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Cortisol/Dehydroepiandrosterone Ratio and Responses to Antipsychotic Treatment in Schizophrenia

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Dehydroepiandrosterone (DHEA) or their sulfate conjugate (DHEAS) (together abbreviated DHEA(S)) exert multiple effects in the central nervous system, and may be involved in the pathophysiological processes in schizophrenia. This prospective study aimed to investigate whether serum cortisol/DHEA(S) molar ratios are associated with response to antipsychotic treatment during the exacerbation of schizophrenia. Serum DHEA(S) and cortisol were determined at baseline, and 2 and 4 weeks later for 43 medicated schizophrenia inpatients with acute exacerbation. The patients were treated with stable doses of antipsychotic agents up to 2 weeks prior to entering the study and for the 4-week duration of the study after which they were classified as either responders or nonresponders to treatment. Findings suggest that responders had significantly higher serum cortisol levels and cortisol/DHEA(S) ratios compared with nonresponders. These differences remained significant at three time points controlling for gender, age, severity of symptoms and emotional distress, benzodiazepines, type or dosage of antipsychotic agents, and background variables. The logistic regression model shows advantages of both cortisol/DHEA(S) molar ratios *vs* serum cortisol and DHEA(S) concentrations for prediction of responsivity to antipsychotic treatment. No significant canonical correlations were observed between changes from baseline through end-of-study in hormonal values and severity of symptoms and emotional distress among responders and nonresponders. Thus, these data provide evidence that elevated serum cortisol and cortisol/DHEA(S) ratios may serve as markers of biological mechanisms that are involved in responsivity of schizophrenia patients to antipsychotic treatment.
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INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate ester, DHEAS (together abbreviated DHEA(S)), are the most abundant adrenal androgens. They serve as a precursor to the sex hormones estradiol and testosterone and their serum levels decrease with age in healthy individuals. DHEA(S) also function as neurosteroids (Morley *et al*, 1997; Nafziger *et al*, 1998; Binello and Gordon, 2003).

Neurosteroids exert multiple effects in the central nervous system mediated through its nongenomic actions on several neurotransmitter systems, such as gamma-aminobutyric acid ($GABA_A$), *N*-methyl-D-aspartate (NMDA), and sigma receptors (Majewska, 1992; Debonnel *et al*, 1996; Wen *et al*, 2001), modulating neuronal excitability and plasticity, as well as presenting neuroprotective properties (Kimonides *et al*, 1998; Cardounel *et al*, 1999; Wolf and Kirschbaum, 1999; Lhullier *et al*, 2004).

In humans, 99% of circulating DHEA is in the sulfate form, serum DHEAS concentrations are 100 or more times higher than DHEA and approximately 5–10 times higher than serum cortisol plasma concentrations (Guazzo *et al*, 1996; Leowattana, 2004). Previous studies investigating DHEA blood levels in concentrations in psychosis or schizophrenia have demonstrated low DHEA levels (Tourney and Hatfield, 1972; Oertel *et al*, 1974; Harris *et al*, 2001) observed by some particularly in the morning (Tourney and Erb, 1979), abnormal DHEA diurnal rhythms (Erb *et al*, 1981), and no differences in DHEA levels (Brophy *et al*, 1983; Ritsner *et al*, 2004) compared to matched healthy controls. DHEAS levels have been reported to possibly be elevated (Oades and Schepker, 1994; Strous *et al*, 2004) and

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no different (Ritsner *et al*, 2004) in schizophrenia patients compared to healthy controls. The inconsistencies in published findings may be due to wide clinical polymorphism, small sample sizes, or differences in the age, and duration of illness of patients enrolled in the studies.

In a previous case–control study of 40 schizophrenia inpatients examined during stable phases of the illness (the Positive and Negative Syndrome Scale (PANSS; Kay *et al*, 1987) total score was 68.4, standard deviation (SD) = 19.8), and 15 healthy subjects, we found that serum cortisol/DHEA(S) ratios were significantly higher in schizophrenia patients than in healthy comparison subjects, and that these elevated ratios were independent of severity of psychopathology and antipsychotic treatment (Ritsner *et al*, 2004). The present prospective study was designed to evaluate whether serum cortisol/DHEA(S) molar ratios would be associated with response to antipsychotic treatment during the exacerbation of schizophrenia. Therefore, this study sought to compare repeated measures of serum DHEA, DHEAS, and cortisol concentrations, as well as the molar ratios of cortisol/DHEA(S) at baseline, and 2 and 4 weeks later, between schizophrenia patients admitted for exacerbation of psychosis who responded and those who did not respond to antipsychotic treatment.

METHODS

Data Collection

Schizophrenia patients with exacerbation (PANSS: P1-Delusions and P3-Hallucinatory Behavior $\geqslant 4$) were recruited from inpatient units at Sha'ar Menashe Mental Health Center, Israel. Patients who had received antidepressants and/or mood stabilizers during the 4 weeks prior to the investigation and patients who suffered from major physical illness, drug or alcohol abuse, epilepsy, and other organic brain syndromes were not included. All patients underwent physical examination and routine laboratory tests to rule out physical illness. Demographic, physical, psychiatric history, and clinical data were then collected. The patients enrolled in the study were assessed, and blood samples were collected at baseline, after 2, and 4 weeks (end-of-study). The Institutional Review Board of Sha'ar Menashe Mental Health Center approved the study. Prior to enrollment, all participants provided written informed consent for participation in the study after having received a detailed explanation of the study procedures.

Participants

A total of 43 medicated schizophrenia patients (40 men, three women) participated in this study. All patients had been admitted to psychiatric inpatient units due to exacerbation of paranoid schizophrenia. Once stabilized enough to provide informed consent for participation in the study, participants remained in hospital for the 4-week duration of the study. The mean baseline PANSS total score was 101.7 (SD = 12.0), positive scale, 16.8 (SD = 4.9), negative scale, 32.4 (SD = 5.9), activation factor, 18.4 (SD = 5.6), dysphoric mood, 13.9 (SD = 4.1), and autistic preoccupation, 23.2 (SD = 4.4). All subjects were free of illicit substances as documented by results of urine toxicologic screening.

Patients were treated with one or more antipsychotics within the same medication group, and were allowed to receive anticholinergic or anti-Parkinsonian medications. Of the patients treated with typical agents, nine received haloperidol ($M = 31.7$ mg/day, SD = 15.8), eight perphenazine ($M = 25.0$ mg/day, SD = 9.0), seven zuclopenthixol ($M = 33.3$ mg/day, SD = 27.2), six clothiapine ($M = 116.7$ mg/day, SD = 107.6), and the remaining seven received either levomepromazine ($N = 2$, $M = 212$, 5 mg/day, SD = 265.2), haloperidol decanoate ($N = 1$, 3 mg/day), zuclopenthixol decanoate ($N = 2$, both 13.3 mg/day), and flupentixol decanoate ($N = 2$, $M = 11.4$ mg/day, SD = 14.1) (total percentages exceed 100% because six patients received more than one antipsychotic medication). Of the patients treated with second-generation agents, eight received olanzapine ($M = 17.5$ mg/day, SD = 7.5), six risperidone ($M = 3.2$ mg/day, SD = 1.8), and five clozapine ($M = 320.0$ mg/day, SD = 144.0) (one patient received both risperidone and olanzapine).

Anti-Parkinson drugs were prescribed for 40% (8/20) of the responders and 43.4% (10/23) of the nonresponders (Fisher's Exact test, $p > 0.05$). Benzodiazepines were prescribed for 50% (10/20) of the responders and 56.5% (13/23) of the nonresponders ($\chi^2 = 0.18$, df = 2, $p = 0.91$). The patients were treated with stable doses of antipsychotics and benzodiazepines up to 2 weeks prior to entering the study and during the course of the study. The patients did not receive other (nonpsychiatric) medications.

Clinical Assessment

Patients were diagnosed with DSM-IV schizophrenia following interviews with senior psychiatrists (AG, YR, MR), using the Structured Clinical Interview for DSM-IV Axis I Disorders, Patients Edition (First *et al*, 1995). Participants were examined for physical and neurological disturbances including EEG.

Clinical evaluation of patients was performed using the Clinical Global Impression Scale (CGI; Guy 1976), and the Positive and Negative Symptom Scale (PANSS; Kay *et al*, 1987). The PANSS five-factor model was used for analysis of schizophrenia psychopathology: positive, negative, activation, dysphoric mood, and autistic preoccupation (White *et al*, 1997). Raters were trained and reached an acceptable level of reliability; interrater reliability for the primary diagnosis, CGI scale, and PANSS was high (κ: 0.95, 0.93, and 0.89, respectively).

The Talbieh Brief Distress Inventory (TBDI), a self-report questionnaire including 24 items, was used to measure the degree of emotional distress (Ritsner *et al*, 1995). Responses are scored on a five-point scale, with higher ratings indicating higher intensity of TBDI distress index. The TBDI was validated for use with psychiatric patients (Ritsner *et al*, 2002). For the present sample, the TBDI distress index demonstrated high reliability (Cronbach's $\alpha = 0.89$).

Steroid Determination

Patients were controlled for time of awakening, morning activity, caffeine consumption and smoking, factors that can

affect morning cortisol levels. All participants were instructed to avoid morning exercises. Serum samples of cortisol, DHEA, and DHEAS were collected between 0800 and 0900 hours after 20 min of rest. Subjects were instructed to abstain from unusual physical activity or stress for a period of 24 h prior to blood sampling. Cortisol was measured by the TKCO1 Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, CA, USA). DHEA was assessed with the DHEA-DSL 9000 Active™ DHEA coated tube radioimmunoassay (RIA) kit (Diagnostic System Laboratories, Webster, TX, USA). DHEA-S was tested with the DHEA-S-DSL-3500 Active™ DHEAS coated tube RIA kit (Diagnostic System Laboratory, Webster, TX. USA), as we previously described (Ritsner *et al*, 2004). Hormone levels in all samples were measured simultaneously to avoid interassay variability.

Data Analysis

Patients who met the following criteria at the end-of-study: (i) had no ratings of >3 (mild) on items P1, P2, P3, P5, and P6 of the PANSS; (ii) had a $\geqslant 30\%$ reduction from baseline in the PANSS total score, and (iii) had a CGI severity score $\leqslant 4$ (moderately ill) were classified as responders (Lieberman *et al*, 2003). Patients who did not reach these criteria were defined as nonresponders.

Values of serum cortisol, DHEA and DHEAS concentrations, molar cortisol/DHEA and cortisol/DHEAS (*100) ratios were analyzed. Results are presented as mean $\pm$ SD. Continuous variables between groups of subjects were compared with multivariate analysis of variance (MANOVA, Hotelling–Lawley Trace test), and by using two-tailed Wilcoxon Rank Sum test (z-value). The general linear model of ANOVA was applied to assess the main effect of responsivity to antipsychotic treatment (groups: responders *vs* nonresponders), by time (across three examinations), on serum DHEA, DHEAS, cortisol levels, and molar cortisol/DHEA(S) ratios controlling for age at examination. To test the roles of serum DHEA, DHEAS, cortisol levels, and molar cortisol/DHEA(S) ratios in predicting response to treatment in schizophrenia, hierarchical logistic regression analysis using gender and baseline hormonal data of responders and nonresponders was applied. Spearman-rank correlation coefficients were estimated between changes in serum values of DHEA(S), cortisol and two ratios and changes in PANSS dimensions, and emotional distress scores across three examinations controlling for age at examination. Finally, in order to avoid the effect of multiple statistical comparisons, we evaluated canonical correlations between two sets of variables: changes from baseline through end-of-study in (a) serum values of DHEA(S), cortisol, and molar ratios and in (b) five PANSS factors and emotional distress scores. Differences in proportions were examined with χ^2-test or Fisher's Exact Test. Statistical significance was tested at the $p<0.05$ level. NCSS-2000 PC program (Hintze 1998) was used for all analyses.

RESULTS

Table 1 shows that responders ($n=20$) and nonresponders ($n=23$) did not differ in terms of gender (men/women: 19/1 and 21/2, respectively; Fisher's Exact Test, $p>0.05$), marital status ($\chi^2=0.46$, df $=2$, $p=0.80$), education, age of onset of illness, and total number of hospitalizations. However, the responders were more likely to be younger in age, with a shorter duration of illness and hospitalization, than nonresponders. Nonresponders received higher daily doses of typical antipsychotic agents and lower daily doses of benzodiazepines compared to responders.

The two patient groups did not differ on PANSS total scores at baseline ($p=0.06$); however, responders scored significantly higher on positive, dysphoric mood and autistic preoccupation factors compared to nonresponders (MANOVA, Hotelling–Lawley Trace test, $F=3.7$, df $=6,35$, $p=0.005$, Figure 1). At the end-of-study, the responders significantly improved on all PANSS factors compared to the nonresponders (Hotelling–Lawley Trace test, $F=10.0$, df $=6,35$, $p<0.001$). Indeed, the responders showed greater reduction in the PANSS total score from baseline to end point than nonresponders (39.7 ± 5.9 *vs* $8.1\pm19.5\%$; odds ratio $=4.9$; $z=3.1$, $p<0.001$). Interaction ('group' $\times$ 'time') was found significant for all PANSS factors (Hotelling–Lawley Trace test, $F=2.1$, df $=10,226$, $p=0.024$).

At baseline, responders had significantly higher serum levels of cortisol ($p=0.007$), DHEAS ($p=0.045$), and cortisol/DHEA ratio value ($p<0.001$) compared with nonresponders (Figure 2). Across all three-assessment points (baseline, 2 weeks, and 4 weeks) the responders had a significantly higher serum cortisol ($p<0.001$), cortisol/DHEA ($p<0.001$), and cortisol/DHEAS ($p=0.019$) ratios compared with nonresponders, while DHEA(S) concentrations did not differ among these groups of subjects (Table 2A). Hormonal values and molar ratios did not change during the study period among responders and nonresponders ($F=1.13$, df $=10,218$, $p=0.33$; Table 2B). Likewise, no significant 'group' $\times$ 'time' interactions were found for all hormonal values and molar ratios ($F=0.74$, df $=10,218$, $p=0.68$; Table 2AB).

Table 3 presents a summary of the hierarchical logistic regression analysis for prediction of responsivity (being responder or nonresponder) to 4 weeks of antipsychotic treatment from basal hormonal measures. The first model ($R^2=0.07$) did not indicate significant predictors among DHEA(S) concentrations, while the second ($R^2=0.16$) and the third ($R^2=0.36$) models revealed cortisol ($p=0.018$) and cortisol/DHEA molar ratios ($p=0.004$), respectively, as significant predictors of responsivity to antipsychotic treatment. The fourth model ($R^2=0.23$) confirmed that the variance in treatment response is explained much better by serum cortisol levels ($p=0.019$) than by DHEA(S) concentrations ($p>0.05$). However, the best-fit fifth model shows clear advantages for both cortisol/DHEA ($p=0.007$), and cortisol/DHEAS ($p=0.036$) molar ratios *vs* serum cortisol concentrations ($p>0.05$) for prediction of responsivity to antipsychotic treatment. This model accounted for 42% of the variance and correctly classified responders and nonresponders; 87.8% of the 43 patients. Gender demonstrated no significant contribution to prediction of responsiveness to antipsychotic treatment in schizophrenia patients.

Table 4 shows the effect of additional variables regarding hormonal differences between responders and nonresponders at three time points. As can be seen, differences

Table 1 Sociodemographic, Background, and Clinical Characteristics of Schizophrenia Patients

	Responders ($N = 20$)		Nonresponders ($N = 23$)		Wilcoxon Rank test	
Variables	**Mean**	**SD**	**Mean**	**SD**	***z***	***p***
Age (years)	30.2	7.3	37.6	9.4	2.4	0.014
Education (years)	9.8	2.3	10.5	2.3	1.7	0.08
Age of onset	23.1	3.8	22.6	7.1	1.4	0.17
Number of admissions	8.8	7.4	6.2	4.8	1.1	0.28
Length of stay in hospital (months)	18.7	17.3	36.4	10.2	3.7	0.001
Duration of illness (years)	7.1	6.8	15.6	10.5	3.2	0.001
PANSS (total)						
Baseline	105.8	9.6	98.0	21.3	1.9	0.06
End-of-study	63.5	5.8	91.8	28.2	3.4	0.001
DDD of antipsychotic agents[a]						
Typical	1.5 (14)[b]	1.4	2.9 (11)	2.3	2.0	0.022
Atypical	0.8 (6)	0.7	1.2 (6)	0.6	1.5	0.13
Combined	—	—	2.7 (6)	1.4	—	—
Total	1.3 (20)	1.2	2.4 (23)	1.9	2.8	0.01
DDD of benzodiazepines[c]	0.7 (10)	0.6	0.5 (13)	0.4	2.1	0.033

[a]Dosages for antipsychotic agents and benzodiazepines were converted into defined daily dose (DDD), which is the average maintenance dosage as defined by the WHO Collaborating Center for Drug Statistics (WHO Collaborating Centre for Drug Statistics Methodology, 2000).
[b]In brackets, the number of patients is shown.
[c]Of the responders treated with benzodiazepines, three received diazepam ($M = 15.0$ mg/d, SD = 5.0), five clonazepam ($M = 2.6$ mg/day, SD = 1.9), two oxazepam ($M = 15.0$ mg/day, SD = 7.0); four nonresponders received diazepam ($M = 8.75$ mg/day, SD = 2.5), seven clonazepam ($M = 1.2$ mg/day, SD = 0.8), one oxazepam (0.2 mg/day), and one lorazepam (1.2 mg/day).

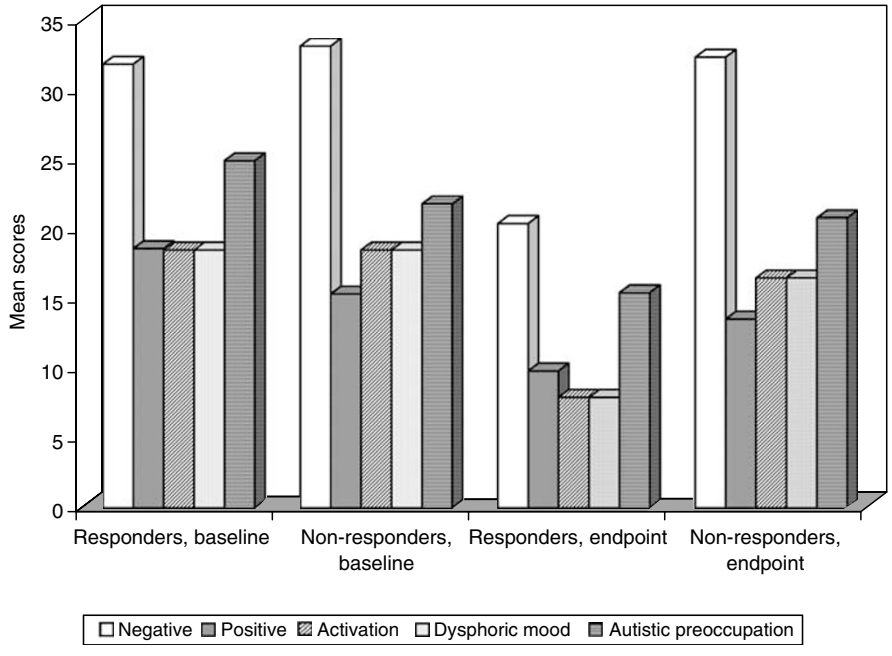

Figure 1 Symptoms severity (PANSS factor scores) at baseline and end point assessments among responders and nonresponders.

between responders and nonresponders in serum cortisol levels and cortisol/DHEA(S) ratios remained significant when background variables, clinical symptoms, and emotional distress were controlled; while between-group differences in cortisol/DHEAS ratios were, at least partly, associated with age of onset. Again, between-group

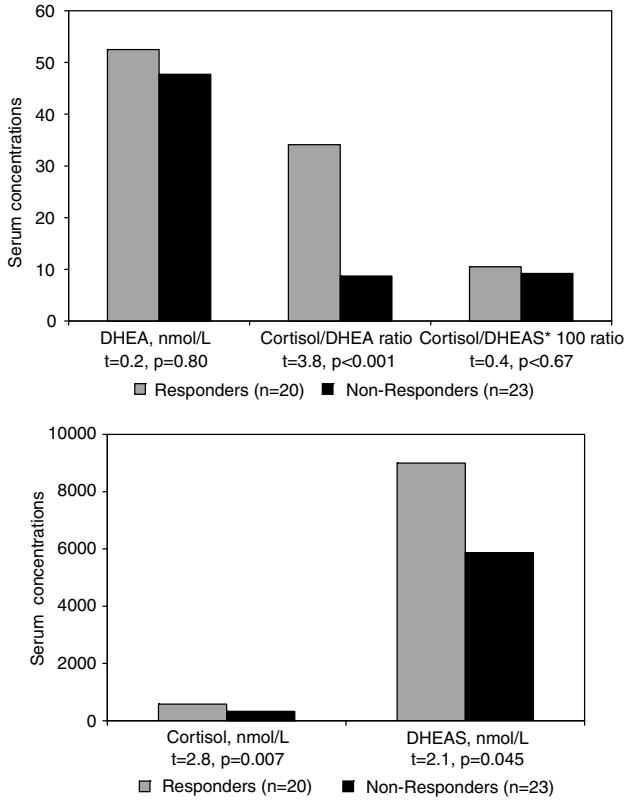

Figure 2 Basal levels of serum steroids and their molar ratios among schizophrenia patients.

differences for serum DHEA and DHEAS concentrations did not reach significant levels.

The possible effect of medication on plasma hormonal values among responders and nonresponders was tested as follows. First, two-way ANCOVA controlling for age and defined daily doses (DDD) of antipsychotic agents or benzodiazepines revealed significantly higher cortisol levels, and cortisol/DHEA(S) ratios among the responders as compared to nonresponders (Table 4). Second, type of antipsychotic agent (first or second generation; six patients with combined therapy were excluded) that was added into this model. The cortisol level ($F=16.4$, df = 1,98, $p<0.001$), cortisol/DHEA ($F=26.1$, df = 1,98, $p<0.001$), and cortisol/DHEAS ($F=6.4$, df = 1,98, $p=0.014$) ratios were again significantly higher among responders than nonresponders. These findings of elevated serum levels of cortisol and higher ratios of cortisol to DHEA(S) among responders persisted after controlling for benzodiazepine treatment (Table 4). Further, no significant correlation was found for serum cortisol, and DHEA(S) concentrations and both molar ratios with daily doses of first- and second-generation antipsychotic agents, as well as with daily doses of benzodiazepines among responders and nonresponders (all $p>0.05$, data not shown).

Spearman-rank correlation coefficients were estimated between changes in serum values of DHEA(S), cortisol and two ratios and changes in PANSS dimensions, and emotional distress scores across three examinations controlling for age at examination. Correlation coefficients indicate that among responders increased serum DHEA and cortisol concentrations significantly correlated with improvement in activation ($r=-0.51$, $p=0.026$ and $r=-0.60$, $p=0.006$, respectively), autistic preoccupations ($r=-0.52$, $p=0.023$ and $r=-0.46$, $p=0.046$, respectively), and PANSS total scores ($r=-0.48$, $p=0.038$ and $r=-0.66$, $p=0.002$, respectively). Likewise, reduction over time of PANSS total scores showed significant association with increased DHEAS levels ($r=-0.49$, $p=0.032$). No significant correlations were found between cortisol/DHEA(S) ratios and other PANSS factors. Among nonresponders, no significant correlation was observed between changes in any hormonal measures and symptom severity. Correlation coefficients of emotional distress scores with serum steroids and ratios were found not significant for both patient groups (all $p>0.05$). No significant correlations were observed between serum levels of DHEA(S) and cortisol or at any of the assessment time points.

In order to avoid the effect of multiple statistical comparisons, we evaluated canonical correlations between two sets of variables: changes from baseline through end-of-study in both (a) serum values of DHEA(S), cortisol and molar ratios and (b) five PANSS factors and emotional distress scores were evaluated. No significant canonical correlations were found between these variables among responders ($r=0.79$, $R^2=0.62$, $F=0.73$, df = 35,36, $p=0.82$) and nonresponders ($r=0.98$, $R^2=0.96$, $F=1.15$, df = 35,11, $p=0.43$). Likewise, no canonical significant

Table 2 Comparison of Hormonal Concentrations and Molar Ratios between Responders ($N=20$) and Nonresponders ($N=23$) Across Three Time Points (MANOVA Controlled for Age)

Serum stress hormones	Test value	Df		F	p
(A) Responders vs non-respoders					
Hotelling–Lawley Trace test	0.6	5	110	12.32	0.001
Cortisol (nmol/l)	3145312.1	1	114	26.96	0.001
DHEA (nmol/l)	1166.0	1	114	0.45	0.50
DHEAS (nmol/l)	69228456.9	1	114	3.10	0.08
Cortisol/DHEA ratio	40045.8	1	114	46.81	0.0001
Cortisol/DHEAS*100 ratio	919.9	1	114	5.65	0.019
(B) Across three examination					
Hotelling–Lawley Trace test	0.1	10	218	1.13	0.33
Cortisol (nmol/l)	276554.6	2	114	2.37	0.10
DHEA (nmol/l)	2433.0	2	114	0.93	0.39
DHEAS (nmol/l)	19364184.8	2	114	0.87	0.42
Cortisol/DHEA ratio	1293.9	2	114	1.51	0.22
Cortisol/DHEAS*100 ratio	251.9	2	114	1.55	0.21
(AB) Interaction					
Hotelling–Lawley Trace test	0.06	10	218	0.74	0.68
Cortisol (nmol/l)	74788.7	2	114	0.64	0.52
DHEA (nmol/l)	1588.3	2	114	0.61	0.54
DHEAS (nmol/l)	26902525.2	2	114	1.21	0.30
Cortisol/DHEA ratio	1064.9	2	114	1.24	0.29
Cortisol/DHEAS*100 ratio	283.5	2	114	1.74	0.18

Table 3 Hierarchical Logistic Regression Analysis Serum Hormonal Concentrations and Molar Ratios between Responders and Nonresponders (at Baseline Data)

Model									
N	***R*²[a]**	**df**	**Log likelihood[b]**	**Correctly classified (%)**	**Parameters**	***β***	**SE**	**Wald z-value**	***p***
1	0.07	4	−26.2	63.4	Intercept	−1.50	1.436	1.05	0.29
					DHEA (nmol/l)	0.001	0.007	0.19	0.84
					DHEAS (nmol/l)	0.0001	0.0001	1.79	0.07
					Gender	0.45	1.365	0.33	0.74
2	0.16	3	−24.4	76.2	Intercept	−1.36	1.57	0.86	0.39
					Cortisol (nmol/l)	0.004	0.002	2.36	0.018
					Gender	−0.45	1.281	0.35	0.73
3	0.36	4	−18.2	85.4	Intercept	−0.78	1.400	0.56	0.57
					Cortisol/DHEA ratio	0.14	0.050	2.87	0.004
					Cortisol/DHEAS*100 ratio	−0.09	0.060	1.57	0.11
					Gender	−0.63	1.440	0.43	0.66
4	0.23	5	−21.8	75.6	Intercept	−3.75	1.839	2.00	0.041
					Cortisol (nmol/l)	0.005	0.002	2.34	0.019
					DHEA (nmol/l)	0.004	0.007	0.52	0.60
					DHEAS (nmol/l)	0.0002	0.0001	1.62	0.10
					Gender	0.45	1.432	0.31	0.75
5	0.42	5	−16.4	87.8	Intercept	−2.13	1.616	1.32	0.18
					Cortisol (nmol/l)	0.006	0.003	1.67	0.09
					Cortisol/DHEA ratio	0.14	0.054	2.67	0.007
					Cortisol/DHEAS*100 ratio	−0.18	0.089	2.09	0.036
					Gender	−0.76	1.452	0.52	0.60

[a]R^2 represents the proportion of variation in the dependent variable accounted for by the independent variables.
[b]The Likelihood Ratio test statistic is −2 times the difference between the log likelihoods of two models, one of which is a subset of the other.

Table 4 Hormonal Differences between Responders and Nonresponders Schizophrenia Patients Across Three Examinations: the Effect of Background, Clinical Variables, Antipsychotic Agents, and Benzodiazepines

	Cortisol, nmol/l		**Cortisol/DHEA ratio**		**Cortisol/DHEAS* 100 ratio**	
Additional variable[a]	***F***	***p***	***F***	***p***	***F***	***p***
Age of onset (years)	8.6	0.005	23.2	0.001	**3.3**	0.073
Number of hospitalizations	20.1	0.001	29.6	0.001	12.6	0.001
Length of stay in hospital	17.0	0.001	35.2	0.001	6.6	0.01
Illness duration (years)	18.2	0.001	35.8	0.001	8.9	0.005
Background variables[b]	5.7	0.019	14.8	0.001	5.6	0.013
PANSS, total	24.9	0.001	38.7	0.001	10.8	0.001
Emotional distress	22.6	0.001	37.0	0.001	10.6	0.001
Daily dosage (DDD) of antipsychotic agents	29.2	0.001	42.5	0.001	12.8	0.001
Receiving benzodiazepines[c]	23.7	0.001	38.4	0.001	12.3	0.001

[a]ANCOVA model: two groups of patients (df = 1,112), three examinations (df = 2,112), covariates—age at examination and additional variables.
[b]Backgound variables: age of onset, number of hospitalizations, length of stay in hospital, and illness duration.
[c]ANCOVA model: two groups of patients (df = 1,112), three examinations (df = 2,112), benzodiazepines (1-yes, 0-no), controlled for age at examination.

correlations were observed between these sets of variables at any of the assessment time points (all $p > 0.05$).

COMMENT

This study investigated the hypothesis that molar ratios of cortisol to DHEA and its sulfate conjugate would be associated with responsiveness to antipsychotic treatment during exacerbation of schizophrenia. To test this assumption, serum DHEA(S) and cortisol concentrations were repeatedly determined for 43 medicated schizophrenia inpatients with acute exacerbation, who were classified as responders and nonresponders following 4 weeks of stable antipsychotic treatment.

The main result of this study is that (a) responders had significantly higher serum cortisol levels and cortisol/DHEA(S) ratios compared with nonresponders. With hierarchical logistic regression analysis, cortisol/DHEA(S) molar ratios were found to be significant predictors of responsivity to antipsychotic treatment; (b) these differences remained significant across three examinations controlling for gender, age, severity of symptoms and emotional distress, benzodiazepines, type or dosage of antipsychotic agents, and background variables; (c) no significant canonical correlations were observed between changes from baseline through end-of-study in hormonal values and PANSS factors and emotional distress scores among responders and nonresponders. Elevated cortisol/DHEA(S) ratios were found in our cross-sectional study of stabilized schizophrenia patients (Ritsner *et al*, 2004).

Five possible explanations concerning these differences were tested: (1) age at examination and age of illness onset, (2) background variables such as number of hospitalizations, length of stay in hospital, and illness duration, (3) symptom severity, (4) emotional distress, and (5) effect of antipsychotic agents and benzodiazepines.

First, in this study, the possible effects of age at examination do not explain differences between patients and controls in hormonal measures since theses differences remained significant after controlling for this variable in two-way ANCOVA (Table 4). However, differences between responders and nonresponders in cortisol/DHEAS ratios revealed an association with age of onset. These findings are supported by investigations that reported that age and DHEA(S) concentrations are inversely correlated (Orentreich *et al*, 1992; Binello and Gordon, 2003; Ritsner *et al*, 2004).

A second possible explanation concerning hormonal differences between responders and nonresponders may be related to background variables. Although, the responders were more likely to have shorter duration of illness and hospitalizations than nonresponders, ANCOVA with number of hospitalizations, length of stay in hospital, and illness duration as covariant variables suggests that between-group differences in serum cortisol and cortisol/DHEA(S) ratios are independent of the effects of these background variables.

Third, high serum cortisol and cortisol/DHEA ratios among responders compared with nonresponders may be explained by differences in symptom severity. Indeed, the responders had significantly higher symptom severity on PANSS factors at baseline examination, and lower psychopathology after 4 weeks compared to the non- responders. However, ANCOVA across three examinations with PANSS scores as a covariant variable suggests that hormonal differences between responders and nonresponders cannot be explained by differences in symptom severity. It should be noted that previous studies investigating cortisol blood levels in schizophrenia revealed inconsistent findings (Muck-Seler *et al*, 1999; Ceskova *et al*, 2001; Lee *et al*, 2001; Kaneda *et al*, 2002; Ritsner *et al*, 2004) possibly because they did not consider responsivity to antipsychotic treatment.

Fourth, several previous cross-sectional studies indicated the controversial role of steroids in the ongoing symptomatology of schizophrenia (Walder *et al*, 2000; Harris *et al* 2001; Shirayama *et al*, 2002). In this study, in order to avoid the effect of multiple statistical comparisons, we applied canonical correlation analysis and no significant correlation was found between changes in hormonal measures, symptom severity, and emotional distress among responders and nonresponders. In addition, since it has been demonstrated that DHEA(S) exhibit antistress properties, the role of emotional distress in hormonal alterations was tested. We found that hormonal differences between responding and non-responding schizophrenia patients remained significant after controlling for emotional distress scores, so this assumption is unlikely.

Another possible reason for hormonal differences between responders and nonresponders may be the effect of antipsychotic agents and/or benzodiazepines. Several preclinical studies have shown that haloperidol failed to alter the brain concentrations of neurosteroids (Barbaccia *et al*, 2001; Nechmad *et al*, 2003), whereas clozapine administered for 8 days reduced the brain concentrations of DHEA and DHEAS (Nechmad *et al*, 2003).

There is evidence that atypical antipsychotics may differentially affect cortisol (Meltzer *et al*, 2001; Lee *et al*, 2001) and neurosteroid (Marx *et al*, 2003) levels. The present study indicates that controlling for daily dosages and types of antipsychotic agents (first or second generation) did not affect differences. Further, consistent with our previous study (Ritsner *et al*, 2004), no significant correlation was found between hormonal measures and daily doses of first- and second-generation antipsychotic agents among both responders and nonresponders. It should be noted that we recruited patients who had been free of antidepressants or mood stabilizers for at least 1 month. Benzodiazepines also can affect concentrations of DHEA(S). For example, alprazolam (GABA agonist) produced significant increases in DHEA, reduction in cortisol, and no change in DHEAS concentrations (Kroboth *et al*, 1999). Findings from the present study suggest that benzodiazepines did not influence the elevation of serum cortisol levels, and molar cortisol/DHEA(S) ratios among the responders compared with nonresponders. Thus, antipsychotic agents and benzodiazepines cannot explain the differences between responders and nonresponders.

Next, a general concern of this study is the importance of the cortisol/DHEA(S) ratios for predicting response to treatment in schizophrenia. However, we may assume that the significant findings for the ratios are largely due to the numerator (cortisol), with no prominent effects for

DHEA(S) concentrations. In order to test this assumption, hierarchical logistic regression analysis was applied to see how much of the variance in treatment response is explained by DHEA(S), *vs* cortisol *vs* the ratios. The best-fit model revealed clear advantages of both cortisol/DHEA ($p = 0.007$), and cortisol/DHEAS ($p = 0.036$) molar ratios *vs* serum cortisol concentrations ($p > 0.05$) for prediction of responsivity to antipsychotic treatment. This model accounted for 42% of the variance and correctly classified responders and nonresponders—87.8% of the 43 patients. Values of serum DHEA(S) and gender demonstrated no significant contribution that accounted for prediction of responsiveness to antipsychotic treatment in schizophrenia patients. In light of the gender differences in concentrations of both DHEA(S) hormones (Carlstrom *et al*, 1988) future studies should include many more women in the study sample.

In view of the above findings, we addressed the central question raised in our study: What is the meaning of these hormonal differences between responders and nonresponders to antipsychotic treatment in schizophrenia? Overall, it is still possible that the endocrine findings are not related to a diagnostic specificity, and the elevated cortisol/DHEA(S) ratios in schizophrenia patients may be associated with impaired stress-response (Wolf and Kirschbaum, 1999; Boudarene and Legros, 2002; Kimonides *et al*, 1999) and may lead to dysregulated neurotransmission (Akwa and Baulieu, 2000), resulting in chronic and progressive deterioration in cognitive, emotional, and psychosocial functions (Wen *et al*, 2001; Johnson *et al*, 2002). Indeed, clinical investigations produced evidence of the involvement of neuroactive steroids in conditions such as fatigue during pregnancy, premenstrual syndrome, postpartum depression, epilepsy, dementia, depressive, and anxiety disorders (Stoffel-Wagner, 2001; Pisua and Serra, 2004).

At present, it is not clear how best to explain these results. We suggest that imbalance in serum DHEA(S) and cortisol may be related to pathophysiological processes in schizophrenia, particularly, to responsivity to antipsychotic treatment. Interestingly, the group of responders showed improvement in symptom severity after 4 weeks of treatment, although cortisol/DHEA(S) ratios and serum cortisol concentrations did not significantly change. Therefore, we may assume that despite improvement, these patients either still remained in the exacerbation stage of illness, or that these hormonal alterations may represent a trait-like marker. It should be noted that responders had significantly higher basal levels of cortisol, DHEAS, and cortisol/DHEA ratio values compared with nonresponders (Figure 2). Further studies are needed to test these assumptions.

The antagonist action of DHEA to cortisol in the brain (Goodyer *et al*, 1988), and the cortisol-lowering effect of DHEA administration (Kroboth *et al*, 2003) may provide a relevant physiological basis for the examination of the ratio of cortisol/DHEA(S) ratios. Indeed, the antiglucocorticoid properties of DHEA (van Broekhoven and Verkes, 2003) may contribute to an upregulation of HPA axis responses as well as mitigate possible deleterious effects of high cortisol levels on the brain in schizophrenia subjects. This study could not show us a clear causal relationship between cortisol/DHEA(S) ratios and responsivity to antipsychotic treatment. The complex relationship between exacerbated chronic schizophrenia, stress, circulatory DHEA(S), HPA activity, and response to antipsychotic treatment merits further investigation.

Limitations of the study include the predominance of men among the schizophrenia patients. Another potential factor relates to assessment of diurnal rhythmicity of hormones. Future studies should collect blood samples taken more frequently during the day. Assessment over a longer period of time also should be performed. The findings of this study indicate the need for further evaluation of the relationship between alteration in metabolism of DHEA(S) and schizophrenia. Future studies should include examination of cortisol/DHEA(S) ratios during the administration of DHEA.

In conclusion, our findings suggest that the molar ratios of cortisol to DHEA and its sulfate conjugate may have predictive value for responding to antipsychotic medications in the context of exacerbation of chronic schizophrenia. Understanding the complex interactions of DHEA(S) with cortisol, and other stress-responsive systems may help to explain the variability in the course and treatment responsivity observed in schizophrenia.

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