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Assessment of the Dexamethasone/CRH Test as a State-Dependent Marker for Hypothalamic-Pituitary-Adrenal (HPA) Axis Abnormalities in Major Depressive Episode: A Multicenter Study

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There is compelling evidence for the involvement of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in depression. Growing evidence has suggested that the combined dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test is highly sensitive to detect HPA axis abnormalities. We organized a multicenter study to assess the DEX/CRH test as a state-dependent marker for major depressive episode in the Japanese population. We conducted the DEX/CRH test in 61 inpatients with major depressive episode (Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV)) and 57 healthy subjects. In all, 35 patients were repeatedly assessed with the DEX/CRH test on admission and before discharge. The possible relationships between clinical variables and the DEX/CRH test were also examined. Significantly enhanced pituitary–adrenocortical responses to the DEX/CRH test were observed in patients on admission compared with controls. Such abnormalities in patients were significantly reduced after treatment, particularly in those who underwent electroconvulsive therapy (ECT) in addition to pharmacotherapy. Age and female gender were associated with enhanced hormonal responses to the DEX/CRH test. Severity of depression correlated with DEX/CRH test results, although this was explained, at least in part, by a positive correlation between age and severity in our patients. Medication *per se* was unrelated to DEX/CRH test results. These results suggest that the DEX/CRH test is a sensitive state-dependent marker to monitor HPA axis abnormalities in major depressive episode during treatment. Restoration from HPA axis abnormalities occurred with clinical responses to treatment, particularly in depressed patients who underwent ECT.
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INTRODUCTION

There is compelling evidence for an important role of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in the pathophysiology of mood disorders (Holsboer, 1995; Plotsky *et al*, 1998). To quantify the dysregulation of HPA axis, the dexamethasone (DEX) suppression test (DST) has

been studied most extensively since Carroll *et al* (1981) standardized it as a biological marker for the diagnosis of melancholia. A major drawback of the DST, however, is its modest sensitivity (rate of nonsuppression of cortisol) of 40–50% (Carroll, 1982; American Psychiatric Association, 1987). Subsequently, a refined laboratory test that combines the DST and corticotropin-releasing hormone (CRH) challenge, the DEX/CRH test, has been introduced (Holsboer *et al*, 1987; von Bardeleben and Holsboer, 1989). In normal subjects, pretreatment with DEX suppresses pituitary–adrenal responses to CRH. In depressed patients, however, the same procedure enhances hormonal responses to CRH, resulting in higher sensitivity of the DEX/CRH test to major depression by up to 80% (Heuser *et al*, 1994b). Furthermore, the DEX/CRH test was shown to be more closely associated with the activity of HPA system (24-h cortisol profiles) than the standard DST in healthy and depressed subjects (Deuschle *et al*, 1998). We have confirmed the relatively high sensitivity of the DEX/CRH test in Japanese patients with depressive disorder (Oshima *et al*, 2000; Kunugi *et al*, 2004). These findings indicate that the DEX/CRH test is a useful laboratory tool to monitor HPA axis abnormalities in depressed patients in clinical practice. However, previous studies have not always provided consistent results as to whether clinical variables, such as age, severity, and diagnosis (unipolar *vs* bipolar), are associated with DEX/CRH test results.

In serial DST studies, conversion from nonsuppressor to suppressor status is temporally associated with clinical responses to antidepressants (eg Holsboer *et al*, 1982; Greden *et al*, 1983). In line with this, hormonal responses to the DEX/CRH test also restored after successful treatment with antidepressants (Holsboer-Trachsler *et al*, 1991; Heuser *et al*, 1996; Baghai *et al*, 2002; Hatzinger *et al*, 2002), suggesting that the DEX/CRH test is a useful state-dependent marker. However, it has been argued that DEX/CRH test results may be trait dependent, particularly in bipolar patients (Holsboer *et al*, 1995; Schmider *et al*, 1995; Watson *et al*, 2004; Ising *et al*, 2005).

Changes in repeated DEX/CRH tests may depend on treatment modality. In responders to repetitive transcranial magnetic stimulation therapy, post-DEX cortisol levels prior to CRH challenge decreased, while no change of CRH-induced ACTH and cortisol release was observed (Zwanzger *et al*, 2003). Similarly, partial changes in DEX/CRH tests were observed in responders to partial sleep deprivation therapy (Schule *et al*, 2002). Concerning electroconvulsive therapy (ECT), there is little information on changes in the DEX/CRH test. Previous studies employing conventional DST have provided inconsistent results: some authors reported a positive correlation between response to ECT and normalization of the HPA axis (Albala *et al*, 1981; Papakostas *et al*, 1981; Grunhaus *et al*, 1987; Devanand *et al*, 1991); an author reported an equivocal result (Devanand *et al*, 1987); and others failed to find such a relationship (Coryell, 1986; Fink *et al*, 1987). These inconsistent results require further investigations.

The present study aimed to assess the DEX/CRH test as a state-dependent marker to monitor HPA axis abnormalities in patients with major depressive episode, including patients receiving ECT. Furthermore, we examined DEX/CRH test results for a possible association with clinical variables, such as age, gender, severity, and medication.

METHODS

Subjects

Subjects were 57 healthy controls (37 men and 20 women; mean age: 31 years, range: 22–48, standard deviation (SD): 7) and 61 inpatients (24 men and 37 women; mean age: 54 years, range 30–82, SD: 13) with major depressive episode according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) (American Psychiatric Association, 1994), who were recruited from eight institutions in Japan. All the patients and controls were biologically unrelated Japanese. In all, 54 patients were diagnosed with unipolar major depression (29 with single episode and 25 with recurrent episodes), and the remaining seven with bipolar I disorder. The following were excluded: patients who had somatic disorders, such as inflammation, endocrine disorders, and neoplasm; patients who were treated with liver-enzyme-inducing drugs, lithium carbonate, or carbamazepine; and patients who were withdrawn from abused illicit drugs and other substances, such as benzodiazepines and alcohol. After full description of the study, written informed consent was obtained from every subject for his/her participation in the study. The study protocol was approved by the ethics committee of each institution.

Among the 61 patients, 35 (13 men and 22 women; 13 unipolar depression with single episode, 18 unipolar depression with recurrent episodes, and four bipolar disorder; mean age: 55 years, SD: 15) were repeatedly assessed with the DEX/CRH test on admission and before discharge, while the remaining 26 were assessed only once on admission. Among the 35 patients, 12 underwent ECT in addition to pharmacotherapy. Based on the effect size reported by Holsboer-Trachsler *et al* (1991), this sample size ($N = 35$) had a power of 96% at the 0.05 level of significance (two-tailed) to detect effects of treatment on DEX/CRH test results.

The severity of index depressive episode was assessed with the 21-item version of the Hamilton depression rating scale (HDRS; Hamilton, 1967) when the DEX/CRH test was conducted. Patients who were scored less than 15 by the HDRS on admission were not enrolled in the study. The majority of the patients were medicated with antidepressants, while seven patients were drug naive on admission. We did not control for class of antidepressant medication, and data were obtained in the ordinary clinical setting in Japan. Thus, a variety of antidepressants were medicated; however, switch in medication was avoided for at least 4 days before conducting the DEX/CRH test. ECT was administered six to 10 times (two or three times a week) for each patient with electrical stimuli of 100–110 V for 5–10 s, discharged with bilateral electrodes placed bifrontotemporally. Patients were anesthetized with propofol, and motor convulsion was suppressed with succinylcholine.

DEX/CRH Test

The DEX/CRH test was conducted as described by Zobel *et al* (2001). Subjects were pretreated with an oral dose of

1.5 mg of DEX (Asahikasei Pharmaceutical Corporation, Tokyo, Japan) at 2300 hours. On the next day, a vein was cannulated at 1430 hours to collect blood at 1500, 1530, 1545, 1600, and 1615 hours via an intravenous catheter. Human CRH (100 μg) (hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1500 hours immediately after the first blood collection (protocol 1). In part of patients ($N=8$), a vein was cannulated at 1330 hours to collect blood at 1400, 1415, 1430, 1500, and 1600 hours (protocol 2). hCRH was administered intravenously immediately after the first blood collection at 1400 hours. Subjects rested supine throughout the test in a calm room. Between the collections of blood specimen, the catheter was kept patent by physiological saline infusion at a rate of 50 ml/h. Blood samples were immediately centrifuged and stored at -20°C. Plasma concentrations of ACTH and cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limits for ACTH and cortisol were 5.0 pg/ml and 1.0 μg/dl, respectively.

Data Presentation for Hormonal Measures

We defined A_0 as the plasma concentration of ACTH in the blood sample obtained at 1500 hours for protocol 1 or 1400 hours for protocol 2, that is, plasma concentrations that were measured at 16 or 15 h after the oral intake of DEX but immediately before the infusion of CRH. A_{30} and A_{60} were defined as the plasma concentrations of ACTH, which were measured at 30 and 60 min after the intravenous infusion of CRH, respectively. A_{dif} denotes the difference between the A_0 and A_{60} values. The C_0, C_{30}, C_{60}, and C_{dif} values were defined similarly for the plasma concentrations of cortisol. These values were available in both protocols 1 and 2, which enabled us to combine data from these protocols and to analyze all the subjects simultaneously. There was no evidence for significant effects on hormonal outcomes due to a 1 h difference between the two protocols (data not shown), combining the data from the two protocols. According to our previous reports (Oshima *et al*, 2000; Kunugi *et al*, 2004), the plasma concentrations of ACTH and cortisol peak at approximately 60 min after infusion of CRH, indicating that the A_{60} and C_{60} values reflect the maximal responses to CRH. The area under the time curve (AUC, arbitrary unit) was calculated according to the trapezoidal rule (A_{auc} for ACTH and C_{auc} for cortisol). A_{auc} was calculated from the A_0, A_{30}, and A_{60} values, and C_{auc} from the C_0, C_{30}, and C_{60} values.

As the plasma cortisol criterion concentration of 5 μg/dl was suggested to be most effective for assessing an abnormal DST result (Carroll, 1982), 'nonsuppressor' was defined to be an individual who showed a C_0 value of $\geqslant 5$ μg/dl irrespective of the C_{60} value. Furthermore, 'intermediate suppressor' was defined *a priori* to be an individual who showed a C_0 value of <5 μg/dl and a C_{60} of $\geqslant 5$ μg/dl. The remaining individuals, who showed both C_0 and C_{60} values of <5 μg/dl, were defined as 'suppressors'. These definitions were almost identical to those described by Kunugi *et al* (2004).

Statistical Analysis

All statistical analyses were made with SPSS for Windows (version 11, SPSS Japan, Tokyo). Intergroup comparisons were made for age and HDRS scores according to *t*-test or one-way ANOVA. The proportion of categorical data, such as gender, was compared according to the χ^2 test for independence. Intergroup comparisons were made for ACTH and cortisol according to the Mann–Whitney *U*-test (comparisons between two groups) or the Kruskal–Wallis *H*-test (comparisons among three groups). Differences between hormonal responses observed on admission and before discharge were tested according to the Wilcoxon paired signed-ranks test. Nonparametric tests were employed because the endocrine values did not always show a normal distribution and part of subjects showed the A_0 and/or C_0 values below detection limits. All *p*-values reported are two-tailed. A value of $p<0.05$ was considered statistically significant.

RESULTS

Hormonal Responses in Patients and Controls

Effects of age and gender. Hormonal responses in patients (on admission) and controls are shown in Table 1. To see the effects of age on hormonal responses, the patient group was dichotomized into the 'young' ($\leqslant 50$ years) and 'aged' (>50 years) patient groups. All controls, whose age ranged between 22 and 48 years, were considered to be 'young' subjects. To see the effects of gender, hormonal values were presented separately by gender. In the patient group, all the mean hormonal values (A_0, A_{60}, A_{dif}, A_{auc}, C_0, C_{60}, C_{dif}, and C_{auc}) were higher in the aged patient group than in the young patient group, although a statistically significant difference was found only for A_0 ($p=0.048$, Mann–Whitney *U*-test) and a nonsignificant trend for C_{auc} ($p=0.055$).

When males and females were compared, hormonal responses to pretreatment with DEX (A_0 and C_0) did not seem to be consistently different in young patients, aged patients, or controls (see Table 1). However, hormonal responses to CRH as manifested by A_{dif} and C_{dif} were consistently higher in females than in males for all the three groups (young patients, aged patients, and controls). Among young patients, C_{dif} was significantly higher in females than in males ($p=0.029$, Mann–Whitney *U*-test), although differences in A_{dif} did not reach statistical significance ($p=0.17$). Similarly, in aged patients, the mean C_{dif} value was significantly higher in females than in males ($p=0.034$). However, differences in mean A_{dif} failed to reach statistical significance ($p=0.14$). Among subjects in the control group, females showed statistically higher A_{dif} values than males, and there was a trend towards an increased C_{dif} value in females than in males.

Patients vs *controls.* Since the above data indicated that age and gender have effects on the DEX/CRH test, young patients (14 men and eight women) only were compared with controls (37 men and 20 women). These two groups were nearly matched for the ratio of males to females ($p=0.91$, χ^2 test), although there was still a significant difference in mean age (41 years SD 7 *vs* 31 years SD 7;

Table 1 Hormonal Responses to the DEX/CRH Test in Patients (on Admission) and Controls (Mean ± SD)

		ACTH (pg/ml)				Cortisol (μg/dl)			
	N	A_0	A_{60}	A_{dif}	A_{auc}	C_0	C_{60}	C_{dif}	C_{auc}
Patients									
Total	61	8.7 ± 8.2	27.4 ± 29.3	18.7 ± 24.8	1227 ± 1230	3.2 ± 3.8	8.4 ± 7.1	5.2 ± 4.9	365 ± 344
Young total	22	6.6 ± 3.5	20.9 ± 19.4	14.3 ± 16.4	958 ± 937	2.1 ± 1.0	6.6 ± 5.8	4.5 ± 5.3	268 ± 243
Young male	14	6.8 ± 4.1	18.3 ± 19.9	11.6 ± 16.3	870 ± 998	1.8 ± 0.8	4.8 ± 4.9	3.0 ± 4.6	190 ± 180
Young female	8	6.4 ± 2.6	25.4 ± 19.1	19.0 ± 16.6	1112 ± 862	2.6 ± 1.1	9.7 ± 6.3	7.1 ± 5.9	404 ± 290
Aged total	39	9.9 ± 9.8	31.1 ± 33.3	21.2 ± 28.4	1379 ± 1356	3.9 ± 4.5	9.4 ± 7.7	5.6 ± 4.7	420 ± 381
Aged male	10	9.9 ± 7.3	21.5 ± 21.0	11.6 ± 14.3	1211 ± 1278	3.8 ± 4.8	6.8 ± 6.6	3.1 ± 3.5	355 ± 385
Aged female	29	9.9 ± 10.6	34.5 ± 36.3	24.5 ± 31.3	1437 ± 1399	3.9 ± 4.5	10.3 ± 7.9	6.4 ± 4.8	442 ± 384
Controls									
Total	57	6.0 ± 1.5	13.8 ± 9.3	7.8 ± 8.6	638 ± 317	1.5 ± 0.8	3.9 ± 3.9	2.4 ± 3.8	155 ± 131
Young male	37	6.1 ± 1.5	12.4 ± 8.9	6.3 ± 8.1	598 ± 321	1.6 ± 0.9	3.2 ± 2.6	1.6 ± 2.4	139 ± 102
Young female	20	5.8 ± 1.6	16.4 ± 9.6	10.6 ± 9.2	713 ± 303	1.3 ± 0.3	5.2 ± 5.4	3.9 ± 5.3	186 ± 170

$p < 0.001$ by t-test). Although the values for ACTH (A_0, A_{60}, A_{dif}, and A_{auc}) were consistently higher in young patients than in controls (see Table 1), none of the differences reached statistical significance ($p = 0.41$ for A_0, $p = 0.27$ for A_{60}, $p = 0.13$ for A_{dif}, $p = 0.74$ for A_{auc}, Mann–Whitney U-test). However, the values for cortisol were significantly higher in young patients than in controls ($p = 0.004$ for C_0, $p = 0.033$ for C_{60}, $p = 0.036$ for C_{auc}), except for C_{dif} ($p = 0.14$).

One might suspect, however, that the significant differences observed in cortisol measures were attributable simply to the differential age distributions between young patients and controls. To resolve this issue, *post hoc* analyses were performed, selecting 18 patients and 18 controls matched for both age and gender (12 males and six females; mean age 38 years (SD 6) for patients and controls). Results in these subjects were essentially unchanged; cortisol measures were significantly higher in patients than in controls ($p = 0.009$ for C_0, $p = 0.044$ for C_{60}, $p = 0.040$ for C_{auc}), except for C_{dif} ($p = 0.21$). This ensures that enhanced cortisol responses to the DEX/CRH test in patients were not attributable simply to differential age distribution between patients and controls.

Clinical variables and DEX/CRH test results. Possible relationships between clinical variables and DEX/CRH test results were examined in the patient group. There was a significant or a nonsignificant trend towards a positive correlation between HDRS scores on admission and some hormone values (A_{60}: Pearson's $r = 0.27$, $p = 0.035$; A_{dif}: $r = 0.27$, $p = 0.038$; A_{auc}: $r = 0.25$, $p = 0.055$; C_{60}: $r = 0.23$, $p = 0.079$; C_{auc}: $r = 0.24$, $p = 0.059$), suggesting that pituitary–adrenal responses to the DEX/CRH test tend to become higher as severity of depression increases. When confounding factors of age and gender were examined, however, there was a highly significant positive correlation between age and HDRS scores ($r = 0.39$, $p = 0.002$), although there was no significant difference in mean HDRS score between males and females ($t = -1.2$, $df = 59$, $p = 0.23$). Thus, the observed correlation between HDRS scores and the DEX/CRH test might be explained, at least in part, by the correlation between HDRS scores and age in our sample. No significant difference was found in hormonal measures between patients on medication and patients without medication on admission (Table 2). When the diagnosis groups (unipolar depression with single episode, recurrent unipolar depression, and bipolar disorder) were compared, ACTH responses differed consistently with a significant difference (A_{dif}) and a nonsignificant trend (A_0 and A_{60}) (Table 2). Against our expectation, ACTH responses were high in the decreasing order of patients with unipolar depression with single episode, those with bipolar disorder, and those with recurrent unipolar depression. Comparisons of confounding factors of age, gender, and HDRS score revealed no significant difference among these three diagnosis groups (age: $p = 0.35$ by one-way ANOVA; gender: $p = 0.37$ by χ^2 test; HDRS score: $p = 0.52$ by one-way ANOVA).

Repeated Assessments of the DEX/CRH Test

Among patients, 35 were repeatedly assessed with the DEX/CRH test on admission and before discharge. The mean HDRS scores as well as plasma concentrations of ACTH and cortisol before and after treatment are shown in Table 3. HDRS scores lowered substantially due to treatment. All values of ACTH and cortisol decreased significantly after treatment. When suppression status was examined, the distributions of nonsuppressors, intermediate suppressors, and suppressors changed significantly after treatment (14, 51, and 34%, respectively, on admission, in contrast to 11, 14, and 74%, respectively, before discharge; $p = 0.004$ by the Wilcoxon paired signed-ranks test). No significant correlation was found between changes in HDRS scores

Table 2 Relationships between Clinical Variables and Hormonal Responses to the DEX/CRH Test in the Patient Group (on Admission) (Mean ± SD)

		ACTH (pg/ml)				Cortisol (μg/dl)			
	N	A_0	A_{60}	A_{dif}	A_{auc}	C_0	C_{60}	C_{dif}	C_{auc}
Medication									
With medication	54	8.7 ± 8.3	27.7 ± 30.4	19.0 ± 25.9	1194 ± 1200	3.1 ± 3.5	8.2 ± 7.1	5.1 ± 4.9	350 ± 326
Without medication	7	8.8 ± 8.0	25.4 ± 21.2	16.7 ± 14.8	1481 ± 1523	4.4 ± 5.6	10.1 ± 7.9	5.6 ± 5.7	483 ± 476
p-value[#]		$p = 0.73$	$p = 0.96$	$p = 0.85$	$p = 0.75$	$p = 0.27$	$p = 0.51$	$p = 0.82$	$p = 0.63$
Diagnosis									
UP single episode	29	10.3 ± 10.5	33.2 ± 31.0	22.9 ± 23.0	1543 ± 1494	3.3 ± 4.0	9.2 ± 7.1	6.0 ± 4.8	399 ± 363
UP recurrent	25	7.1 ± 5.5	20.5 ± 28.7	13.3 ± 28.0	849 ± 790	3.4 ± 3.9	7.1 ± 7.7	3.7 ± 4.8	321 ± 355
BP	7	7.7 ± 4.3	28.3 ± 21.3	20.7 ± 12.2	1269 ± 1067	2.5 ± 1.3	9.6 ± 5.5	7.1 ± 5.1	380 ± 221
p-value[##]		$p = 0.086$	$p = 0.071$	$p = 0.036$	$p = 0.10$	$p = 0.89$	$p = 0.29$	$p = 0.095$	$p = 0.39$

UP = unipolar; BP = bipolar.
[#]Mann–Whitney *U*-test.
[##]Kruskal–Wallis *H*-test.

Table 3 HDRS Scores and Hormonal Measures in 35 Patients who were Repeatedly Assessed (Before and After Treatment) with the DEX/CRH Test (Mean ± SD)

		ACTH (pg/ml)				Cortisol (μg/dl)			
	HDRS	A_0	A_{60}	A_{dif}	A_{auc}	C_0	C_{60}	C_{dif}	C_{auc}
Total (N = 35)									
Before treatment	28.8 ± 9.1	8.1 ± 5.5	27.5 ± 28.2	19.4 ± 26.5	1176 ± 971	3.3 ± 3.4	8.9 ± 7.1	5.6 ± 5.0	384 ± 325
After treatment	6.5 ± 4.1	5.7 ± 2.3	14.9 ± 12.4	9.2 ± 11.8	682 ± 529	2.1 ± 1.8	5.3 ± 6.5	3.2 ± 5.4	236 ± 273
p-value[#]	$p < 0.001$	$p = 0.022$	$p = 0.007$	$p = 0.012$	$p = 0.007$	$p = 0.005$	$p = 0.002$	$p = 0.013$	$p = 0.002$
Pharmacotherapy alone (N = 23)									
Before treatment	29.3 ± 9.6	7.6 ± 4.8	28.7 ± 32.0	21.1 ± 31.0	1171 ± 989	3.2 ± 3.7	8.7 ± 7.7	5.6 ± 5.3	374 ± 344
After treatment	6.8 ± 4.7	5.9 ± 2.9	16.4 ± 14.1	10.5 ± 13.3	746 ± 622	2.4 ± 2.2	6.3 ± 7.7	3.9 ± 6.3	277 ± 323
p-value[#]	$p < 0.001$	$p = 0.14$	$p = 0.078$	$p = 0.083$	$p = 0.048$	$p = 0.19$	$p = 0.048$	$p = 0.13$	$p = 0.042$
ECT and pharmacotherapy (N = 12)									
Before treatment	27.8 ± 8.4	9.0 ± 6.8	25.0 ± 19.8	16.0 ± 15.1	1186 ± 979	3.6 ± 2.7	9.3 ± 6.2	5.7 ± 4.6	403 ± 299
After treatment	6.1 ± 2.9	5.3 ± 0.7	12.2 ± 8.1	6.8 ± 8.2	559 ± 261	1.7 ± 0.5	3.5 ± 2.9	1.8 ± 2.9	158 ± 115
p-value[#]	$p = 0.002$	$p = 0.11$	$p = 0.041$	$p = 0.050$	$p = 0.041$	$p = 0.006$	$p = 0.021$	$p = 0.038$	$p = 0.012$

[#]*p*-value was obtained by the Wilcoxon paired signed-ranks test.

and changes in any of hormonal measures in the 35 patients.

Restoration from HPA axis abnormalities was examined separately in patients who underwent ECT and in patients who underwent pharmacotherapy alone. Figure 1 shows time-course changes of the mean plasma concentrations of cortisol in patients who underwent ECT ($N = 12$) and in patients who did not ($N = 23$). As shown in Figure 1, the magnitude of hormonal response reduction appeared to be greater in patients who underwent ECT in addition to pharmacotherapy compared with those who underwent pharmacotherapy alone. As shown in Table 3, indeed, changes in hormone concentrations between pre- and post-treatment were significant only for A_{auc}, C_{60}, and C_{auc} in the pharmacotherapy group, while all values of both ACTH and cortisol, except for A_0, changed significantly in the ECT group.

DISCUSSION

We assessed the DEX/CRH test as a state-dependent marker to monitor HPA axis abnormalities in depression. We found that elderly and female individuals tended to show increased pituitary–adrenal responses to the DEX/CRH test compared to young and male individuals, respectively. We

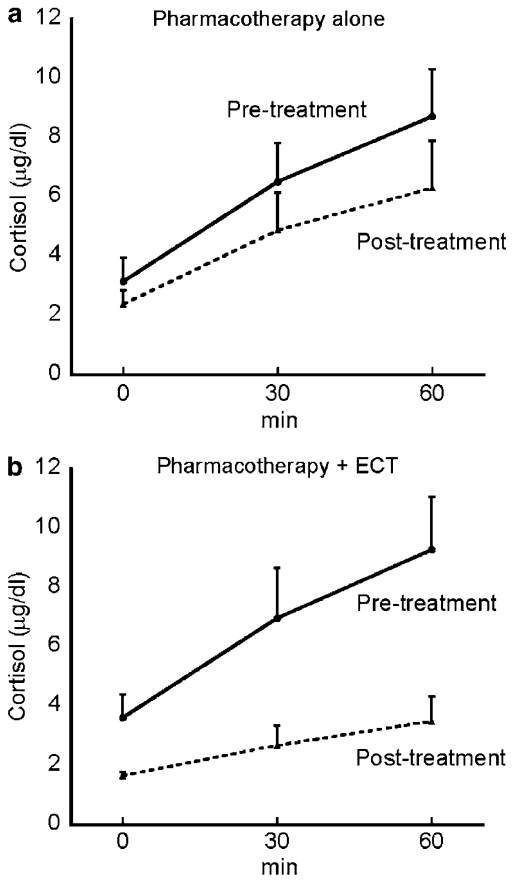

Figure 1 Time-course curves of cortisol responses to the DEX/CRH test before and after treatment in the pharmacotherapy group (a) and the ECT group (b). The *X*-axis represents time after CRH infusion. Error bars represent SEs.

confirmed that pituitary–adrenal responses to the DEX/CRH test are enhanced in depressed patients compared to controls. Severity of depression correlated with the DEX/CRH test results, although this was explained, at least in part, by a positive correlation between age and severity in our patients. Medication *per se* was unrelated to DEX/CRH test results. For patients who were repeatedly assessed with the DEX/CRH test, hormonal responses were reduced after treatment. This reduction was observed predominantly in patients who underwent ECT in addition to pharmacotherapy rather than in those who underwent pharmacotherapy alone.

Effects of Age and Gender

In our patients, all the mean plasma concentrations of hormones were higher in the aged patient group (>50 years) than in the young patient group ($\leqslant 50$ years), although a statistically significant difference was found only for A_0 and a nonsignificant trend for C_{auc}. Previous studies have provided conflicting results as to the possible effects of age on hormonal responses to the DEX/CRH test. von Bardeleben and Holsboer (1991) reported that the cortisol response to the DEX/CRH test increased with age in patients with depression but was absent in controls. Heuser *et al* (1994a) examined healthy subjects and found that hormonal responses to the DEX/CRH test were enhanced in older subjects (aged 60–84 years) compared to younger subjects (22–48 years). In line with this, Kudielka *et al* (1999) found that healthy elderly women (60–75 years) exhibited a markedly enhanced cortisol response to the DEX/CRH test compared to young controls (20–31 years). In contrast, a more recent study found no significant effect of age on hormonal responses to the DEX/CRH test in acutely depressed inpatients (Kunzel *et al*, 2003). Studies in rats have provided evidence that aged animals tend to show enhanced hormonal responses to the DEX/CRH test compared to young animals, and that vasopressin is involved in these age-associated changes (Hatzinger *et al*, 1996, 2000; Revskoy and Redei, 2000). Our results support the possibility that older age is associated with enhanced pituitary–adrenal responses to the DEX/CRH test, at least in depressed patients.

With respect to gender, female depressed and normal subjects were reported to have increased hormonal responses to the DEX/CRH test in comparison with male counterparts (Heuser *et al*, 1994a; Kunzel *et al*, 2003). Our results give further support for this gender difference. Based on our data, responses to pretreatment with DEX (A_0 and C_0) did not seem to be consistently different between males and females among young patients, aged patients, and controls (Table 1). However, hormonal responses to the subsequent injection of CRH as manifested by A_{dif} and C_{dif} were consistently higher in females than in males for all the three groups. These results suggest that gender difference in hormonal responses to the DEX/CRH test might be attributable predominantly to that in hormonal responses to CRH.

Patients *vs* Controls

When young patients were compared with controls, all hormonal responses of both ACTH and cortisol were consistently higher in patients on admission than in controls (Table 1). The differences in C_0, C_{60}, and C_{auc} were statistically significant. The differences in A_0, A_{60}, A_{dif}, A_{auc}, and C_{dif} failed to reach statistical significance, which was probably attributable to the lack of statistical power due to the small number of young patients ($N=22$). Even when *post hoc* analyses were performed in 18 patients and the same number of controls, matched for both age and gender, results were similar. Overall, our data provide further evidence for enhanced HPA activity detected with the DEX/CRH test in depressed patients.

Clinical Variables and DEX/CRH Test

We found a positive correlation between HDRS scores on admission and hormonal values, suggesting that pituitary–adrenal responses to the DEX/CRH test tend to become higher as severity of depression increases. This observation is in accordance with previous studies (von Bardeleben and Holsboer, 1991; Rybakowski and Twardowska, 1999; Kunzel *et al*, 2003). In our sample, however, there was a highly significant correlation between HDRS scores and age ($p=0.002$), which was stronger than the correlation between HDRS scores and hormonal measures, indicating

that the observed correlation between HDRS scores and the DEX/CRH test might be explained, at least in part, by the correlation between HDRS scores and age. Zobel *et al* (2004) recently reported that a decrease in cortisol response to the DEX/CRH test was more closely related to an improvement in specific brain functions (especially working memory) than to the global severity of depression.

Hormonal measures were not significantly different between patients on medication and patients without medication on admission, indicating that the fact of being on medication *per se* was unrelated to DEX/CRH test results. This observation is in line with the finding of Kunzel *et al* (2003), that is, the presence or absence of antidepressant treatment, the type of antidepressant treatment, or the number of ineffective antidepressant treatment trials during the index episode had no effect on hormonal responses to the DEX/CRH test. One exceptional drug is mirtazapine, an antidepressant known to acutely inhibit cortisol secretion in healthy subjects; the drug was shown to attenuate rapidly HPA axis hyperactivity in depressed patients via direct pharmacoendocrinological effects, which was not necessarily related to clinical improvement (Schule *et al*, 2003).

With respect to possible differences in DEX/CRH test results among the diagnosis groups (unipolar depression with single episode, recurrent unipolar depression, and bipolar disorder), ACTH responses were high in the decreasing order of patients with unipolar depression with single episode, those with bipolar disorder, and those with recurrent unipolar depression. This finding was unexpected because at least three research groups had obtained evidence that HPA abnormalities as detected with the DEX/CRH test became more pronounced as the number of previous episodes increased (Hatzinger *et al*, 2002; Kunzel *et al*, 2003; Gervasoni *et al*, 2004). Inconsistency between previous studies and ours might be attributable to the effects of confounding factors of age, gender, and HDRS score. However, we found no significant difference in any of these variables among the three diagnosis groups. Alternatively, differences in ACTH responses may have occurred by chance, considering that there was no significant difference in cortisol responses among the diagnosis groups. Further investigations are required to draw any conclusion.

Repeated Assessments of the DEX/CRH Test

When depressed patients were repeatedly assessed with the DEX/CRH test on admission and before discharge, all the measures of ACTH and cortisol were significantly reduced after treatment. Our results are consistent with previous studies reporting a reduction in hormonal responses to the DEX/CRH test after successful pharmacotherapy with antidepressants (Holsboer-Trachsler *et al*, 1991; Heuser *et al*, 1996; Deuschle *et al*, 1997; Baghai *et al*, 2002; Frieboes *et al*, 2003), suggesting that alterations in the HPA axis as detected with the DEX/CRH test are, at least in part, state dependent.

When suppression status was examined, the distributions of nonsuppressors, intermediate suppressors, and suppressors significantly changed after treatment (14, 51, and 34%, respectively, on admission; 11, 14, and 74%, respectively, before discharge) ($p = 0.004$). However, the difference in the rate of nonsuppressors (defined as C_0 of $\geqslant 5\,\mu g/dl$) alone (14 *vs* 11%) was not statistically significant in our patients. This finding indicates that pretreatment with DEX alone (ie conventional DST) lacks sensitivity to monitor changes in HPA abnormalities during the treatment course of depressed patients and that the combination of CRH with DEX (ie DEX/CRH test) is much more sensitive. The definition of 'intermediate suppressors' (defined as C_0 of $<5\,\mu g/dl$ and C_{60} of $\geqslant 5\,\mu g/dl$) might be useful in future studies and in the clinical setting.

When patients who underwent pharmacotherapy alone and patients who underwent ECT in addition to pharmacotherapy were examined separately, the magnitude of hormonal response reduction appeared to be greater in the latter than in the former, suggesting that ECT has an additional effect on restoration from HPA axis abnormalities besides the effect of pharmacotherapy. This finding is consistent with the results from several conventional DST studies that reported a positive correlation between hormonal responses to ECT and the normalization of the HPA system (Albala *et al*, 1981; Papakostas *et al*, 1981; Grunhaus *et al*, 1987; Devanand *et al*, 1991). However, controversial results have also been reported (Coryell, 1986; Fink *et al*, 1987). This inconsistency in the literature may be due in part to the immediate effects of ECT, which activates HPA axis (Aperia *et al*, 1984; Swartz and Chen, 1985). Devanand *et al* (1991) measured plasma cortisol concentrations in depressed patients who had undergone ECT three times, that is, pre-ECT, immediately post-ECT, and 1 week after ECT. They found that plasma cortisol concentrations decreased significantly from pretreatment to immediately post-treatment and then further decreased during the first week after the ECT course. Based on this observation, Devanand *et al* (1991) pointed out that it may be necessary to wait 1 or more weeks in repeated DST studies in patients receiving ECT. We conducted the post-treatment DEX/CRH test before discharge, but not immediately after ECT, which might have reduced the possible effects of postictal increases in plasma ACTH and cortisol concentrations.

Proposal for a Simpler Test Protocol

In our comparisons between patients and controls, cortisol responses were more clearly different from ACTH responses between the two groups. Furthermore, ACTH and cortisol responses generally tend to parallel as pointed out by Heuser *et al* (1994b). Therefore, the measurement of ACTH might add little information. In our study, core results were derivable from the C_0, C_{60}, and C_{dif} values. A simpler test protocol might hence be available which measures plasma cortisol concentrations only twice at 1500 hours (immediately before CRH injection) and 1600 hours (1 h later). Such a simpler protocol might save time and cost without losing essential information to be obtained from the DEX/CRH test and might be practical in the clinical setting. However, the current results were based on observations on in-patients with major depressive episode who were acutely ill, and data on chronic depression or minor depression are unavailable in the current study. Further studies are warranted to draw conclusions as to the appropriateness of such a simple test protocol.

Limitations

The present study involves several limitations. First, the sample size was not very large, which might have given rise to type II errors. Second, all patients were in-patients and acutely ill; hence, severe forms of depression might be over-represented in our sample. It is possible that the sensitivity of the DEX/CRH test might be lower in outpatients and patients with chronic depression as, respectively, reported by Gervasoni *et al* (2004) and Watson *et al* (2002). Third, the majority of patients were medicated with antidepressants, which might have influenced the DEX/CRH results. Although antidepressants *per se* have been shown not to influence test results (Heuser *et al*, 1996; Kunzel *et al*, 2003), it is possible that the effects of drug treatment already emerged in part of patients. Furthermore, drugs may have exerted some effects on the degradation rate of DEX and in turn influenced DEX/CRH test results. However, a recent study of Watson *et al* (2004) compared DEX levels between patients and controls and found that there was no difference in DEX levels between the two groups, suggesting that an effect of psychotropic medication on cortisol output via DEX metabolism appears unlikely. Finally, the detection limits for the hormone assay may have reduced the sensitivity, particularly ACTH responses, of the DEX/CRH test.

Conclusions

In conclusion, we obtained additional evidence for enhanced HPA axis activity, which was detected with the DEX/CRH test when Japanese patients with major depressive episode were compared with controls. Age, gender, but not the fact of being on medication *per se*, are shown to be associated with DEX/CRH test results. Repeated DEX/CRH tests on admission and before discharge provided clear evidence that the DEX/CRH test is a sensitive test as a state-dependent marker to monitor HPA axis abnormalities in Japanese patients with depression. Furthermore, our results suggest that normalization of HPA axis occurs with clinical responses to ECT as well as pharmacotherapy in depressed patients.

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