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Hormonal Response Pattern in the Combined DEX-CRH Test Is Stable over Time in Subjects at High Familial Risk for Affective Disorders

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One of the major neurobiological alterations in depressive disorders consists in a disturbed regulation of the hypothalamic-pituitary-adrenocortical (HPA) system. This is reflected by a pathological increase in the adrenocorticotropin (ACTH) and cortisol release after pretreatment with 1.5 mg dexamethasone (DEX) the previous night and a challenge with 100 μg corticotropin-releasing hormone (CRH) the next day. The changes evoked by this combined DEX-CRH test recede partially with an improvement of the psychopathological symptoms of depressed patients. It is still unclear, however, whether this long-lasting disturbance of the HPA system is due to acquired changes in the acute illness or whether it plays a causal role and could be considered as a trait or vulnerability marker for depression. In a previous study we have examined the HPA function of healthy probands with a high genetic load for affective disorders. We found that this group of high-risk probands (HRPs) showed abnormal DEX-CRH test results with a cortisol release that was between that of a control group and a group of patients with depression. In a follow-up study we now reexamined 14 of the 47 HRPs about 4 years after the index investigation and found surprisingly constant DEX-CRH test results, so that one of the requirements for a vulnerability marker is fulfilled.
[Neuropsychopharmacology 18:253–262, 1998]

KEY WORDS: *Affective disorders; Dexamethasone-CRH test; Vulnerability; Corticotropin*

The most prominent pathobiological deviations in depression involve an altered function of the hypothalamic-pituitary-adrenocortical (HPA) system. During the depressive state, the cortisol secretion is increased and most of the patients with severe depression display an insufficient suppression of cortisol secretion after administration of the synthetic glucocorticoid dexamethasone (DEX) (Carroll 1981) and a blunted adrenocorticotropin (ACTH) response to ovine or human corticotropin-releasing hormone (CRH) (Holsboer et al. 1986; Gold et al. 1986).

The DEX suppression test (DST) using 1 to 2 mg DEX as a test dose became one of the most frequently used neuroendocrine tests to assess HPA system function. Ultimately, the DST proved to be valuable for longitudinally monitoring depressed patients rather than for diagnostic purposes. Although the DST is easily performed in a clinical setting, its value is limited by its low sensitivity, which ranges between 20% and 50% (Arana et al. 1991).

For this reason, a HPA function test that combines the suppression with 1.5 mg DEX and a challenge of 100 μg CRH has been introduced, demonstrating that

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Received March 10, 1997; revised August 6, 1997; accepted August 8, 1997.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/98/$19.00
PII S0893-133X(97)00144-9

depressed patients show an increased cortisol release compared with controls (Holsboer et al. 1987; von Bardeleben and Holsboer 1989). This cortisol release is influenced by age and the severity of depression (von Bardeleben and Holsboer 1991). If these variables are considered, the sensitivity of the DEX-CRH test can be increased to above 90% (Heuser et al. 1994).

Successful treatment of depression leads to a gradual normalization of most of the altered parameters of the HPA system, yet a slightly increased cortisol release remains even in the DEX-CRH test of already remitted patients. The persistency of a completely abnormal test response is seen if the clinical outcome is poorer, and those patients have an increased risk of relapse (Holsboer et al. 1987; Holsboer-Trachsler et al. 1991, 1994). The HPA function of healthy controls, however, is not influenced by the administration of antidepressants over weeks, suggesting that the antidepressive effect is mediated by the normalization of the HPA feedback control in depression (Heuser et al. 1996).

So far, it is not yet clear whether the changes in the DEX-CRH test of remitted patients should be regarded as a state marker, because they are due to long-term consequences of central neurotransmitter changes responsible for the development of depression. Alternatively, these changes in the HPA regulation may play a causal role and therefore could be considered as a trait marker for depression.

Several family and twin studies provide evidence that a genetic predisposition is one of the major factors for the development of an affective disorder (Bertelsen et al. 1977; Crowe et al. 1983; Weisman et al. 1984; Wender et al. 1986; Kendler et al. 1993, 1994; Sadovnick et al. 1994). The neurobiological equivalent of this "predisposition", however, is still completely unclear and has not been examined so far. If the alteration of the HPA system attributes to an increased risk for the development of depression, it should then be present in healthy members of families with a high load of affective disorders. For this reason we investigated healthy subjects with a high genetic load for affective disorders. As recently reported (Holsboer et al. 1995), this group of so-called high-risk probands (HRPs) showed abnormal DEX-CRH test results with a cortisol release that was between that of the control group and the depressed group. Moreover a discriminant analysis identified 32% of the HRPs as showing a hormonal response pattern indistinguishable from that of the depressed patients. We interpreted these findings in terms of a genetically transmitted risk factor influencing the HPA system and rendering the HRPs vulnerable to the development of an affective disorder.

In the present investigation, we reexamined a subgroup of 14 HRPs after a mean time interval of 4 years, supposing that if the previously found pathobiological deviations in the function of the HPA system might serve as a vulnerability marker, they would have to require a certain stability over time.

MATERIAL AND METHODS

Subjects

High-Risk Probands. As the general selection procedure of the initial sample of high-risk probands (HRPs) is introduced elsewhere in more detail (Holsboer et al. 1995; Lauer et al. 1995), only a brief description will be given here. A total of 431 consecutively admitted inpatients with a diagnosis of major depression, bipolar disorder, or "bipolar II" disorder (bipolar disorder NOS) were screened to identify those patients who had (1) at least one first-degree relative with an affective disorder or schizophrenia, and (2) at least one first-degree relative with no current or lifetime DSM-III-R diagnosis of a psychiatric disorder (HRPs). All diagnoses including the "negative" ones of the HRPs were verified by the Structured Clinical Interview for DSM-III-R (German version; Wittchen et al. 1990). Of the 72 inpatients fulfilling the study inclusion criteria (1) and (2), 37 refused to let their relatives participate in the study. The relatives of three further patients did not participate in the neuroendocrine part of the study. Of the remaining 32 families, a total of 47 HRPs agreed to participate in the neuroendocrine protocol at index assessment (t_1).

At follow-up investigation (t_2), we had lost contact to 18 HRPs, because of a change of address or because we were not allowed to recontact them due to the German law of personal data protection. Nine HRPs refused to participate, two HRPs had developed an affective disorder, and three HRPs could not be investigated due to continuous shift work ($n = 1$), frequent transmeridian flights ($n = 1$), and pregnancy ($n = 1$). In one HRP, the cortisol plasma concentrations measured at t_2 could not be analyzed due to technical reasons. Therefore, at follow-up investigation, the study sample consisted of 14 HRPs, who had remained in good health over the follow-up period (seven female, seven male; mean age at t_1: 31.4 $\pm$ 2.0 years; mean age at t_2: 35.2 $\pm$ 2.7 years; mean duration of follow-up period: 46.4 $\pm$ 3.5 months; range: 22 to 70 months). These 14 HRPs are members of 11 families (one family provided two HRPs and one family three HRPs). The respective diagnoses of the index patients were bipolar disorder ($n = 3$), major depression, recurrent episode ($n = 5$), and major depression, single episode ($n = 3$). The affected first-degree relatives of the index patients had the following diagnoses: major depression, recurrent episode ($n = 8$) and major depression, single episode ($n = 3$). To assess stressful life events that had happened between the index and follow-up investigation, the HRPs had to fill in a life event questionnaire (Sulz 1992) before the follow-up investigation.

Subjects with Depression and Healthy Controls. To keep the reference groups constant, we used the data of the same subjects already assessed at t_1. Briefly, one comprised 18 inpatients with major depression (in accordance with DSM-III-R; 14 women, four men; mean age: 34.3 ± 1.9 years; age range: 22 to 45 years) who had been drug-free for a least 2 weeks and suffered from severe depression (mean score on the Hamilton Rating Scale for Depression: 26.1 ± 6.6 points). The control group comprised 20 normal volunteers without any personal or family history of psychiatric disorders (seven women, 13 men; mean age: 30.9 ± 1.6 years; age range: 21 to 45 years).

All HRPs and CPs had been free of any prescription or nonprescription drug for at least 2 months. Possible concurrent medical disorders and drug abuse were ruled out by thorough medical examination and laboratory tests (including electrocardiography, blood analysis, and urinary screening for drugs such as amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates).

Neuroendocrine Protocol

After careful exclusion of acute illness (e.g., infection) and a lifestyle that could interfere with the test (e.g., sleep deprivation, shift work, transmeridian flights), all subjects received an oral dose of 1.5 mg dexamethasone at 23:00 h. The following day they were given a calorie- and electrolyte-balanced lunch before an indwelling intravenous forearm catheter was inserted at 13:00 h and connected to a long tube that ran through a soundproof lock into the adjacent laboratory . The subjects rested in a supine position and were monitored via a video system to exclude their falling asleep. Blood samples were drawn through the long tube at the time points given in Figure 1. At 15:00 h 100 μg hCRH (Bissendorf Peptide, Wedemark, Germany) reconstituted in 1 ml 0.02% HCl in 0.9% saline solution were infused with 30 s.

Hormone Analysis

Cortisol plasma concentrations were analyzed using a commercially available radioimmunoassay kit with a coated tube technique (ICN Biomedicals, Carson, CA). The detection limit was 0.3 ng/ml plasma; intra- and interassay coefficients of variation for 20 and 40 ng/ml were <7%.

For ACTH measurements an immunoradiometric assay without extraction was used (Nichols Institute, San Juan Capistrano, CA). The detection limit for plasma ACTH concentrations was 4.0 pg/ml and the intra- and interassay coefficients of variation at 20 pg/ml plasma were <8%.

The mean values for the samples drawn between 14:00 h and 15:00 h are reported as the basal concentration. The maximal hormone responses after CRH administration (between 15:00 h and 18:00 h) are reported as peak values. Furthermore, the maximal post-CRH increase corrected for baseline values was calculated (delta). After CRH infusion the ACTH and cortisol responses were computed as the area under the time course curve (AUC) using trapezoidal integration. They are reported at AUC_{total} (not baseline-corrected) and as AUC_{net} (baseline-corrected). The dexamethasone suppression test (DST) status was determined on the basis of the highest plasma cortisol concentration between 14:00 h and 15:00 h, with a cutoff value of 110 nmol/L to define DST nonsuppression. Finally, adrenocortical responsivity to ACTH was assessed by calculating two pituitary-adrenal ratios (PAR; AUC_{total} ACTH/AUC_{total} cortisol values and AUC_{net} ACTH/AUC_{net} cortisol values).

Statistical Methods

Group means were compared using analysis of variance (ANOVA) and analysis of covariance (ANCOVA, with an age and time interval between index assessment and follow-up investigation as the respective covariables). When there were significant main group effects, their subeffects on group differences were analyzed via parameter estimates for contrasts (High-Risk Probands (HRPs) versus Control Probands (CPs), HRPs versus Depressed Patients (DPs)) or via ANCOVA. To compare the HRPs' data at t_1 and t_2, a repeated measurements ANCOVA (with the time interval between index assessment and follow-up investigation as the covariable) was performed. In addition, linear canonical discriminant analysis (method: minimizing Wilks lambda) was performed to determine which subjects of the HRP group belonged to the DP group and which to the CP group according to their hormonal response. As nominal level of significance $\alpha = 0.05$ was accepted. Because the acceptance of the null hypothesis appeared to us to be important for our study, we accepted $\beta = 0.10$ as type II error.

RESULTS

As shown in Figure 1, the combined DEX-CRH test induced plasma ACTH and cortisol response curves in the HRPs that did not differ between the index assessment and the follow-up investigation. At both time points, the ACTH response curves were comparable between the HRPs and the CPs, but were lower than those of the DPs, whereas the cortisol response curves were different for each one of the three groups studied.

Index Assessment (t_1)

At index assessment, the hormonal measurements assessed in the HRPs who participated in the follow-up

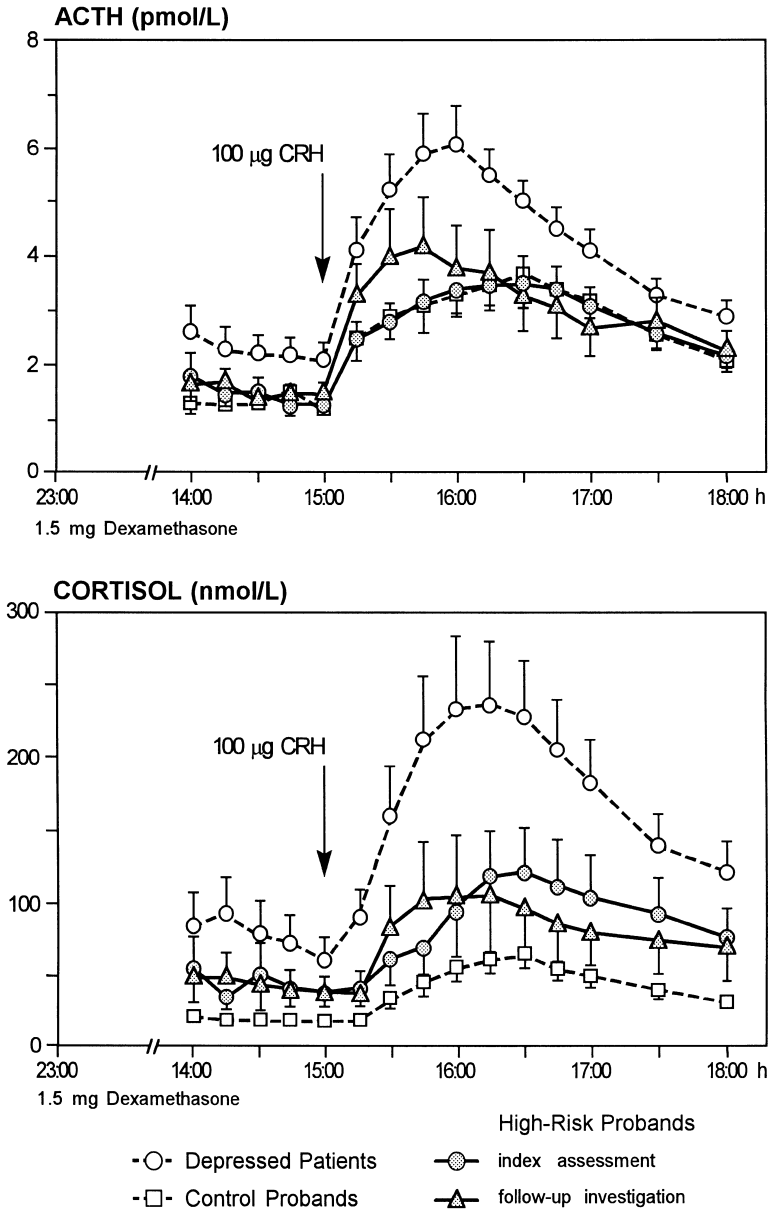

Figure 1. Plasma ACTH and cortisol concentrations (mean ± SEM) in dexamethasone-pretreated subjects before and after intravenous CRH injection.

investigation ($n = 14$) were similar to those observed in the HRPs not participating at follow-up ($n = 33$; $F(1,44) < 0.80$, $p > .38$; data not shown). Furthermore, the comparison of the 14 "follow-up" HRPs, the CPs and the DPs yielded results that were comparable to those earlier reported of the total group of 47 HRPs (Holsboer et al. 1995). In more detail, significant main group effects were found with respect to the dexamethasone-pretreated (basal) ACTH and cortisol levels, the ACTH and cortisol peak levels, the cortisol delta value, the AUC_{total} for ACTH and cortisol, and the AUC_{net} value for cortisol (Table 1). In order to locate these differences, contrasts were applied. No significant differences in the ACTH parameters were found between the HRPs and the CPs. The cortisol basal and peak values as well as the AUC_{total} were significantly higher in the HRPs than in the CPs. Compared with the DPs, the HRPs tended to have lower ACTH basal values, but they had significantly less pronounced ACTH peak and delta values and a lower AUC_{total} for ACTH. In addition, their cortisol peak and delta values were less pronounced, resulting in a smaller AUC_{total} for cortisol. On the other hand, the cortisol AUC_{net} only tended to be decreased in the HRPs when compared to the DPs, and the cortisol basal values were similar in both groups. Calculation of PARs revealed that the adrenocortical responsiveness of the HRPs to ACTH was between that of the controls and the DPs.

Because the gender distribution differed significantly in the three study samples $\chi^2(2) = 7.09$, $p < .05$, the data were analyzed by a two-factor ANOVA with gender as the second factor. However, none of the pa-

Table 1. Results (mean ± SEM) of the DEX-CRH Challenge-Test at Index Assessment in 14 High-Risk Probands, 20 Control Probands, and 18 Depressed Patients

	High-Risk Probands HRP (n = 14)	Control Probands CP (n = 20)	Depressed Patients DP (n = 18)	ANOVA F(2,49)	t-Tests p values	
					HRP vs. CP	HRP vs. DP
Age (years)	31.4 ± 2.0	30.4 ± 1.5	34.3 ± 1.9	1.06	—	—
ACTH (pmol/L)						
Basal	1.50 ± 0.27	1.32 ± 0.13	2.28 ± 0.31	4.60[b]	NS	=0.07
Peak	4.03 ± 0.66	4.31 ± 0.60	6.54 ± 0.75	4.43[b]	NS	<0.05
Delta	2.53 ± 0.45	2.99 ± 0.60	4.26 ± 0.66	2.50[a]	NS	<0.05
AUC_{total}	498 ± 78	497 ± 48	747 ± 73	5.20[c]	NS	<0.05
AUC_{netto}	228 ± 51	258 ± 44	336 ± 60	1.24	—	—
Cortisol (nmol/L)						
Basal	43.8 ± 13.7	19.3 ± 1.8	77.3 ± 20.1	4.87[c]	<0.05	NS
Peak	132.9 ± 35.5	75.3 ± 10.7	278.2 ± 46.9	11.20[d]	<0.05	<0.05
Delta	89.0 ± 27.9	56.0 ± 10.4	201.0 ± 44.2	7.28[c]	NS	<0.05
AUC_{total}	15064 ± 3947	7773 ± 1071	29928 ± 5251	10.73[d]	<0.05	<0.05
AUC_{netto}	7178 ± 2578	4301 ± 1027	16022 ± 4994	5.71[c]	NS	=0.08
PAR_{total}	0.06 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	2.61[a]	=0.09	NS
PAR_{netto}	0.02 ± 0.04	0.04 ± 0.05	−0.70 ± 0.66	0.97	—	—

Basal: Mean value for the samples between 14:00 and 15:00 h (baseline value); Peak: Maximal hormone response after CRH administration; Delta: Basal-corrected peak; AUC_{total}: Area under the response curve between 15:00 and 18:00 h; AUC_{net}: Basal-corrected area under the response curve between 15:00 and 18:00 h; PAR: Pituitary-adrenal ratio; ratio of AUC for ACTH to AUC for cortisol.

ANOVA: [a] $p < .10$.

[b] $p < .05$.

[c] $p < .01$.

[d] $p < .001$.

t-Tests: — = not tested; NS = not significant.

rameters investigated was significantly affected by the subjects' gender and, therefore, the results already presented were not affected either.

Follow-Up Investigation (t_2)

ANCOVA, repeated measurement design, with the time period between index assessment and follow-up investigation as the covariable, revealed no systematic changes in the hormonal response pattern of the 14 "follow-up" HRPs (Table 2a, Table 2b; Figure 2). Furthermore, no significant effects of the covariable "time period" on any of the investigated measurements were found ($F(1) < 2.49$, $p > .15$).

At follow-up, the HRPs used to be of higher age than the CPs ($F(1, 32) = 3.64$, $p = .06$). Therefore, we performed analyses of covariance (ANCOVA) with age being the covariable to compare the HRPs, the CPs, and the DPs. After controlling for possible age-related effects, significant main group differences were found in the dexamethasone-pretreated (basal) ACTH and cortisol levels, the ACTH and cortisol peak levels, the cortisol delta value, the AUC_{total} for ACTH and cortisol and the AUC_{net} value for cortisol (Table 2a, b). To locate these significant differences, subsequent group by group comparisons were performed (HRPs versus CPs; HRPs versus DPs). Compared to the CPs, the HRPs had significantly higher cortisol basal values, cortisol peaks and cortisol AUC_{tot} values. The remaining parameters did not differ between both groups. These findings were well comparable with those obtained at index assessment. As opposed to the DPs, the HRPs showed lower ACTH basal values and a lower ACTH AUC_{total}; these findings nearly reached a level of significance. In contrast to the index assessment, the ACTH peak value was no longer significantly less pronounced in the HRPs at follow-up. The results of the cortisol measurements, however, were completely identical with those obtained at the index assessment: the cortisol basal values were similar in the HRPs and the DPs, the cortisol peak and delta values were significantly less pronounced in the HRPs, again resulting in a significantly decreased cortisol AUC_{total}, whereas the cortisol AUC_{net} only tended to be decreased in the HRPs compared to the DPs.

Calculation of PARs revealed that the adrenocortical responsiveness of the HRPs to ACTH continued to range (nonsignificantly) between that of the controls and the DPs. Again, these findings obtained at follow-up were not affected by the subjects' gender.

Classificational Observations

Before CRH stimulation at 15:00 h, none of the CPs escaped cortisol suppression by dexamethasone, whereas 4 DPs (22%) were identified as dexamethasone nonsup-

Table 2a. Results (mean ± SEM) of the DEX-CRH Challenge-Test at Index Assessment and at Follow-up Investigation in 14 High-Risk Probands

	High-Risk Probands HRP			
	Index Assessment (n = 14)	**Follow-up Investigation (n = 14)**	**Control Probands CP (n = 20)**	**Depressed Patients DP (n = 18)**
Age (years)	31.4 ± 2.0	35.2 ± 2.7	30.4 ± 1.5	34.3 ± 1.9
ACTH (pmol/L)				
Basal	1.50 ± 0.27	1.53 ± 0.24	1.32 ± 0.13	2.28 ± 0.31
Peak	4.03 ± 0.66	4.62 ± 0.96	4.31 ± 0.60	6.54 ± 0.75
Delta	2.53 ± 0.45	3.09 ± 0.75	2.99 ± .060	4.26 ± 0.66
AUC_{total}	498 ± 78	537 ± 99	497 ± 48	747 ± 73
AUC_{netto}	228 ± 51	261 ± 67	258 ± 44	336 ± 60
Cortisol (nmol/L)				
Basal	43.8 ± 13.7	43.6 ± 12.9	19.3 ± 1.8	77.3 ± 20.1
Peak	132.9 ± 35.5	121.8 ± 40.9	75.3 ± 10.7	278.2 ± 46.9
Delta	89.0 ± 27.9	78.2 ± 33.4	56.0 ± 10.4	201.0 ± 44.2
AUC_{total}	15064 ± 3947	13805 ± 4734	7773 ± 1071	29928 ± 5251
AUC_{netto}	7178 ± 2578	5958 ± 3365	4301 ± 1027	16022 ± 4994
PAR_{total}	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.01
PAR_{netto}	0.02 ± 0.04	0.04 ± 0.06	0.04 ± 0.05	−0.70 ± 0.66

The reference data of the control probands and the depressed patients are also indicated.

Basal: Mean value for the samples between 14:00 and 15:00 h (baseline value); Peak: Maximal hormone response after CRH administration; Delta: Basal-corrected peak; AUC_{total}: Area under the response curve between 15:00 and 18:00 h; AUC_{net}: Basal-corrected area under the response curve between 15:00 and 18:00 h; PAR: Pituitary-adrenal ratio; ratio of AUC for ACTH to AUC for cortisol.

pressors according to our normative data base (Holsboer et al. 1986). Two HRPs (14%) were nonsuppressors at index assessment and another HRP (7%) was identified as nonsuppressor at the follow-up investigation.

During the 3 h after CRH stimulation (15:00 h to 18:00 h) four CPs (20%) and 14 DPs (78%) escaped dexamethasone suppression of cortisol. At index assessment, this was the case in six HRPs (43%; including the two HRPs identified as nonsuppressors prior to CRH stimulation), and four of these HRPs as well as one further HRP were classified as nonsuppressors at follow-up investigation (including the HRP identified as nonsuppressor before CRH stimulation).

We earlier reported (Holsboer et al. 1995) that 15 of

Table 2b. Results of the Statistical Analyses

	ANCOVA Repeated Measurements Index vs Follow-up		**ANCOVA at Follow-Up HRP-CP-DP**		**ANCOVA at Follow-Up p-Value**	
	F(1,13)	**p**	**F(2,48)**	**p**	**HRP vs. CP**	**HRP vs. DP**
ACTH (pmol/L)						
Basal	0.08	NS	4.64	0.015	NS	=0.08
Peak	1.95	NS	3.19	<0.05	NS	NS
Delta	2.71	NS	1.50	NS	—	—
AUC_{total}	0.90	NS	4.31	<0.05	NS	=0.09
AUC_{netto}	0.90	NS	0.95	NS	—	—
Cortisol (nmol/L)						
Basal	1.81	NS	4.78	<0.05	<0.05	NS
Peak	1.19	NS	9.63	<0.001	<0.05	<0.05
Delta	0.47	NS	7.41	<0.01	NS	<0.05
AUC_{total}	1.14	NS	8.78	<0.001	<0.05	<0.05
AUC_{netto}	0.07	NS	5.13	<0.01	NS	=0.08
PAR_{total}	0.19	NS	1.95	NS	—	—
PAR_{netto}	0.02	NS	0.91	NS	—	—

ANCOVA: — = not tested; NS = not significant.

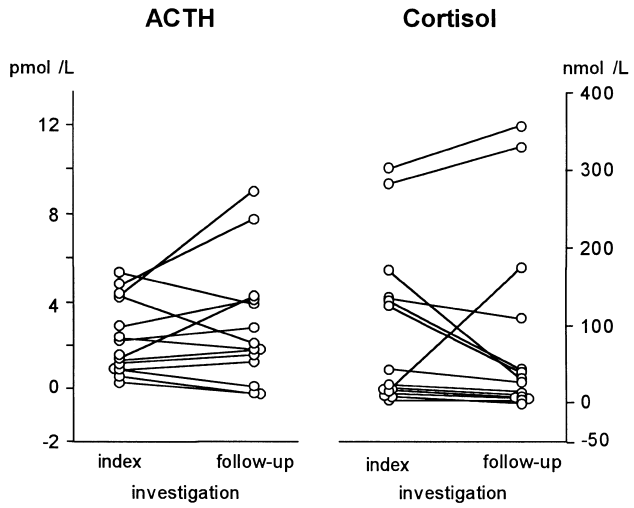

Figure 2. Changes in the ACTH and cortisol delta values between index assessment and follow-up investigation in 14 HRPs.

the 47 HRPs (32%) were identified as showing a depression-like hormonal response to CRH stimulation by using a multivariate approach (discriminant equation in which the plasma concentrations of ACTH and cortisol at 15:00 h, 15:30 h, 15:45 h, 16:00 h, and 16:15 h were used as the independent variables and the CPs and DPs as the grouped cases, according to the proposal of Heuser et al. 1994). On applying the same discriminant equation to the present subsample of 14 HRPs, four subjects (29%) were identified who showed such a depression-like hormonal response pattern at index assessment. At follow-up investigation, however, only two of these HRPs (14%) continued to show this response pattern.

DISCUSSION

The hormonal response pattern in the combined DEX-CRH challenge test of the present group of high-risk probands remained stable over time between the index assessment and the follow-up investigation that took place about 4 years later. At both time points the response pattern of the HRPs, on average, was between that of the healthy controls and the patients with depression.

The finding that our 14 HRPs—as a group—showed comparable hormonal response patterns at index assessment and at follow-up investigation could also be confirmed on an individual level. As indicated in Figure 2, the rise in ACTH and cortisol levels after CRH injection (expressed as delta-values) was only slightly changed in 12 HRPs. In one HRP, however, there was a sharp increase in cortisol release. In this case, the change in the secretory response may be attributed to a recent stressful life-situation, because during the 3 months preceding participation in the follow-up investigations, this proband had completed her university education, had moved back to her parents' home accompanied by her husband and her little child, and was anxious about her unemployment and the difficulty of finding a position in her profession. A second HRP showed a sharp decline in cortisol release as well. However, no stressful life events had been obvious at index assessment, and the life-situation of this HRP had remained stable until follow-up investigation, rendering underlying neuroadaptive processes unlikely.

Our classificational observations appear to be less robust. The two HRPs who had been identified as DEX-nonsuppressors at index assessment did not escape cortisol suppression at follow-up. However, several studies have demonstrated that the sensitivity of this "test phase", which is similar to the dexamethasone suppression test, is relatively low (Arana et al. 1991). A more stable classification result was obtained when plasma cortisol levels were considered after CRH injection. At follow-up, four of the initial six HRPs continued to correspond to their earlier classification as cortisol nonsuppressors. One has to keep in mind, however, that the applied "critical" cortisol value (110 nmol/L) is an arbitrarily chosen reference value, although it was derived from our large normative data base. Finally, based on a discriminant equation for which we had demonstrated a high specificity (95%) and a sufficient sensitivity in our CPs and DPs (83%) (Holsboer et al. 1995), four HRPs were classified as showing a conspicuous and depression-like hormonal response pattern at index assessment and two of them at follow-up. At the moment, this finding indicates that a conspicuous hormonal response profile in the DEX-CRH test is not as stable over time. This may lessen the probability that this particular profile indicates vulnerability for affective disorders, a suggestion that we raised earlier (Holsboer et al. 1995). However, a definite answer to the question of which of the measurements assessed in the DEX-CRH test indeed represents a vulnerability marker can only be found when we are aware of those HRPs who developed an affective disorder during the observation period.

Unfortunately we had to deal with a very high dropout rate in our follow-up study. We were only able to reexamine 14 of the 47 HRPs of the index investigation. One reason for this was the high mobility of the HRPs, especially of those living in larger cities, who frequently moved, the address unknown. A second reason for the high dropout rate was the tightening of the personal data law in Germany in the beginning of the 1990s that forbid us to contact again those HRPs who had been recruited in another county. Moreover, some HRPs show a special personality pattern (Lauer et al. 1997) that requires a close, permanent, stable, and therefore very time-consuming way of keeping contact and permits only a reduced flexibility with regard to changes related

to the examining person or to minor deviations in the arranged time protocol, which also sometimes leads to dropouts.

Although the HPA system shows interindividual variations (Mason 1968), good evidence of heritability is derived from studies in which monozygotic twin pairs were examined. Maxwell et al. (1969) found a resemblance of unstimulated plasma cortisol levels in samples of female monozygotic twin pairs. Meikle et al. (1988) reported of a genetic impact on morning plasma cortisol levels in male and female monozygotic twins. Also, the response to an acute stimulation with 100 μg CRH showed a strong genetic influence comparing mono- and dizygotic twin pairs (Kirschbaum et al. 1992).

Stability over time of both normal and abnormal results in the dexamethasone suppression test (DST) has also been shown in healthy subjects who underwent three DSTs a month (Coryell and Zimmerman 1987). Moreover, the authors demonstrated that relatives of nonsuppressors were at a significantly higher morbid risk of developing an affective disorder.

The mechanism underlying the enhanced cortisol response to the DEX-CRH test in depressed patients and in high-risk-probands is not yet clear. Studies in which varying doses of dexamethasone were administered showed a dose-dependent release of ACTH and cortisol in healthy controls (Heuser et al. 1994) as well as in depressed patients (Modell et al. 1997). A higher cortisol and ACTH surge was seen in patients versus controls in comparable dosage groups. These findings suggest that a pathological DEX-CRH test reflects a decreased central corticosteroid receptor function in depression.

The reason for this corticosteroid receptor dysfunction is still unknown. Maybe, the homo- or heterodimerization pattern of gluco- and mineralocorticoid receptors (Trapp et al. 1994) and affiliated components such as heat shock proteins and transcription factors (Truss and Beato 1993) are changed. A genetically transmitted vulnerability factor could also result in a receptor polymorphism.

In the presence of a decreased corticosteroid receptor function, the suppression of vasopressin release is incomplete (Fink et al. 1991). As a result, the administration of CRH leads to an exaggerated ACTH and cortisol release (Hermus et al. 1986) due to the increased AVP secretion, because DEX does not add to the suppressing effect of corticosteroids at the hypothalamic level.

These results have been demonstrated in healthy human controls (von Bardeleben et al. 1985). They are further supported by postmortem findings of an increased number of CRH neurons with AVP colocalization and expression in the hypothalamic paraventricular nucleus of depressed patients (Raadsheer et al. 1993, 1994; Purba et al. 1996). Moreover, transgenic mice with an altered glucocorticoid receptor function show "depressive symptoms" (Richard et al. 1993). These could be improved by a long-term antidepressant treatment resulting in an enhanced corticosteroid receptor function with a more effective negative HPA feedback (Montkowski et al. 1995, Reul et al. 1993, 1994).

But even if a genetically altered HPA regulation seems to be present in HRPs: what is, finally, the cause for the actual development of an affective disorder? Depression is probably not caused by changes in a single gene; more likely, it is the result of many genes acting together. Even if all of them could be identified, environmental factors cannot be ruled out.

We assume that the HPA feedback disturbance lowers the threshold for the disease and results in increased liability to develop an affective illness, e.g., in response to specific life events that are often preceding the onset of an acute episode (Post 1992). This effect could also be observed in one of our HRPs who showed a marked increase in the hormonal response in the DEX-CRH test in the presence of a stressful life situation. However, it is still premature to deduct any predictive value from this result. For this reason we will follow-up our HRPs for the next years and try to characterize the premorbid features of those HRPs who actually develop an affective disorder, because the determination of a genetic factor for an increased susceptibility to depression in an individual would then open the possibility of taking preventive measures (Merikangas et al. 1989).

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