants were considered to have no/minimal maltreatment according to the CTQ.

Genotyping

DNA was extracted from frozen whole blood using standard methods. Two of the individual *CRHR1* single nucleotide polymorphisms (SNPs) that have previously been reported to interact with reported childhood maltreatment in the prediction of depression [2], rs110402 located in intron 1 and rs242924 from intron 2 (which are about 5 kb apart), were genotyped in the present study. Markers were genotyped using a fluorogenic 5' nuclease assay (the Taqman method; [23]). All samples were genotyped in duplicate for quality control. Genotyping failed or provided ambiguous genotype results from seven subjects for the rs110402 SNP and three subjects for rs242924; these participants were therefore missing from the relevant analyses.

Dex/CRH Test

The night before the test, participants took dexamethasone 1.5 mg orally at 11:00 p.m. The following day, participants arrived at 12:00 p.m. and were given lunch. A topical anesthetic cream containing lidocaine 2.5% and prilocaine 2.5% was applied to the forearm between 12:30 and 12:45 p.m. At 1:00 p.m., an indwelling intravenous (IV) catheter was inserted in the forearm by a research nurse. Subjects then remained in a semi-recumbent position throughout the procedure except to use the bathroom. They were permitted to read or watch pre-selected movies that did not contain emotionally-charged material. Vital signs were

monitored throughout the test. At 3 p.m., CRH (corticotropin ovine triflutate, Acthrel®, Ferring Pharmaceuticals, Inc.) 100 µg reconstituted in 2 ml 0.9% sodium chloride was infused intravenously over 30 seconds. Blood samples were withdrawn at 2:59 p.m. (baseline) and every 15 minutes thereafter until 5:00 p.m. Samples were immediately stored on ice, centrifuged within 45 minutes, and then stored at -80° C. Cortisol assays were performed on samples from 2:59 p.m., 3:30 p.m., 3:45 p.m., 4:00 p.m., 4:15 p.m., and 5:00 p.m. The GammaCoat cortisol I-125 coated-tube radioimmunoassay (RIA) kit (INCSTAR Corp., Stillwater, Minn.) was used to measure cortisol in duplicate at each time point. The intra-assay and inter-assay CVs observed for quality assessment samples (5 and 20 µg/dl) were less than 5% and 10%, respectively.

Statistical Analysis

Data were analyzed using SPSS 16.0 for Windows. All statistical tests were two-tailed, and alpha was set at 0.05. Haploview 4.1 [24] was used to compute minor allele frequency for the SNPs, compute D' , and test for Hardy-Weinberg Equilibrium. In order to assess potential confounding factors, the maltreated group was compared to the nonmaltreated group with respect to demographic factors, genotype, and past Axis I diagnoses using correlation and chi square tests. A logistic regression model, controlling for age and sex was used to determine whether there was any association between genotype and past history of MDD, PTSD, or alcohol abuse/dependence. General linear models controlling for age and sex were used to test for effects of genotype on changes in heart rate and blood pressure during the test.

Cortisol values were positively skewed and were therefore log-transformed for statistical analyses; raw values are displayed in the Figures. Repeated measures general linear models, controlling for age and sex, and testing genotype, childhood maltreatment, and their interaction as predictors of cortisol response to the test, were conducted to test the primary hypothesis. Additional models were conducted to assess the effect of potential confounding factors. The general linear models were repeated controlling for use of oral contraceptives or estrogen replacement in women, BMI, and for effects of lifetime MDD, lifetime PTSD, lifetime alcohol abuse or dependence, and any major lifetime Axis I psychiatric disorder.

RESULTS

Sample Characteristics

Both SNPs were in Hardy-Weinberg equilibrium; D' for the two markers was 0.98. Minor allele frequency (MAF) for rs110402 was 0.46, and for rs242924, 0.44. Characteristics of the sample are shown in Table 1. Participants reporting a history of maltreatment were significantly older, had higher BMIs, and were more likely to meet the criteria for lifetime MDD and for lifetime alcohol abuse/dependence than those with none or minimal maltreatment. CRHR1 genotypes were not significantly associated with past history of MDD, PTSD, or alcohol abuse/dependence, for either SNP. Genotype was also not significantly associated with changes in heart rate or blood pressure during the Dex/CRH test.

Dex/CRH Cortisol Response

The repeated measures general linear models, controlling for age and sex, and testing genotype, childhood maltreatment, and their interaction as predictors of cortisol response to the test, showed a significant gene $\times$ maltreatment interaction for both SNPs. For rs110402, there was a significant within-subjects interaction effect ($F(5, 264)=3.18, p<.01$). As shown in the right panel of Fig. 1, for participants with no history of moderate to severe maltreatment, cortisol response to the Dex/CRH test did not vary according to genotype.

However, for those reporting a history of moderate to severe maltreatment (Fig. 1, left panel), subjects with the GG genotype had higher cortisol responses to the test over time than those with a T allele. There was a similar significant within-subjects interaction for rs242924 ($F(5, 276)=2.24$, $p<.05$, Fig. 2). There were no main effects of maltreatment or genotype after accounting for the interaction effect for each SNP.

This interaction remained significant for both SNPs after controlling for use of oral contraceptives or estrogen replacement in women. In addition, the interaction between genotype and maltreatment remained for each SNP in models that controlled for BMI, and for effects of lifetime major depression and PTSD, lifetime alcohol abuse or dependence, and any major lifetime Axis I psychiatric disorder.

DISCUSSION

Results of this study indicate that both *CRHR1* SNPs moderate the effect of childhood maltreatment on cortisol responses to the Dex/CRH test. The finding that the GG genotype of both SNPs was linked to higher cortisol responses to the test extends recent findings of an increase in depressive symptoms among subjects with the GG genotype of these SNPs who reported a history of childhood maltreatment [2]. In both cases, the major allele was the stress-responsive allele, suggesting that the minor allele may play a protective role and confer resilience against stress and adversity. Maltreated subjects with the minor allele had attenuated cortisol responses to the Dex/CRH test.

Given the role of the type I receptor in the activation of the HPA axis as well as involvement in extra-hypothalamic brain regions and the behavioral response to stress, these findings suggest that this genotype may increase neuroendocrine sensitivity to stress. This study is limited by the modest sample size and the sample may have been skewed by excluding individuals with current MDD and PTSD. In addition, it is not known whether the SNPs studied here have a functional role or whether they are in linkage disequilibrium with a functional variant. However, enhanced sensitivity of the *CRHR1* receptor or altered receptor feedback regulation could account for our findings of increased cortisol responsivity in the Dex/CRH test among those with the GG genotype of these SNPs.

Increased CRH neurotransmission and cortisol reactivity, which previously have been associated with both early-life stress and mood and anxiety disorders, may be an intermediate phenotype for these disorders. Given that excessive exposure to CRH and glucocorticoids can have neurotoxic effects on brain regions implicated in the pathophysiology of depression and anxiety disorders [9,10], enhanced CRH and HPA axis reactivity could represent a mechanism for the association between the *CRHR1* gene, stressful life experiences, and major depression. However, the literature on the links of HPA axis reactivity with depression and with childhood maltreatment has been variable, with both exaggerated and attenuated cortisol reactivity in these conditions [5,7,8,25]. Gene-by-environment interactions such as those seen in the present study may account for a portion of the variability in the effects of childhood maltreatment on HPA axis function.

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Figure 1.

Repeated measures general linear model showed a significant within-subjects interaction effect ($F(5, 264)=3.18, p<.01$) between rs110402 genotype and maltreatment. Influence of genotype on cortisol response to the Dex/CRH test is displayed for subjects reporting A) moderate/severe maltreatment and B) no/minimal maltreatment.

Figure 2.

Repeated measures general linear model showed a significant within-subjects interaction effect ($F(5, 276)=2.24, p<.05$) between rs242924 genotype and maltreatment. Influence of genotype on cortisol response to the Dex/CRH test is displayed for subjects reporting A) moderate/severe maltreatment and B) no/minimal maltreatment.

Table 1

Characteristics of Participants According to Childhood Maltreatment

	No/Minimal Maltreatment N = 91	Moderate/Severe Maltreatment N = 38	P value
Age, Mean (SD)	26.7 (8.78)	35.9 (11.65)	< 0.001
Range	18-61	19-58	
Sex, N (%)			ns
Male	40 (44.0)	11 (28.9)	
Female	51 (56.0)	27 (71.1)	
BMI, Mean (SD)	25.1 (3.3)	27.0 (5.1)	< 0.05
Range	20.1-34.8	19.4-40.8	
Genotype, N (%)			ns
SNP rs242924 (N=126)			
GG	27 (30.3)	11 (29.7)	
GT	46 (51.7)	19 (51.4)	
TT	16 (18.0)	7 (18.9)	
SNP rs110402 (N=119)			ns
GG	22 (26.8)	10 (27.0)	
AG	44 (53.7)	20 (54.1)	
AA	16 (19.5)	7 (18.9)	
Type of Maltreatment, N (%)*			—
Emotional abuse	0	19 (50.0)	
Physical abuse	0	8 (21.1)	
Sexual abuse	0	17 (44.7)	
Emotional neglect	0	21 (55.3)	
Physical neglect	0	14 (36.8)	
SCID "Probable" Lifetime Axis I Diagnoses, N (%)			< 0.05
Major Depressive Episode	14 (15.4)	12 (31.6)	
Dysthymic Disorder	2 (2.2)	3 (7.9)	
Alcohol Abuse/Dependence	7 (7.7)	8 (21.1)	
Drug Abuse/Dependence	6 (6.6)	3 (7.9)	
Post-traumatic Stress Disorder	1 (1.1)	2 (5.3)	
Panic Disorder or Social Phobia	1 (1.1)	2 (5.3)	ns

Note: Percentages for types of maltreatment do not sum to 100% because participants could report more than one type of maltreatment. "ns" designates p values that are not significant. Subjects were excluded if they met the criteria for lifetime psychotic disorder or bipolar disorder, or current alcohol or drug abuse or dependence, major depression, and post-traumatic stress disorder. No participant had a lifetime diagnosis of generalized anxiety disorder.