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Elevated Cortisol Level and Cortisol/DHEAS Ratio in Schizophrenia as Revealed by Low-Dose Dexamethasone Suppression Test

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Abstract: Earlier studies have used the dexamethasone (DEX) suppression test (DST) to investigate the hypothalamic-pituitary-adrenal (HPA) function in schizophrenia, although the findings are controversial. Recently there has been an increased interest in the role of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) in HPA axis function. Several studies have investigated basal DHEA(S) levels and cortisol/DHEA(S) ratios in schizophrenia patients, while no attempts have been made to investigate DHEA(S) level in response to the DEX administration. We aimed to compare the post-DEX cortisol and DHEAS levels and the cortisol/DHEAS ratio between schizophrenia patients and healthy controls. Here we administered the DST to 43 patients with schizophrenia and 37 age- and sex-matched healthy controls. Plasma cortisol levels, serum DHEAS levels, and cortisol/DHEAS ratio after administration of 0.5 mg of DEX were compared between the two groups. Schizophrenia patients showed significantly higher cortisol level and cortisol/DHEAS ratio than controls, while DHEAS levels were not significantly different between groups. These results suggest that besides the cortisol level, cortisol/DHEAS ratio as assessed with the DST might reflect abnormal HPA axis function in schizophrenia.

Keywords: Cortisol, dehydroepiandrosterone, dehydroepiandrosterone sulfate, dexamethasone suppression test, hypothalamic-pituitary-adrenal axis, schizophrenia.

INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis is activated by all sorts of stressors, and this fact has represented the rationale for investigations into HPA axis function in schizophrenia, a disorder where stress could play a pivotal role in its onset and exacerbation. A large number of earlier studies have used the dexamethasone (DEX) suppression test (DST) to explore negative feedback inhibition of cortisol in schizophrenia patients. The findings were, however, controversial such that the rate of non-suppression to the DST varied from 0% to 73% [1]. A meta-analysis of 26 DST studies [2] revealed that non-suppression rates of schizophrenia patients (19%) were significantly lower than those of patients with major depression (51%) and significantly higher than those of healthy controls (7%). Indeed, HPA axis dysfunction is less consistently reported in schizophrenia than in depression [3,4]. On the other hand, studies employing psychosocial challenge paradigms have reported blunted cortisol responses in schizophrenia patients [5,6]. Individuals who are at risk for developing psychosis [7,8], those with first episode psychosis [9,10], and those with schizotypal personality [11,12] have also been shown to be associated with altered HPA axis function, although findings are again not uniform, that is, both hyper- and hypo-cortisolism are reported. All these findings indicate that although schizophrenia would be associated with altered HPA axis function, its precise nature is yet to be established. More recently, there has been an increasing interest in the role of dehydroepiandrosterone (DHEA) and its more stable sulphated conjugated metabolite, DHEAS, in the neuroendocrinology of schizophrenia.

Like cortisol, DHEA and DHEAS (herein jointly referred to as ''DHEA(S)''), are secreted by the adrenal cortex, at least partly in response to adrenocorticotropin-releasing hormone. DHEAS is the most abundant adrenal steroid in humans and its serum concentration is much higher than DHEA. DHEA(S) has neuroprotective actions, and is a precursor to more potent androgens and estrogens, such as testosterone and estradiol [13]. DHEA(S) is also classified as a neurosteroid, meaning that this hormone does not only cross the blood brain barrier to exert effects within central nervous system (i.e., a neuroactive steroid) but is synthesized *de novo* in the brain from its sterol precursor [14]. DHEA(S) acts as a gamma-aminobutyric acid type A receptor antagonist and is shown to be involved in the regulation of neuronal survival and differentiation [15]. It is also shown to act as a sigma-1 receptor agonist [16].

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Importantly, DHEA(S) is known to possess anti-glucocorticoid properties [17] because it antagonizes the effect of cortisol [13] and inhibits glucocorticoid-induced enzyme activity [18]. In healthy adults, acute administration of DHEA rapidly reduces cortisol levels [19]. Hence, DHEA(S) is assumed to be involved in stress responses [20] as well as in a broad range of behavioral functions [21]. Due to these characteristics, DHEA(S) has been implicated in the pathophysiology of various psychiatric disorders including major depression [22], bipolar disorder [23], dysthymic disorder [24], panic disorder [25], borderline personality disorder [26], eating disorder [27], posttraumatic stress disorder (PTSD) [28], and schizophrenia [29,30]. Several studies have investigated baseline DHEA(S) levels and cortisol/DHEA(S) ratios in schizophrenia patients, although their findings are not necessarily consistent. Nevertheless, based on the fact that the DHEA(S) level is influenced by the negative feedback of DEX administration, some studies have shown that DHEA(S) and/or cortisol/DHEA(S) ratio in response to the DST could be a sensitive marker for HPA axis function [27,31]. However, no studies to date have investigated DHEA(S) as assessed by the DST in schizophrenia.

In this context, the present study aimed to compare the post-DEX cortisol and DHEAS levels and the cortisol/DHEAS ratio between schizophrenia patients and age- and sex-matched healthy controls. We hypothesized that, among these measures, the cortisol/DHEAS ratio would be the most sensitive marker that distinguishes patients from healthy controls. We also expected that schizophrenia patients would be more likely to be associated with either or both of hyper- and hypo-cortisolism as compared to controls.

METHODS

Participants

Forty-three patients with schizophrenia (age range: 16-70 years), who were under treatment at the National Center of Neurology and Psychiatry Hospital or at a nearby hospital or psychiatric clinic, were enrolled. Of the 43 patients, 23 were hospitalized in the emergency ward of the National Center of Neurology and Psychiatry Hospital for the acute treatment of their psychotic symptoms at the time of the neuroendocrine testing. Consensus diagnoses for DSM-IV schizophrenia [32] were made by psychiatrists based on clinical interviews, observations and case notes. Thirty-seven age- and sex-matched healthy volunteers (age range: 23-70 years) were recruited from the community, through advertisements in free local magazines and our website announcement. At the first visit, the healthy participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview [33] by a research psychiatrist, and only those who demonstrated no current Axis I psychiatric disorders were enrolled in this study. In addition, those who demonstrated one or more of the following conditions during a non-structured interview performed by an experienced psychiatrist were excluded from the healthy control group: past or current contact to psychiatric services, and other obvious self-reported signs of past primary psychotic and mood disorders as well as PTSD. Additional exclusion criteria from both the patient and control groups were: having a prior medical history of central nervous system disease or severe head injury, having a history of substance dependence or substance abuse within the past six months, having major systemic medical illnesses, or taking corticosteroids, antihypertensive medications, oral contraceptives or estrogen replacement therapies. The present experiments on our subjects were conducted in accordance with the Declaration of Helsinki. After the nature of the study procedures had been explained, written informed consent was obtained from all subjects. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry.

Dexamethasone Suppression Test

First, participants took 0.5 mg tablet of DEX orally at 23:00 h. For inpatients, a ward nurse gave this tablet to each patient. For the remaining subjects, compliance was monitored at the time of the blood collection by asking them whether they took the tablet as directed on the previous night. On the next day, plasma and serum samples were collected at 10:00 h. Plasma concentrations of cortisol were measured by radioimmunoassay and serum concentrations of DHEAS were measured by chemiluminescent enzyme immunoassay at SRL Corporation (Tokyo, Japan). The detection limit for cortisol was 27.59 nmol/l (= 1.0 μg/dl). Cortisol values under the detection limit were treated as 0 nmol/l. As our hypothesis was that the two extreme ends of cortisol values (i.e., both exaggerated and blunted cortisol reactivity) would be related to schizophrenia, in the main analysis we also adopted the categorical division of participants based on *a priori* defined cut-off values of cortisol, i.e., 27.59 nmol/l (= 1.0 μg/dl) and 137.95 nmol/l (= 5.0 μg/dl), which were derived from several previous studies [12,34-36]. 'Non-suppressors' were defined to be individuals whose cortisol level was equal to or more than 137.95 nmol/l. 'Enhanced-suppressors' were defined as those individuals whose cortisol level was less than 27.59 nmol/l which corresponded to the cortisol level under the detection limit. The remaining individuals were considered to be 'moderate-suppressors'.

Clinical Assessment, Antipsychotic Medication, and Psychological Distress

For schizophrenia patients, symptoms were assessed by an experienced research psychiatrist in 41 of the total 43 patients using the Positive and Negative Syndrome Scale (PANSS) [37]; this yields a total score in addition to scores on positive, negative, and general psychopathology subscales. All patients with schizophrenia were receiving antipsychotics at the time of the neuroendocrine testing. Daily doses of antipsychotics, including depot antipsychotics, were converted to chlorpromazine equivalents using guidelines [38,39].

For healthy controls, subjectively perceived psychological distress during one week preceding the neuroendocrine test was assessed *via* the Hopkins Symptom Checklist (HSCL) [40], a self-report questionnaire consisting of 58 (or 54) items which are scored on five underlying symptom dimensions, i.e., somatization, obsessive-compulsive, interpersonal sensitivity, anxiety, and depression symptoms. In the present study a validated Japanese version of the HSCL [41] comprising 54 items was

used, as described in our previous study [35]. In this questionnaire, subjects were instructed to rate each item based on the distress perceived during the previous week, using a four-point likert scale, with "not-at-all" being scored 1, "occasionally", 2, "sometimes", 3, and "frequently", 4.

Statistical Analysis

Averages are reported as means ± standard deviation (SD). To compare categorical variables, χ^2 test was used. The t-test or Mann-Whitney U-test was used to examine differences between two groups. Plasma cortisol levels, serum DHEAS levels, and cortisol/DHEAS ratio were compared between two groups using the Mann-Whitney U test because these hormonal data did not satisfy the assumptions for parametrical testing, which was revealed by the Kolmogorov-Smirnov test. Correlation between hormonal measures and clinical variables were calculated using the Spearman's rank correlation test. Statistical significance was set at two-tailed $p < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

RESULTS

Relationships of Hormonal Measures with Demographic Characteristics

Table **1** shows the demographic and clinical characteristics of patients with schizophrenia and healthy controls. Patients and controls were well matched for age and sex. In the patient group, age significantly correlated with the DHEAS level ($\rho = -0.39$, $p = 0.009$), but not with cortisol level ($\rho = 0.05$, $p = 0.76$) or cortisol/DHEAS ratio ($\rho = 0.26$, $p = 0.09$). In the control group, age significantly correlated with the DHEAS level ($\rho = -0.55$, $p < 0.001$) and cortisol/DHEAS ratio ($\rho = 0.41$, $p = 0.013$), but not with cortisol level ($\rho = 0.12$, $p = 0.49$). Schizophrenic males and females did not significantly differ in cortisol level (Mann-Whitney U = 259.5, p = 0.44) or cortisol/DHEAS ratio (Mann-Whitney U = 298.0, p = 0.09), whereas DHEAS was significantly higher in males than in females (Mann-Whitney U = 120.0, p = 0.008). Similarly, control males and females did not significantly differ in cortisol level (Mann-Whitney U = 154.0, p = 0.62) or cortisol/DHEAS ratio (Mann-Whitney U = 181.0, p = 0.74), whereas DHEAS was significantly higher in males than in females (Mann-Whitney U = 96.5, p = 0.025). Patients were significantly more likely to be smokers than controls; however, smokers and non-smokers did not significantly differ in the cortisol level, DHEAS level, or cortisol/DHEAS ratio for both patients (all $p > 0.1$) and controls (all $p > 0.3$). Patients were significantly more likely to have a family history of psychiatric disorders than controls, although the presence *vs* absence of such a history did not significantly impact on any of the three hormonal measures for both patients (all $p > 0.4$) and controls (all $p > 0.1$). In addition, all demographic and clinical variables were compared between males and females within each of the two diagnostic groups, and found no significant sex differences in any of the variables examined (all $p > 0.05$).

Relationships of Hormonal Measures with Clinical Variables and Symptoms

Clinical variables including age at onset of schizophrenia, antipsychotic dosage, duration of antipsychotic medication, and duration of hospitalizations were not significantly correlated with any of the three hormonal measures, i.e., cortisol level, DHEAS level, and cortisol/DHEAS ratio (all $p > 0.05$). In total, 5 patients were

Table 1. Demographic Characteristics and Clinical Variables of Schizophrenia Patients and Control Subjects

Variable	Schizophrenia Patients ($n = 43$)	Healthy Controls ($n = 37$)	Statistics	*P*
Sex, male/female (% female)	24/19 (44%)	20/17 (46%)	$\chi2(1) = 0.02$	0.87
Age, years	42.7 ± 11.9	41.1 ± 14.8	t = 0.54, df = 78	0.59
Smoking status, smokers/non-smokers	18/25	6/31	$\chi2(1) = 6.23$	0.013
Family history of psychiatric disorder, yes/no	18/25	5/32	$\chi2(1) = 7.80$	0.005
Age at onset, years	25.4 ± 9.2			
Duration of illness, years	16.1 ± 11.5			
Duration of antipsychotic medication, years	14.9 ± 11.5			
Chlorpromazine equivalents of antipsychotics, mg/day	634.2 ± 615.7			
Number of hospitalizations	3.6 ± 3.3			
Duration of total hospitalizations, months	12.9 ± 21.2			
Numbers of out-/in-patients	20/23			
PANSS total score	62.9 ± 21.0			
PANSS positive score	13.9 ± 5.2			
PANSS negative score	17.1 ± 7.8			
PANSS general psychopathology score	31.9 ± 10.4			

PANSS, Positive and Negative Syndrome Scale.

taking typical antipsychotic(s), 29 were taking atypical antipsychotic(s) and 9 were taking both types at the time of testing. No significant differences were seen between these three medication groups in any of the three hormonal measures (all $p > 0.6$). Duration of illness was significantly negatively correlated with DHEAS level ($\rho = -0.33$, $p = 0.033$), which was considered a reflection of a confounding effect of age because age was significantly correlated negatively with DHEAS level and positively with illness duration ($p < 0.001$). Number of hospitalizations was significantly correlated negatively with DHEAS level ($\rho = -0.36$, $p = 0.020$) and positively with cortisol/DHEAS ratio ($\rho = 0.33$, $p = 0.031$). Outpatients and inpatients did not differ in any of the three hormonal measures (all $p > 0.2$). Symptom dimensions as assessed with the PANSS, i.e., positive symptoms, negative symptoms, general psychopathology and total score of PANSS, were not significantly correlated with any of the hormonal measures (all $p > 0.2$). In healthy controls, no significant associations were seen between the three hormonal outcomes and any of the five symptom dimensions of the HSCL (all $p > 0.1$).

Comparisons of Hormonal Measures between Patients *vs* Controls

Results of cortisol and DHEAS levels and cortisol/DHEAS ratio (multiplied by 100) for schizophrenia patients and healthy controls are provided in Fig. (**1**). Patients showed significantly higher cortisol level and cortisol/DHEAS ratio, while no significant difference was seen in the DHEAS level. As for the suppression pattern, patients showed a greater ratio of non-suppression of cortisol than controls at a non-significant trend level ($\chi^2(1) = 3.35$, $p = 0.067$) while no significant differences were seen between the two groups in the ratio of enhanced-suppression ($\chi^2(1) = 1.55$, $p = 0.21$). Compared to controls, outpatients showed significantly higher cortisol level (Mann-Whitney $U = 503.0$, $p = 0.025$) but not cortisol/DHEAS ratio (Mann-Whitney $U = 460.0$, $p = 0.13$) while inpatients showed significantly higher cortisol level (Mann-Whitney $U = 598.0$, $p = 0.009$) and cortisol/DHEAS ratio (Mann-Whitney $U = 563.5$, $p = 0.036$).

Table **2** shows a comparison of findings from studies that examined both cortisol and DHEA and/or DHEAS levels in schizophrenia/first-episode psychosis. Of these nine studies, three [42-44] did not provide cortisol/DHEA(S) ratio. It is clear from this table that previous findings of these hormonal indices in schizophrenia have been variable, such that some studies reported an elevation of these hormonal indices in schizophrenia while others not.

DISCUSSION

The present study found that the cortisol level and cortisol/DHEAS ratio, in response to the 0.5mg DEX administration, were significantly higher in schizophrenia patients as compared to healthy controls. The DHEAS level was not significantly different between the two groups. These hormonal indices were not significantly associated with the antipsychotic dosage or symptom dimensions, while cortisol/DHEAS ratio was to some extent associated with the number of hospitalizations and outpatient/inpatient status.

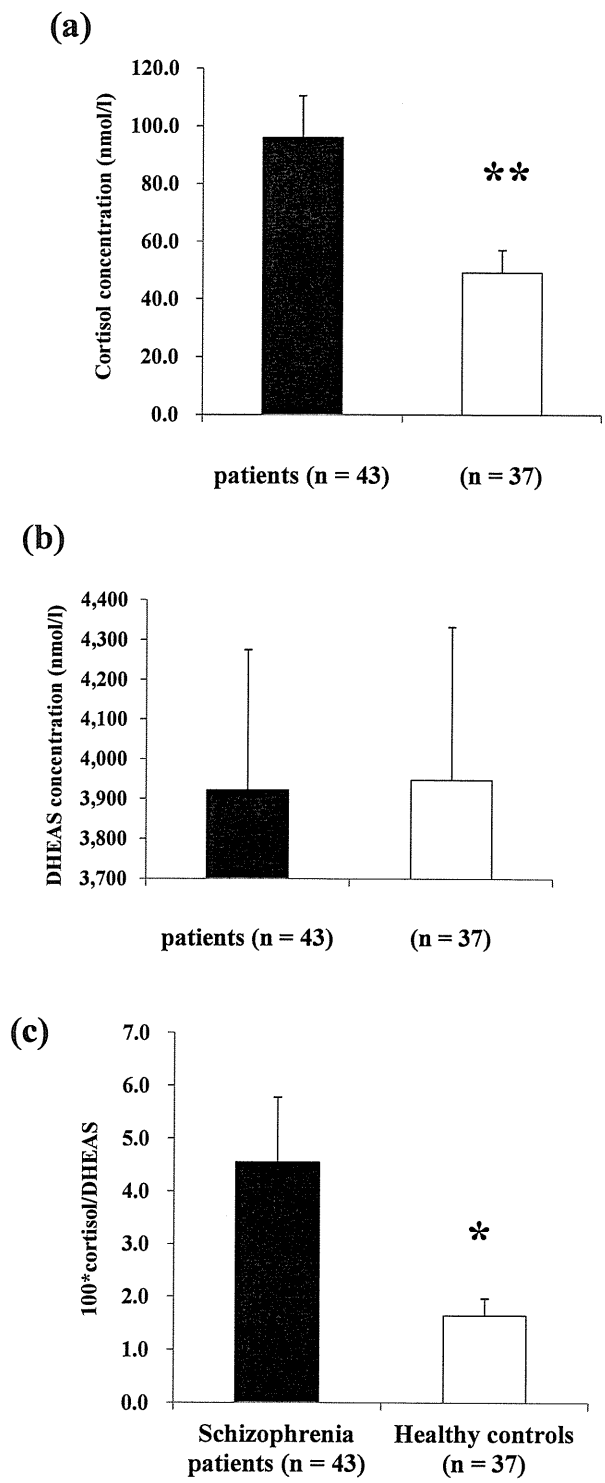

Fig. (1). Hormonal data, including cortisol level (a), DHEAS level (b) and 100*cortisol/DHEAS ratio (c), of schizophrenia patients (black bars) and healthy control subjects (white bars). *$p < 0.05$; **$p < 0.01$ (by Mann-Whitney U test). Error bars represent standard errors of the mean.

Table 2. Summary of Studies that Investigated Both Cortisol and DHEA(S) Levels in schizophrenia Patients and Healthy Controls

	Number of Sample		Matching Status	Patient Characteristics	Sampling Method	Hormonal Outcomes (Patients *vs* Controls)		
	Patients	Controls				Cortisol	DHEA(S)	Cortisol/DHEA(S)
The present study	43	37	age/sex	chronic	after DEX	↑↑	N.S.	↑↑
Shirayama *et al.* (2002)	14	13	males only[c]	moderate negative symptoms	basal	↑↑[e]	N.S.	N.A.
Ritsner *et al.* (2004)	40	15	age/sex	chronically ill, hospitalized	basal	N.S.	N.S.	↑↑
Goyal *et al.* (2004)	10	10	age/sex(males only)	N.A.	basal	N.S.	N.S.	N.A.
Strous *et al.* (2004)	37	25	age/sex	first-episode	basal	N.S.	↑↑	N.S.
Ritsner *et al.* (2007)[a]	43	20	age/sex	chronically ill, hospitalized	basal	↑↑	↑↑[f]	↑↑
Yilmaz *et al.* (2007)	66	28	age/sex(males only)	chronic	basal	↑↑	N.S.	N.A.
Gallagher *et al.* (2007)	20	20	age/sex	probably chronic	basal[d]	↑↑	↑↑	N.S.
Garner *et al.* (2011)[b]	39/20	25/15	none	first-episode	basal	N.S./N.S.	N.S./N.S.	N.S./N.S.

↑↑: significantly higher in schizophrenia patients
N.A.: not applicable
N.S.: not significant
[a]Samples were collected at baseline, after 2, and 4 weeks.
[b]Hormones were measured at baseline and after 12 weeks (baseline/after 12 weeks).
[c]Age not matched.
[d]Serial sampling from 13:00 h to 16:00 h.
[e]Not significant between schizophrenia patients with low negative symptoms and controls.
[f]DHEAS level was significantly higher in schizophrenia patients, while no significant difference was observed in DHEA level.

As described earlier, impaired negative feedback of HPA axis has been implicated in the pathophysiology of schizophrenia, yet findings on cortisol levels from DST studies were not consistent. As shown in Table **2**, studies that investigate both cortisol and DHEA(S) basal levels in schizophrenia patients have again yielded mixed findings, not only for cortisol and DHEA(S) levels but also for cortisol/DHEA(S) ratio. This controversy might be attributable, at least in part, to a number of differential demographic and/or clinical characteristics across studies, such as age, symptom severity, medication status, and comorbid psychiatric disorders, given that these variables are shown to affect cortisol and DHEA(S) levels. For instance, Ritsner *et al.* [45] first investigated cortisol/DHEA(S) ratio in schizophrenia patients in comparison to healthy controls and found this ratio to be significantly elevated in the patient group. This research group confirmed the elevated cortisol/DHEA(S) ratio in their subsequent study where hormonal levels were measured three times every two weeks [46]. In contrast, Gallagher *et al.* [30] did not find such a significant difference in the cortisol/DHEA ratio between schizophrenia patients and healthy controls. In addition, two studies that examined this ratio in individuals with first-episode psychosis did not observe significant differences in this ratio between these individuals and controls [9,47].

Against this background, we administered the DST to measure DHEAS as well as cortisol levels in chronic schizophrenia patients. The significantly elevated cortisol levels in patients than in controls, together with similar levels of DHEAS between the two groups, resulted in the significantly higher cortisol/DHEAS ratio in patients. Similar to the robust effect of oral DEX administration on cortisol suppression, the suppressive effect of DEX on DHEA(S) has been demonstrated [27,31]. Taken together, whereas our result of the elevated cortisol level in schizophrenia patients indicates overall impaired negative feedback inhibition of HPA axis in schizophrenia, the similar level of DHEAS in the two diagnostic groups might suggest that circulating DHEAS level is regulated by several other mechanisms as well as the negative feedback inhibition. However, the mechanism of this dissociation between cortisol and DHEA(S) levels is unclear. Nevertheless, our findings imply that HPA axis hyperactivity as indexed by the elevated cortisol level in schizophrenia is not compensated by the putative anti-glucocorticoid effect of DHEAS, thereby possibly leading to the persistent stress vulnerability in patients with chronic schizophrenia. It may also be worth noting that, among the five studies that have investigated cortisol/DHEAS ratio in patients with schizophrenia/psychosis, three studies including ours that examined chronically ill schizophrenia patients showed significantly higher cortisol/DHEAS ratio in patients than in controls [45,46] while two studies examining individuals with first-episode psychosis found no significant differences [9,47]. The cortisol/DHEAS ratio might thus vary depending on disease stages of schizophrenia, with this ratio becoming higher as the stage progresses.

With respect to the relationship between clinical variables and hormonal levels in schizophrenia patients, we found that, among a number of clinical variables, only those associated with hospital admission (i.e., the number of hospitalizations and the present status of in-/out-patient) were significantly related to altered HPA axis function; hospitalization was associated with higher cortisol/DHEAS ratio. It may be that although an alteration in HPA axis function is present independent of clinical states of schizophrenia patients, which is in line with a previous finding [46], acutely ill inpatients would exhibit even greater alteration. To draw any conclusion, further studies, longitudinal follow-up studies in particular, are required.

Another important issue that should be taken into consideration is the protocol for DST, i.e., the dose of DEX used for the pretreatment as well as the time of hormonal

assays after DEX administration. As for the dose of DEX, most of the earlier studies in schizophrenia have used 1.0 mg of DEX to assess negative feedback of HPA axis [1]. However, we herein administered a lower dose of DEX (i.e., 0.5 mg) to make the sensitivity high for both incomplete and enhanced suppression of cortisol, taking into account a growing body of evidence indicating that HPA axis abnormalities consist of hyper- and hypo-cortisolism. This approach, that is the DST using a low dose DEX, has successfully been employed in the studies of patients with PTSD to detect their enhanced negative feedback of cortisol [48,49]. Although we did not find any excess of enhanced suppression in schizophrenia patients as compared to controls, the fact that we were able to detect the altered HPA axis function in schizophrenia would point to the usefulness of DST with a low dose DEX for this population as well. Regarding the time of hormonal measurements, we decided to draw blood samples at 10:00 h because previous DST studies that have looked at enhanced suppression of cortisol using low-dose DEX draw blood in the morning [48,49]. However, such differences in the dose of DEX and the time of hormonal measurements should be taken into account when comparing the present results with previous ones.

Several limitations need to be commented upon. First, as we did not include baseline measurements of cortisol and DHEAS levels, the extent to which each participant suppressed his/her cortisol and DHEAS in response to the 0.5 mg of DEX cannot be determined. Second, as we sampled blood for hormone measurements only at a single point, the diurnal variation of the hormone levels was unknown. Third, since all patients were receiving antipsychotics, such medication may have influenced HPA axis function as has been demonstrated [50,51]. Although we did not find any significant correlations between antipsychotic dosage and hormonal measures, we have to acknowledge that our data on the chlorpromazine equivalents are themselves limited in that the inpatients were in the midst of their acute treatment and thus the prescription of antipsychotics tended to be frequently changed around the time of the hormone measurement. It should also be noted that chlorpromazine equivalents are an approximate indicator of D2 receptor antagonist activity and most antipsychotics have multiple effects that could have variable influence on the HPA axis function. Fourth, HPA axis abnormalities have been reported in a variety of psychiatric disorders, particularly in mood disorders, it is not known whether the altered cortisol level and cortisol/DHEAS ratio observed here represent schizophrenia-specific HPA axis dysfunction or rather common HPA axis alteration in relation to stressful conditions in general. Finally, we did not collect data on the menstrual cycle or history of childhood trauma, both of which are shown to moderate HPA axis function.

To sum, the present study found that HPA axis function in schizophrenia is altered, as indicated by the elevated cortisol level and cortisol/DHEAS ratio in response to the low-dose DEX administration. In addition to the cortisol level, cortisol/DHEAS ratio may reflect some aspect of HPA axis abnormalities in schizophrenia. Future studies that examine these hormones both before and after the DEX administration are needed to disentangle the baseline and feedback components of the HPA axis alteration in schizophrenia.

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CONFLICT OF INTEREST

None declared.

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Schizotypal Personality in Healthy Adults Is Related to Blunted Cortisol Responses to the Combined Dexamethasone/ Corticotropin-Releasing Hormone Test

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Key Words
Cortisol · Dexamethasone/corticotropin-releasing hormone test · Hypothalamic-pituitary-adrenal axis · Schizotypal personality

Abstract
Background/Aims: Schizotypy is viewed as a dimensional trait ranging from healthy people to schizophrenic spectrum patients. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, and accumulated evidence suggests that schizophrenia is associated with altered HPA axis function; however, HPA axis function in relation to schizotypal personality has not been well documented. ***Methods:*** We examined the relationship between schizotypal traits as assessed with the Schizotypal Personality Questionnaire (SPQ) and cortisol responses to the combined dexamethasone/corticotropin-releasing hormone test in 141 healthy volunteers. Subjects were divided into three groups based on their cortisol responses to the dexamethasone/corticotropin-releasing hormone test: incomplete suppressors, moderate suppressors, and enhanced suppressors. SPQ scores were compared between these three groups using the analysis of covariance, controlling for age and sex. ***Results:*** The analysis of covariance showed significant main effects of the suppressor status on the ideas of reference and suspiciousness/paranoid ideation subscales and cognitive-perceptual factor. Post-hoc analyses with Bonferroni correction revealed that the enhanced suppressors scored significantly higher than the moderate suppressors on these SPQ indices. ***Conclusion:*** These results indicate that nonclinical schizotypal traits in healthy adults are associated with blunted cortisol reactivity, potentially suggesting a shared neuroendocrinological mechanism across schizophrenia spectrum pathology.

Introduction

Research into schizotypal personality is important as it provides key insights into our understanding of schizophrenia [1]. The dimensional model of schizotypal personality posits that the degree of schizotypal traits varies on a continuum where normality lies on one extreme,

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nonclinical schizotypy in the middle, and clinically expressed schizophrenia on the opposite extreme [2, 3]. The putative distinction between schizotypal personality disorder (SPD) and nonclinical schizotype is that the former, like schizophrenia, is considered as an outgrowth of the latter, with additional modifying influences such as environmental stressors that cause frank psychotic symptoms. Although some investigators have argued for the competing theory, namely the categorical approach to schizotypy [4, 5], a large number of schizophrenia/schizotypy researchers have substantiated the dimensional model of schizotypy [6, 7]. Supporting this notion, a growing body of evidence indicates that schizotypal traits at a nonclinical level are associated with a range of endophenotypic characteristics of schizophrenia. More specifically, nonclinical schizotypes have been shown to display neurophysiological [8, 9], neurocognitive [10–13] and neuroimaging [14–16] abnormalities that are intrinsically similar to, albeit less severe than, those in schizophrenia patients. There is also some evidence that SPD may represent a risk factor for developing schizophrenia [17].

Hypothalamic-pituitary-adrenal (HPA) axis is activated by all sorts of stressors, and this fact constitutes the foundation of investigations into HPA axis function in schizophrenia, a disorder where stress could play a pivotal role in its onset and precipitation. A large number of earlier studies have used the dexamethasone (DEX) suppression test (DST) to investigate negative feedback inhibition of cortisol in schizophrenia patients, yielding controversial findings; the rate of nonsuppression to the DST varies from 0 to 73% [18]. A meta-analysis of 26 DST studies [19] revealed that nonsuppression rates of schizophrenia patients (19%) were significantly lower than those of patients with major depression (51%) and significantly higher than those of healthy controls (7%). Subsequently, Ismail et al. [20] found that less than 2% of their schizophrenia patients were nonsuppressors. On the other hand, studies employing psychosocial challenge paradigms have, rather consistently, reported blunted cortisol responses in schizophrenia patients [21–24]. All these findings indicate that schizophrenia would be associated with alteration in HPA axis function, with some suggesting hyperactive HPA axis function while others the opposite, i.e. hypoactive one.

In patients with SPD or nonclinical schizotypes, two studies have been conducted to examine the cortisol reactivity to pharmacological challenge paradigms; Schweitzer et al. [25] found schizotypal traits to be associated with enhanced suppression to DST, and Mitropoulou et al. [26] observed blunted cortisol responses to an acute metabolic stressor (i.e. 2-deoxyglucose) in patients with SPD. Findings from these studies were consistent with each other in that they have demonstrated blunted cortisol reactivity in schizotypal subjects. Paucity of research, however, has precluded any definitive conclusions to be drawn concerning the relation between schizotypy and cortisol activity/reactivity. Given that findings on cortisol levels of schizophrenia patients are not unequivocal, nonclinical schizotypes, who are free of medication, hospitalization, and psychosocial consequences of psychiatric diagnoses [27], would be a suitable target for investigating the HPA axis function in schizophrenia spectrum disorders. Indeed, a number of studies have suggested that cortisol levels are considerably affected by antipsychotic drugs [28–30].

Apart from the viewpoint of dimensional model of schizophrenia, it has been suggested that personality itself would confer sensitivity to stressors [31, 32]. Several lines of research in the nonclinical population have demonstrated that a variety of personality traits are related to altered HPA axis function. While there exist several (challenge) paradigms to assess HPA axis function, an increasing number of studies have used the DEX/corticotropin-releasing hormone (CRH) test [33, 34], a sensitive test for HPA axis function that combines DEX pretreatment with CRH challenge on the following day, to examine the association of personality traits with HPA axis reactivity. For example, using a questionnaire that measures Cloninger's temperamental dimension, Tyrka et al. [35] have shown that low novelty seeking, particularly when combined with high harm avoidance [36], is associated with exaggerated cortisol responses to the DEX/CRH test. Using other personality measures, McCleery and Goodwin [37] observed a relationship between lower neuroticism and greater cortisol responses to this pharmacological challenge test, whereas Zobel et al. [38] found the opposite relation, i.e. higher neuroticism and greater cortisol response. Rinne et al. [39] observed exaggerated cortisol responses to the DEX/CRH test in female subjects with borderline personality disorder who had a history of sustained childhood abuse. It would thus be intriguing to examine the association between schizotypy and cortisol reactivity by using the DEX/CRH test.

In this context, we aimed to explore the possible relationship between schizotypal traits at a nonclinical level and cortisol responses to the DEX/CRH test. To disentangle potential confounding effects of other personality dimensions, we also examined the association of temper-

ament/character with schizotypal personality and with cortisol responses. We predicted that nonclinical schizotypy on its own would be related to either of the two extremes of cortisol responses (i.e. exaggerated or blunted) to the DEX/CRH test.

Methods

Participants

One hundred and forty-one volunteers (35 males and 106 females; age range: 20–70 years) participated in this study, which was conducted in the western part of Tokyo between 2006 and 2010. They were recruited from the community through advertisements in free local magazines and our website announcement. At the first visit, participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview [40, 41] by a research psychiatrist, and only those who demonstrated no current axis I psychiatric disorders were enrolled in this study. In addition, those who demonstrated one or more of the following conditions during a non-structured interview performed by an experienced psychiatrist were excluded from this study: past or current contact to psychiatric services, taking psychotropic drugs, having a history of regular use of psychotropics or substance abuse/dependence, and other obvious self-reported signs of past primary psychotic and mood disorders as well as posttraumatic stress disorder. Additional exclusion criteria were: having a prior medical history of central nervous system disease or severe head injury, having major systemic medical illnesses, or taking corticosteroids, antihypertensive medications, oral contraceptives or estrogen replacement therapies. The present experiments on our subjects were conducted in accordance with the Declaration of Helsinki. After the nature of the study procedures had been fully explained, written informed consent was obtained from all subjects. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

DEX/CRH Test Procedure and Presentation for Neuroendocrine Data

The DEX/CRH test was administered to all subjects according to a protocol proposed in our previous report [42], and this protocol was identical to that employed in our recent report [43]. First, they took 1.5 mg of DEX (Banyu Pharmaceutical Corporation, Tokyo, Japan) orally at 23:00 h. On the next day, they attended our laboratory and sat on a comfortable couch in a calm room. A vein was cannulated at 14:30 h to collect blood at 15:00 h and 16:00 h via an intravenous catheter. Human CRH (100 μg; hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 15:00 h, immediately after the first blood collection. Subjects fasted and rested semi-supine throughout the testing. Blood samples were immediately centrifuged and stored at –20°C. Plasma concentrations of cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limit for cortisol was 1.0 μg/dl. Cortisol values under the detection limit were treated as 0 μg/dl. The intra-assay coefficients of variation at 2.37, 13.02, and 36.73 μg/dl were 6.90, 4.94, and 5.78%, respectively. The interassay coefficients of variation at 2.55, 13.04, and 34.17 μg/dl were 8.91, 6.03, and 6.44%, respectively (SRL Corporation). Outcome measures of this neuroendocrine test were the DST-cortisol (i.e. the concentration of cortisol [μg/dl] at 15:00 h) and DEX/CRH-cortisol (i.e. the concentration of cortisol at 16:00 h). To dissect the extent to which the subject's HPA axis responded to the CRH challenge, the magnitude of change from DST-cortisol to DEX/CRH-cortisol, namely Δcortisol, was calculated for each subject.

As our hypothesis was that the two extreme ends of cortisol values (i.e. both exaggerated and blunted cortisol reactivity) would be related to greater schizotypal traits, in the main analysis we adopted the categorical division of participants based on two cutoff values of cortisol (i.e. 1 and 5 μg/dl), by referring to several previous studies [42, 44, 45]. The cutoff criterion employed here was identical to that used in our recent report [43]. Briefly, incomplete suppressors were defined to be individuals where either or both of DST- and DEX/CRH-cortisols were ≥5 μg/dl. Enhanced suppressors were defined as those individuals whose DST-cortisol was <5 μg/dl and DEX/CRH-cortisol was <1 μg/dl, because this DEX/CRH-cortisol value corresponded to its detection limit and can therefore be regarded as an extremely low cortisol level. The remaining individuals were considered to be moderate suppressors.

Schizotypal Personality Questionnaire

The Schizotypal Personality Questionnaire (SPQ) [46] is a 74-item validated self-report questionnaire with a 'yes/no' response format that incorporates DSM-III-R [47] criteria for a diagnosis of SPD. The questionnaire consists of nine subscales, which have been found to load onto three factors: cognitive-perceptual, interpersonal, and disorganized factors [48]. In the present study the Japanese version of the SPQ translated by Fujiwara [49] was used. This questionnaire had been administered to 258 Japanese college students in a validation study [50], and the reliability and validity of this Japanese version of SPQ were demonstrated to be similar to those of the original version of Raine [46].

Temperament and Character Inventory

Temperament and Character Inventory (TCI) [51] is a 240-item (including 14 items which are not analyzed) self-report questionnaire; each item requires a true/false answer. Four dimensions of temperament (i.e. novelty seeking, harm avoidance, reward dependence, and persistence) and three dimensions of character (i.e. self-directedness, cooperativeness, and self-transcendence) are distinguished. The Japanese version of the TCI translated and validated by Kijima et al. [52, 53] was used in the present study.

Statistical Analysis

Averages are reported as means ± SD (standard deviation). The analysis of variance was used to examine differences between three groups. Pearson's r or Spearman's ρ was used to examine correlations. Partial correlation analysis, controlling for confounding variables, was used to examine correlations among psychological measures. The analysis of covariance (ANCOVA), controlling for confounders, was performed to compare scores of the two questionnaires between the three participant groups. Since age and sex have been shown to significantly influence cortisol levels [42, 54, 55] and schizotypal personality [56], these two vari-

Table 1. Demographics, schizotypal personality, and temperament/character dimensions for the three groups based on the suppression pattern

	Incomplete suppressors (n = 69)[a]	Moderate suppressors (n = 61)[b]	Enhanced suppressors (n = 11)[c]	Statistics	p
Age, years	49.9 ± 12.7	44.4 ± 14.9	41.9 ± 14.9	F(2, 138) = 3.29	0.04
Sex, male/female	10/59	20/41	5/6	$\chi^2(2) = 8.53$	0.014
Temperament and character inventory[d]					
Novelty seeking	20.4 ± 4.2	20.5 ± 4.3	21.9 ± 4.0	F(2, 136) = 0.52	0.59
Harm avoidance	18.3 ± 5.8	19.1 ± 6.0	20.6 ± 7.5	F(2, 136) = 0.51	0.60
Reward dependence	15.0 ± 3.4	15.3 ± 3.6	16.4 ± 2.7	F(2, 136) = 2.25	0.11
Persistence	4.4 ± 1.8	4.1 ± 1.8	4.0 ± 1.4	F(2, 136) = 1.89	0.15
Self-directedness	29.2 ± 6.3	28.4 ± 6.4	27.1 ± 9.5	F(2, 136) = 0.15	0.86
Cooperativeness	29.2 ± 5.6	29.1 ± 4.9	27.8 ± 5.9	F(2, 136) = 0.21	0.81
Self-transcendence	11.3 ± 6.0	9.9 ± 6.0	12.7 ± 8.1	F(2, 136) = 1.40	0.25
Schizotypal personality questionnaire[d]					
Total score	13.7 ± 11.0	12.4 ± 9.3	19.5 ± 17.4	F(2, 136) = 1.89	0.16
Cognitive-perceptual factor	4.6 ± 4.5	3.1 ± 3.3	7.5 ± 7.4	F(2, 136) = 5.48	0.005
Interpersonal factor	6.5 ± 5.8	7.0 ± 5.8	9.0 ± 8.8	F(2, 136) = 0.31	0.74
Disorganized factor	3.8 ± 3.7	3.3 ± 3.2	5.5 ± 4.7	F(2, 136) = 2.02	0.14

[a] Defined as DST-cortisol ≥5 or DEX/CRH-cortisol ≥5. [b] Defined as DST-cortisol <5 and 1 ≤ DEX/CRH-cortisol <5. [c] Defined as DST-cortisol <5 and DEX/CRH-cortisol <1. [d] Scores of the three suppressor groups were compared using the ANCOVA with age and sex as covariates.

ables were considered as potential confounders regardless of the present data. To confirm the results obtained by the ANCOVA, Kruskal-Wallis test was used to examine differences in SPQ scores between the three groups. Statistical significance was set at two-tailed $p < 0.05$ unless otherwise specified. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

Results

Demographic Characteristics and SPQ Scores of the Subjects

Scores on the total SPQ, cognitive-perceptual factor, interpersonal factor, and disorganized factor of the total 141 participants were 13.6 ± 11.0 (range: 0–57, median: 11), 4.2 ± 4.4 (range: 0–24, median: 3), 6.9 ± 6.0 (range: 0–25, median: 5), and 3.7 ± 3.6 (range: 0–16, median: 3), respectively. The numbers of incomplete, moderate, and enhanced suppressors were 69, 61, and 11, respectively. These three groups significantly differed in age and sex; suppression status tended to become incomplete with advancing age, and females demonstrated less suppression than males (table 1). Age was significantly negatively correlated with eccentric/odd behavior and appearance ($r = -0.23$, $p = 0.006$) and odd speech ($r = -0.18$, $p = 0.03$) subscales and their resulting disorganized factor ($r = -0.23$, $p = 0.006$). Males and females were significantly different in social anxiety ($t = 3.27$, $p = 0.001$), no close friends ($t = 2.46$, $p = 0.02$) and constricted affect ($t = 2.76$, $p = 0.01$) subscales and their resulting interpersonal factor ($t = 3.19$, $p = 0.002$); males scored significantly higher than females in all of these four indices.

Correlations between SPQ and TCI Scores

Table 2 shows the partial correlations, controlling for age and sex, between nine subscales of the SPQ and seven dimensions of the TCI. In general, those subscales which load onto the cognitive-perceptual factor of SPQ were significantly negatively correlated with self-directedness and significantly positively correlated with self-transcendence. Those subscales which load onto the interpersonal factor were significantly positively correlated with harm avoidance and significantly negatively correlated with reward dependence, self-directedness and cooperativeness. Similarly to the subscales belonging to the cognitive-perceptual factor, those subscales which belong to the disorganized factor were significantly negatively correlated with self-directedness and significantly positively correlated with self-transcendence.

Table 2. Partial correlations between schizotypal personality and temperament/character dimensions, controlling for age and sex (n = 141)

	Novelty seeking	Harm avoidance	Reward dependence	Persistence	Self-directedness	Cooperativeness	Self-transcendence
Ideas of reference (C-P)	0.03	0.12	0.03	0.07	−0.35***	0.01	0.31***
Social anxiety (I)	−0.14	0.50***	−0.004	0.01	−0.37***	−0.06	0.01
Odd beliefs/magical thinking (C-P)	−0.03	−0.09	0.10	0.26**	−0.10	0.14	0.50***
Unusual perceptual experiences (C-P)	−0.05	0.10	0.01	0.14	−0.22**	0.13	0.38***
Eccentric/odd behavior and appearance (D)	−0.06	0.05	−0.18*	0.10	−0.20*	−0.12	0.26**
No close friends (I)	−0.24**	0.31***	−0.44***	−0.06	−0.31***	−0.30***	−0.01
Odd speech (D)	−0.03	0.25**	−0.09	0.04	−0.52***	−0.08	0.19*
Constricted affect (I)	−0.09	0.34***	−0.21*	−0.01	−0.51***	−0.15	0.06
Suspiciousness/paranoid ideation (C-P, I)	−0.13	0.18*	−0.27**	0.03	−0.39***	−0.26**	0.17*

Each figure represents partial correlation coefficient (d.f. = 137). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. C-P = Cognitive-perceptual factor; I = interpersonal factor; D = disorganized factor.

Table 3. Plasma cortisol concentrations [mean ± SD (range)] for the three groups based on the suppression pattern

	Incomplete suppressors (n = 69)[a]	Moderate suppressors (n = 61)[b]	Enhanced suppressors (n = 11)[c]
DST-cortisol[d]	1.3 ± 1.4 (0–5.8)	0.3 ± 0.6 (0–1.9)	0.1 ± 0.3 (0–1.1)
DEX/CRH-cortisol[e]	10.3 ± 4.8 (5.0–25.1)	2.5 ± 1.1 (1.1–4.9)	0 ± 0
Δcortisol[f]	9.0 ± 4.6 (2.3–20.2)	2.2 ± 1.1 (−0.3 to 4.8)	−0.1 ± 0.3 (−1.1 to 0)

[a] Defined as DST-cortisol ≥5 or DEX/CRH-cortisol ≥5. [b] Defined as DST-cortisol <5 and 1 ≤ DEX/CRH-cortisol <5. [c] Defined as DST-cortisol <5 and DEX/CRH-cortisol <1. [d] The concentration of cortisol (μg/dl) at 15:00 h (i.e. immediately before the CRH challenge). [e] The concentration of cortisol (μg/dl) at 16:00 h (i.e. 1 h after the CRH challenge). [f] Defined as DEX/CRH-cortisol minus DST-cortisol.

Relationships between TCI Scores and DEX/CRH Outcomes

Results of the three cortisol indices for the three suppressor groups are provided in table 3.

No significant correlations were seen between the seven dimensions of TCI and the three cortisol indices (all $p > 0.05$ by Spearman's ρ). As shown in table 1, the ANCOVA controlling for age and sex showed no significant main effect of the suppressor group on any of the seven dimensions of TCI. These results indicated that personality dimensions as assessed with the TCI did not significantly affect the cortisol responses to the DEX/CRH test. Therefore, we decided not to control for this personality dimension when examining the association between schizotypal personality and cortisol responses.

Relationships between SPQ Scores and DEX/CRH Outcomes

No significant correlations were seen between the SPQ scores (including nine subscales, three factors and the total score) and the three cortisol indices (all $p > 0.05$ by Spearman's ρ), indicating that there was no simple linear correlational relationship between SPQ scores and cortisol levels.

Figure 1 illustrates the relationships between nine subscales of the SPQ and DEX/CRH suppression status. The ANCOVA on the nine subscales, controlling for age and sex, showed a significant main effect of the suppressor group on ideas of reference [$F(2, 136) = 5.92$, $p = 0.003$] and suspiciousness/paranoid ideation [$F(2, 136) = 4.50$, $p = 0.013$] subscales. Post-hoc analyses with Bonferroni correction revealed that the enhanced suppressors scored

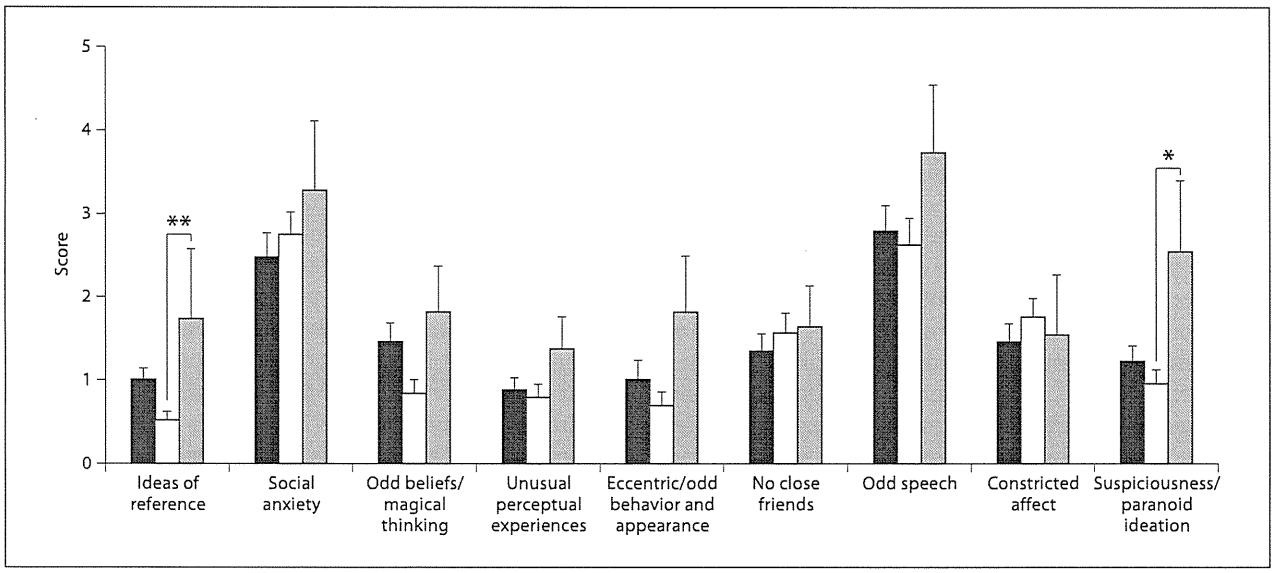

Fig. 1. Comparisons of scores on the nine subscales of the SPQ between the three suppressor groups. Black, white, and gray bars are incomplete suppressors (defined as DST-cortisol ≥5 or DEX/CRH-cortisol ≥5; n = 69), moderate suppressors (defined as DST-cortisol <5 and 1 ≤ DEX/CRH-cortisol <5; n = 61), and enhanced suppressors (defined as DST-cortisol <5 and DEX/CRH-cortisol <1; n = 11), respectively. * $p < 0.05$; ** $p < 0.01$. Error bars represent standard errors of the mean.

significantly higher than the moderate suppressors on these two subscales (fig. 1). Relationships of the SPQ total score and its three factors with DEX/CRH suppression status are provided in table 1. The ANCOVA on the SPQ total score and the three factors, controlling for age and sex, showed a significant main effect of the suppressor group on the cognitive-perceptual factor. Post-hoc analyses with Bonferroni correction revealed that the enhanced suppressors scored significantly higher than the moderate suppressors on this factor. To confirm the results obtained by the ANCOVA, we further performed Kruskal-Wallis test between the three suppressor groups. Similarly to the ANCOVA results, the Kruskal-Wallis test revealed that the three groups differed significantly on ideas of reference ($p = 0.012$) and odd beliefs/magical thinking ($p = 0.049$) subscales and the cognitive-perceptual factor ($p = 0.033$).

Discussion

We examined the relationship between schizotypal personality as assessed with the SPQ and cortisol reactivity to the DEX/CRH test in healthy adults. To our knowledge, this is the first report where HPA axis function in relation to schizotypal personality was investigated by using the DEX/CRH test. The main finding was the significant association between greater schizotypal traits, in particular the cognitive-perceptual factor, and the blunted cortisol response to CRH administration.

Although only two of nine subscales of SPQ were significantly different between the three suppressor groups (i.e. higher scores in the enhanced suppressor group), it is visually clear from figure 1 that most of the other subscales of SPQ were also higher in the enhanced suppressor group than in the other two groups. Consistent with this finding, previous studies have found schizotypal traits or SPD to be associated with enhanced suppression to DST [25] and with blunted cortisol responses to an acute metabolic stressor [26]. Studies on basal cortisol levels, by contrast, have indicated HPA axis hyperactivity in individuals with similar characteristics, such as elevated baseline cortisol levels in individuals with SPD [57] and a positive correlation between cortisol levels and schizotypal symptoms in adolescents at risk for developing schizophrenia [58]. Taken together, baseline studies and challenge studies may reveal different aspects of HPA axis alteration in relation to schizotypal traits, with the former representing the amount of biosynthesis/release of glucocorticoids while the latter reflecting sensitivity to

negative feedback (due to altered glucocorticoid receptor function) and/or downregulation of target receptors for HPA axis secretagogues. It might be of importance that, among the three factors of SPQ, the cognitive-perceptual factor, which is considered as an attenuated equivalent of positive symptoms of schizophrenia, was most strongly associated with the blunted cortisol response. Since this issue has been little studied, the direct comparison of the present findings with previous data cannot be made.

As for schizophrenia patients, findings on HPA axis alteration have not been uniform, and HPA axis dysfunction is less consistently reported in schizophrenia than in depression [59, 60]. In addition to the aforementioned controversies from DST studies, findings on basal HPA activity in schizophrenia patients have been mixed such that some investigators reported elevated baseline cortisol levels [61, 62], whereas others did not confirm such findings [22, 63, 64]. Findings on cortisol levels in first-episode psychosis patients are also inconsistent; Ryan et al. [65] reported elevated basal cortisol levels in drug-naïve first-episode patients with schizophrenia, while McGorry et al. [66], using a DST with 0.25 mg of DEX, showed that cortisol levels of drug-naïve or minimally treated patients with first episode psychosis were significantly lower than those of healthy controls. A study that administered the DEX/CRH test to schizophrenia patients found their cortisol responses to be exaggerated compared with those of healthy controls, albeit to a lesser extent compared to what has been reported in melancholic depression [28]. In contrast, Thompson et al. [67] showed that, among young people at ultra-high risk for developing psychosis, cortisol response to the DEX/CRH test of those who made the transition were lower than that of their counterparts who did not make the transition. Overall, it may be that cortisol responses of individuals with schizophrenia and/or those with high schizotypal traits depend on types of the cortisol measurements (e.g. basal cortisol vs. provoked cortisol) or sample characteristics (e.g. at risk/recent-onset vs. chronic), and our results together with previous data point to the importance of taking account of hypocortisolism as well as hypercortisolism in investigating HPA axis function in relation to schizophrenia-spectrum pathology. Still, the present finding could be considered as supporting the dimensional model of schizophrenia, in that schizotypal traits, like schizophrenia, are associated with alterations in HPA axis function, be they hyperactive or hypoactive.

A plausible explanation for the relationship between schizotypy and blunted cortisol reactivity observed here would be that greater schizotypal traits, its cognitive-perceptual factor in particular, are likely to lead to chronic stress and thus result in the hypoactive HPA axis function, given the well-established association between chronic stress and blunted HPA axis responsiveness [68, 69]. The alternative possibility of this causation (i.e. hypoactive HPA axis causes schizotypal personality) is unlikely partly because this personality trait is demonstrated to have genetic and neurodevelopmental bases [1]. Indeed, preclinical studies have found blunted cortisol reactivity in primates [70] and rodents [71] exposed to chronic social stress. Furthermore, recent clinical studies have shown that blunted cortisol responses to the combined DEX/CRH challenge are associated with stressful conditions that persist [43, 72–74]. As it is now clear that the ability to mobilize cortisol adequately in response to stressors is crucial for coping with stressful conditions [75], the blunted cortisol response observed in the present study may somehow be related to the increased sensitivity to stress in schizophrenia spectrum disorders. In the present study, the significant association between the enhanced suppression to the DEX/CRH test and greater schizotypal traits, coupled with the absence of significant correlations between DST-cortisol and SPQ scores, suggests that hyporeactive HPA axis in relation to nonclinical schizotypy might be accounted for, at least in part, by the downregulation of CRH receptors on the level of the pituitary (thereby leading to the failure to mount an adequate cortisol response to the CRH administration). It should be noted here that blunted cortisol responses to the DEX/CRH test have also been shown in nonclinical adults who have a history of childhood trauma or maltreatment [73, 76, 77]. Therefore, although we have excluded individuals with posttraumatic stress disorder from our subjects, the possibility cannot be ruled out that the association of schizotypal traits with blunted cortisol reactivity observed here might be confounded by such a history of early life adversity.

The present study may also be of relevance to previous studies that have linked nonclinical personality traits to altered HPA axis function. The impacts of neuroticism [37, 38] and novelty seeking [35, 36] on HPA axis function have been well documented, although findings on the association between neuroticism and cortisol responses to the DEX/CRH test are mixed, as described earlier [37, 38]. Interestingly, O'Leary et al. [78] have recently reported that nonclinical students characterized by high psychopathic personality traits lacked psychosocial stress-induced cortisol increases. Thus, the present finding of an association between schizotypal traits and the blunted cortisol response lends support to the evidence that per-

sonality, irrespective of whether or not it is related to schizophrenia-spectrum pathology, can influence HPA axis function. These psychopathic and schizotypal personality traits, like other personality traits, might explain some part of the HPA axis hypoactivity that has been observed in various chronic stress conditions [79, 80]; however, the issue as to what personality trait is related to hypercortisolism (or hypocortisolism) requires further investigations.

Concerning the association of schizotypal personality with temperament and character dimensions in the nonclinical population, two previous studies investigated this association by simultaneously using SPQ and TCI [81, 82]. Findings from these studies have been fairly consistent: cognitive-perceptual and disorganized factors of SPQ correlated negatively with self-directedness and positively with self-transcendence, and interpersonal factor correlated positively with harm avoidance and negatively with self-directedness [81, 82]. All of these significant correlations have been replicated in the present study (see table 2). We further examined the association between the TCI and DEX/CRH outcomes, and found no significant relationships between these outcomes, which was not in line with the finding of Tyrka et al. [35, 36]. This inconsistency might have resulted from different sample characteristics between the present and the previous two studies (e.g. Japanese vs. American or mean age of mid-40s vs. mid-20s), given that age and culture are shown to influence TCI scores [83, 84]. Nevertheless, the absence of significant association between TCI and DEX/CRH outcomes indicates that the relationship between schizotypy and blunted cortisol response observed here was not significantly confounded by temperament/character dimensions.

Several limitations to this study should be acknowledged. First, since the DEX/CRH test used here was based on a simple test protocol (i.e. measuring hormones only twice and omitting the ACTH measures), it may have provided less information on HPA axis function (e.g. inability to calculate the AUC). Moreover, we did not measure baseline cortisol levels, i.e. the cortisol level before the DEX administration, which hindered us from knowing the extent to which each participant suppressed his/her cortisol in response to the 1.5 mg of DEX. Second, this cross-sectional study cannot address the natural history of the alteration in HPA axis function. Third, we did not collect data on menstrual cycle in the female participants, which may have affected HPA axis function. Finally, as the participants were predominantly women, it is possible that the current findings are applicable only to women.

In summary, the present study found that greater schizotypal traits in healthy adults were significantly associated with the blunted cortisol response to the DEX/CRH test, indicating that schizotypy itself could affect HPA axis function. This finding, coupled with findings from previous studies in nonclinical schizotypes as well as schizophrenia patients, supports the possibility that schizophrenia-spectrum pathology would be related to altered HPA axis function.

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Expression of Ca^{2+}-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients

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ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and the release of neurotrophins including brain-derived neurotrophic factor (BDNF) which have been implicated in psychiatric disorders. Furthermore, the expression of CADPS2deltaExon3, a defective splice variant of CADPS2, has been reported to be associated with autism. Based on these observations, we examined whether expression levels of CADPS2 and CADPS2deltaExon3 are altered in psychiatric disorders. Quantitative polymerase chain reaction analysis was performed for postmortem frontal cortex tissues (BA6) from 15 individuals with schizophrenia, 15 with bipolar disorder, 15 with major depression, and 15 controls (Stanley neuropathology consortium). The mean CADPS2 expression levels normalized to human glyceraldehyde-3phosphate dehydrogenase (GAPDH) or TATA-box binding protein levels was found to be significantly increased in the brains of the schizophrenia group, compared to the control group. On the other hand, the ratio of CADPS2deltaExon3 to total CADPS2 was similar in the 4 diagnostic groups. We then analyzed CADPS2 expression in blood samples from 121 patients with schizophrenia and 318 healthy controls; however, there was no significant difference between the two groups. Chronic risperidone treatment did not alter the expression of CADPS2 in frontal cortex of mice. The observed increase in the expression of CADPS2 may be related to the impaired synaptic function in schizophrenia.

1. Introduction

Ca^{2+}-dependent activator protein for secretion (CADPS) family, which consists of two members, CADPS1 and CADPS2, is a secretory granule-associated proteins involved in Ca^{2+}-dependent exocytosis of large dense-core vesicles containing diverse array of modulators including neurotrophines, monoamines and neuropeptides (Liu et al., 2008; Sadakata et al., 2004). CADPS2 mediates the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3. Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). BDNF release is significantly reduced in cultured neurons prepared from the brain of CADPS2 deficient mice (Sadakata et al., 2007a,b).

A number of findings suggest that BDNF action is impaired in psychiatric disorders including schizophrenia, bipolar disorder and depression. Several studies have shown decreased levels of BDNF or its receptor, TrkB, in the postmortem brains of patients with schizophrenia (Hashimoto et al., 2005; Iritani et al., 2003; Weickert et al., 2003), although there are contradictive reports (Chen et al., 2001; Dunham et al., 2009; Durany et al., 2001; Takahashi et al., 2000). The contribution of BDNF in depression has been suggested from animal studies that demonstrated stressful environments decrease, and antidepressive treatments increase BDNF levels in the brain (Duman and Monteggia, 2006; Martinowich et al., 2007). Also, centrally administered BDNF has an antidepressant-like effect in rat models (Siuciak et al., 1997). Thus, the molecules that contribute to the trafficking and release of BDNF may be a culprit of these disorders.

CADPS family also mediate monoamine transmission. Both CADPS1 and CADPS2 mediate the refilling of catecholamine to the releasable vesicles, and catecholamine secretion is significantly suppressed in the CADPS1/2 double deficient cells. (Liu et al., 2008). Another study supports that CADPS family are involved in monoamine storage as antibodies against CADPS1 or 2 inhibit monoamine

Abbreviations: ANCOVA, Analysis of covariance; BDNF, Brain-derived neurotrophic factor; CADPS2, Ca^{2+}-dependent activator protein for secretion 2; CCK, Cholecystokinin; DSM–IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; FST, Freezer storage time; M.I.N.I., Mini-International Neuropsychiatric Interview; NT, Neurotensin; PCR, Polymerase chain reaction; PMI, Postmortem interval; SD, Standard deviation; TBP, TATA-box binding protein.

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sequestration by synaptic vesicles from mice brain (Brunk et al., 2009).

Dysregulation of monoamine neurotransmission has been hypothesized to play a central role in the etiology of psychiatric disorders including schizophrenia and mood disorders. In schizophrenia, not only classical evidence that dopamine agonists induce and dopamine D2 receptor antagonists ameliorate psychoses but also brain imaging studies on drug naïve patients have suggested that dopamine transmission is affected in this disorder (Lyon et al., 2011). In major depression, reduced monoamine transmission hypothesis was derived from the finding that most anti-depressants increase monoamine levels in the synaptic cleft and that reserpine, a monoamine-depleting drug, worsen depressive symptoms in a subset of patients with mood disorder (Krishnan and Nestler, 2008), although imaging, postmortem, or cerebrospinal fluid studies have yet to find the definitive evidence for altered monoamine neurotransmission in this disorder (Belmaker and Agam, 2008; Nikolaus et al., 2009).

While, to our knowledge, CADPS2 expression in schizophrenia or mood disorders have not yet been examined, aberrant splicing of CADPS2 mRNA was reported in autism (Sadakata et al., 2007b). In this study, an exon-3 skipped isoform, CADPS2ΔExon3, was detected in the bloods of several autistic patients but not in those of healthy controls. They also showed that CADPS2ΔExon3 was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. Though the CADPS2ΔExon3 expression in the brains of patients with autism is unclear, the aberrant splicing of CADPS2 could contribute to susceptibility to autism by affecting neurotrophin release.

Based on above findings, the present study was aimed to examine whether the expression of CADPS2 transcripts is altered in the frontal cortex of patients with psychiatric disorders including schizophrenia, major depression and bipolar disorder. The CADPS2 expression levels in the blood of schizophrenia were also examined.

2. Materials and methods

2.1. Brain samples

Frozen postmortem samples of frontal cortex (BA6) were obtained from the Stanley Foundation Neuropathology Consortium (Torrey et al., 2000). The collection consists of 60 subjects: 15 with schizophrenia, 15 bipolar disorder, 15 major depression and 15 unaffected controls. All groups were matched for age, sex, race, pH and hemispheric side (Table 1), although postmortem interval (PMI) and freezer storage time differed across the groups. The brain tissues obtained were coded. Once our blind study was complete, we sent the data to the Stanley Foundation who then returned the codes, demographic and clinical data. In a cold-room, each frozen brain tissue was broken into powder in the plastic bag using dry-ice block and dry-ice-cold hammer. The powder was then transferred and kept in dry-ice-cold tubes. Temperature of the tubes and instruments that directly contacted to the samples was frequently measured by infrared-thermometer (AD-5613A, A&D Company, Japan) and kept under −20 °C. Then, 30 to 40 mg of brain powder was used for cDNA synthesis. RNA was extracted using RNAqueous (Applied biosystems, Foster City, CA) according to manufacturer's instructions with a slight modification, i.e., after homogenization, samples were washed twice with 500 μl of chloroform, and then applied to the spin-column. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Then, the obtained RNA (14 μl) was used for cDNA synthesis using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

Table 1
Demographic information on brain specimens of Stanley Neuropathology Consortium.

	Control	Schizophrenia	Bipolar disorder	Major depression
Age (years)	48.1 (29–68)	44.2 (25–62)	42.3 (25–61)	46.4 (30–65)
Gender (M/F)	9/6	9/6	9/6	9/6
Race	14 C, 1 AA	13 C, 2 A	14 C, 1 AA	15 C
PMI (hours)	23.7 (8–42)	33.7 (12–61)	32.5 (13–62)	27.5 (7–47)
pH	6.3 (5.8–6.6)	6.1 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.6–6.5)
Side of brain frozen (R/L)	7/8	6/9	8/7	6/9
Freezer storage time (months)	11.3 (1–26)	20.7 (2–31)	20.7 (7–28)	14.5 (3–31)

AA, African American; A, Asian; C, Caucasian; F, female; M, male; and PMI, postmortem interval.

2.2. Blood samples

Subjects were 121 patients with schizophrenia (84 males and 37 females; age 44.1 ± 13.7 (mean ± SD) years) and 318 controls (90 males and 228 females; age 43.1 ± 15.3 years). All subjects were biologically unrelated Japanese and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM–IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from the community, through advertisements in free local magazines and our website announcement. Control individuals were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) and those who had a current or past history of psychiatric treatment were not enrolled in the study. After the nature of the study procedures had been fully explained, written informed consent was obtained from all subjects. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

Blood collection and RNA isolation were performed using the PAXgene blood RNA system (Qiagen, Valencia, CA). Blood samples were collected around 11 A.M. Extracted RNA was quantified as described above. Samples that contained more than 40 ng/μl of total RNA were used for analysis; 8 μl from each sample was reverse transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

2.3. Chronic risperidone treatment to mice

C57BL/6J male mice aged 10 weeks were purchased from Crea Japan. Chronic oral risperidone treatment was performed according to Belforte et al., (Belforte et al., 2010). In brief, 2.5 mg/kg/day of risperidone (Rispadal liquid, Janssen Pharmaceutical, Tokyo, Japan) in drinking water freshly made every 72 h had been administered continuously for 3 weeks. Control mice received solvent (1.4 mM tartaric acid neutralized to pH 6–7). All experimental procedures were in accordance with the guidelines of the United State's National Institutes of Health (1996) and were approved by the Animal Care Committee of the National Institute of Neuroscience, NCNP.

2.4. Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed in triplicate (5 μl volume) on 384-well plates using ABI prism 7900HT (Applied Biosystems, Foster City, CA). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. human CADPS2 (Hs01095968_m1 at Exon 4–5 on NM_017954.9), mouse CADPS2 (Mm00462577_m1), human CADPS2ΔExon3 (Forward primer: GTAGCTGACGAAGCATTTTGCA,