Research Article

ABNORMAL NEUROENDOCRINE RESPONSE TO CLOMIPRAMINE IN HEREDITARY AFFECTIVE PSYCHOSIS

Joachim Cordes, M.D.,[1]* Rolf Larisch, M.D.,[2] Uwe Henning, M.D.,[1] Johanna Thünker,[1] Christian Werner, M.D.,[1] Guillermo Orozco, M.D.,[3] Fermín Mayoral, M.D.,[3] Fabio Rivas, M.D.,[3] Georg Auburger, M.D.,[4] Marco Tosch, M.D.,[2] Marcella Rietschel, M.D.,[5] Wolfgang Gaebel, Ph.D.,[1] Hans-Wilhelm Müller, M.D.,[2] and Ansgar Klimke, M.D.[1]

Background: *Blunting of prolactin response after serotonergic stimulation during a major depressive episode has been described by several investigators. In this study, the neuroendocrine responses to clomipramine were assessed in remitted patients suffering from hereditary depression.* **Methods:** *Twenty remitted patients from 11 large families with multigenerational, multiple cases of major affective disorder (bipolar disorder n = 15, recurrent depression n=5, according DSM-IV) and 12 healthy relatives were investigated. After intravenous application of 12.5 mg of the serotonin re-uptake inhibitor clomipramine, serum prolactin and cortisol levels were analysed.* **Results:** *Patients and comparison group did not differ significantly with respect to age, baseline prolactin and cortisol concentrations. A gender effect was found in an exploratory analysis for prolactin but not for cortisol and therefore the data for prolactin were analysed seperately. After clomipramine infusion, the increase of cortisol was significantly lower in patients than in the comparison group (P = .046). For prolactin, this effect could be found in the male (P = .012) as well as in the female (P = .007) subsample.* **Conclusions:** *These results suggest that blunted prolactin and cortisol responses to serotonergic stimulation are characteristic for remitted depressive patients with previous episodes of major affective disorders. Depression and Anxiety 26:E111–E119, 2009.*

Key words: *depression; affective disorder; challenge; response; prolactin; cortisol*

INTRODUCTION

The substantial contribution of genetic factors in the etiology of bipolar disorder has repeatedly been shown by family and twin studies. As the disorder is genetically heterogeneous, a most promising strategy to reduce this heterogeneity is to search for the vulnerability genes and the biological underpinnings in genetically homogeneous populations[1] and in patients from certain geographically restricted regions, e.g., in Scotland,[2] Iceland,[3] Finland,[4] or Costa Rica.[5] There is evidence that the neurotransmitter serotonin plays an important role in the etiology of affective disorders as well as in treatment response.[6,7] Experimental depletion of the serotonin precursor tryptophan induces a transient lowering of mood in normal subjects[8,9] and can induce an acute deteriora-

[1]Department of Psychiatry, Heinrich-Heine-University, Düsseldorf, Germany
[2]Department of Nuclear Medicine, Heinrich-Heine-University Düsseldorf, Germany
[3]Hospital General Carlos Haya, Málaga, Spain
[4]Department of Neurology, Heinrich-Heine-University, Düsseldorf, Germany
[5]Department of Psychiatry, University of Bonn, bonn, Germany

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*Correspondence to: Dr. Joachim Cordes, M.D., Psychiatric Department of the Heinrich-Heine-University Duesseldorf, Bergische Landstraße 2, D-40629 Germany.
E-mail: joachim.cordes@lvr.de

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tion in remitted depressive patients.[10,11] Sobczak and colleagues investigated the effects of an intravenous tryptophan challenge and placebo on cognitive performance in 30 healthy first-degree relatives of bipolar patients and 15 matched controls in a double-blind crossover design. After administration of tryptophan, planning and attention were impaired in bipolar subjects, but not in controls.[12] Cognitive deficits in bipolar subjects, after tryptophan administration may reflect a central 5-hydroxytryptophan (5-HT) vulnerability in frontal brain areas. Tryptophan-triggered relapse rate in recovered depressed patients, who were selective serotonin reuptake inhibitor (SSRI) treated, is not higher than 50–60%.[11] Acute tryptophan depletion (ATD) in currently depressed patients treated with serotonergic antidepressants possibly provides important information about the mechanism of action of SSRIs.[13] Data of six ATD studies were pooled to investigate the mediating role of clinical, demographic, and biochemical characteristics in mood response to ATD. Recurrent depressive episodes, in female gender, before exposure to SSRI antidepressant treatment and previous serious suicidal thoughts/attempts all appear to be independent predictors of mood response to ATD. Chronicity of illness is the most powerful predictor.[8] Furthermore, prolactin release is reduced[14–18] and regional brain responses are blunted[19] in major depression after the serotonin releasing agent fenfluramine, the serotonin reuptake inhibitor clomipramine[20] or the serotonin precursor tryptophan.[21,22] Clomipramine is a potent inhibitor of 5-HT uptake at concentrations that have little effect on norepinephrine, although its demethylated metabolite does inhibit norepinephrine reuptake.[23] However, the lack of specificity of clomipramine which certainly has noradrenergic activites should be considered in the interpretation of the study results. A serotonin reuptake inhibitor like citalopram is more specific to 5-HT function, but the clomipramine infusion (CMI) challenge test has a dose–response relationship for the neuroendocrine response to CMI challenge.[24] Intravenous administration of clomipramine minimizes the problems seen with oral pharmacologic challenges regarding interindividual differences in rate and degree of absorption.[20] Furthermore, this type of administration avoids the "first pass" effect of hepatic metabolism and thereby delays the formation of demethylated clomipramine and minimizes its effect on norepinephrine during the period of hormonal measurements.[24] Several studies have described abnormal neuroendocrine responses to CMI challenge in depression, in particular, blunted prolactin responses.[20,25] A clomipramine challenge induces a decrease of the binding potential of altanserin for 5-HT-2 receptors in subjects with a history of recurrent major depression.[26] It remains to be clarified, whether genetic predisposition for affective disorders is associated with a serotonergic dysregulation. There is evidence that a transient lowering of mood after tryptophan depletion is especially pronounced in subjects with positive family history of affective psychosis.[27] A report suggests a significant association between tryptophan hydroxylase genotypes and manic-depressive illness.[28] Genetic factors impairing the serotonergic system have also been suggested by the finding that prolactin response in opioid addicts was linked to comorbidity with familial depression.[29] Using a placebo-controlled crossover design, clomipramine-induced prolactin release was significantly greater in subjects with a polymorphism in the promotor region of the human serotonin transporter (5-HTT) gene. A variant is associated with lower expression of 5-HTT sites and a reduced efficiency of 5-HT reuptake.[30] These results are consistent with clinical data indicating that subjects with this genotype may have a poorer therapeutic response to SSRI monotherapy. However, there are controversial findings as to whether blunted prolactin response to a serotonergic challenge is a *state* marker during the depressive episode[18,31–33] or a *trait-like* disturbance after remission of depression[34,35] or present in absence of depressive symptoms, e.g., in patients with a history of suicide attempts and aggressive and impulsive traits.[36] Therefore, this study investigates whether a blunted prolactin response to serotonergic stimulation is also present outside acute depressive episodes. For this purpose, we applied the clomipramine challenge paradigm to remitted patients and their relatives from large andalucian families with multiple cases of multigenerational affective disorder.

METHOD

SUBJECTS

After systematic screening of bipolar patients in the psychiatric department of the University of Düsseldorf, we identified two index patients from a large andalucian group of families with multiple cases of affective psychosis. Collaboration with the psychiatric hospital in Málaga and personal interviews of associated psychiatrists revealed evidence, in 11 large families, for the existence of multiple, multigenerational cases of bipolar disorder or recurrent depression within a geographic area of 50 km^2 in northern Málaga (Table 1). An example of one representative family is given in Figure 1. An extensive interview, using the schedule for affective disorders and schizophrenia-lifetime[14] and the family history questionnaire (family informant schedule and criteria)[37] was performed by an experienced genetic (G.O.) and psychiatric (M.R.) investigator. A lifetime diagnosis of an affective disorder according to DSM-IV criteria was assessed in all patients and excluded in relatives. Furthermore, comorbidity with any other axis I or II diagnosis, including alcoholism or drug dependency, was excluded in both groups. On this occasion, all family members were asked for participation in this study. Twenty patients from 11 families and 12 non-affected relatives from seven families (for details, see Table 2) gave their written informed consent after the experimental procedure had been fully explained to them. According to DSM-IV criteria, the subtype of major affective disorder was bipolar I in 14 patients, bipolar II in one patient, and recurrent major depression in five patients. From the 12 comparison subjects, six were first-degree relatives of the investigated patients, and six were non-consanguineous spouses. Hamilton

TABLE 1. Number of family members and characterization of the included families

ID	Total/ generation	Alive	Dead (suicide)	Assessed so far	Non-affected	BP I	BP II	Major depression recurrent	Major depression single episode	% MAD/ assessed
A	84/4	73	11(0)	72	56	8	2	6	0	22.2
B	27/3	18	9(0)	12	7	1	0	4	0	41.6
C	95/5	85	10(1)	47	39	5	0	3	0	17.0
D	36/5	31	5(1)	24	19	4	1	0	0	20.8
E	107/5	84	23(0)	102	73	12	6	9	2	28.4
F	107/7	68	39(5)	92	71	13	2	4	2	22.8
G	34/4	18	16(0)	26	22	3	1	0	0	15.4
H	68/4	45	23(5)	59	41	10	2	3	3	30.5
I	37/4	26	11(0)	33	26	6	0	1	0	21.2
J	20/3	9	11(0)	13	9	4	0	0	0	30.8
K	7/2	5	2(1)	7	4	0	0	3	0	42.9
Σ	620	462	160 (13)	487	367	66	14	33	7	24.6

BP, bipolar; MAD, major affective disorder.

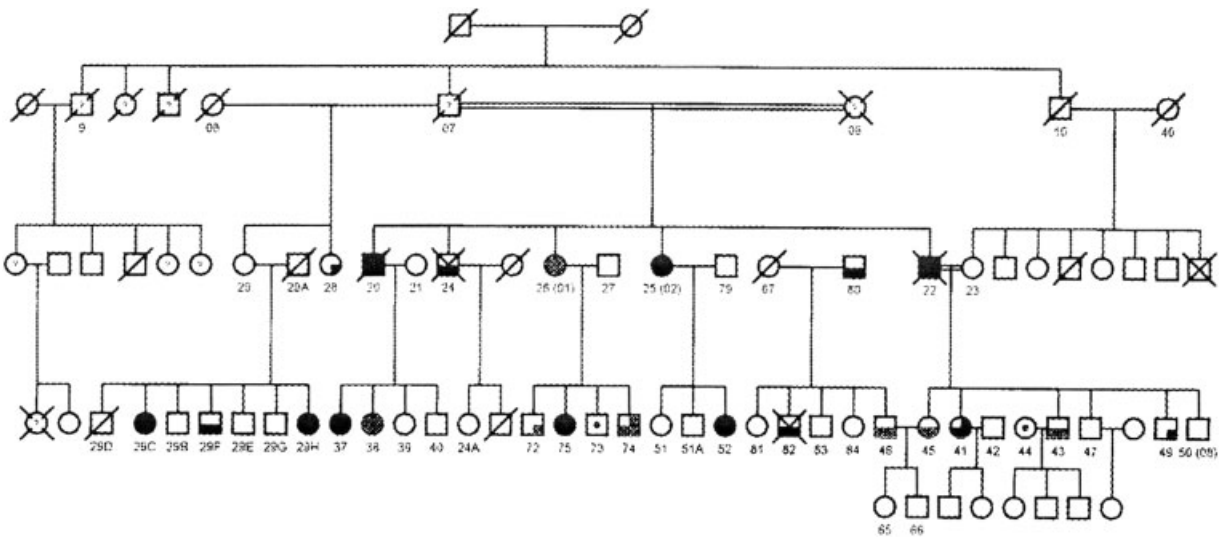

Figure 1. Representative family tree of kindred H. Square: male; circle: female; single oblique strike: natural death; cross: death by suicide; filled symbol: bipolar I; three-quarter filled: bipolar II; half filled: major depression; recurrent; one-quarter filled: major depression; single episode; patients and comparisons subjects included in the study were no. 26(1), 38, 72, 73, 74, 43, 44, 45, and 46.

depression rating scale (HAMD) total scores were assessed immediately before the clomipramine challenge. Patients had a median HAMD score of 1.5 (mean = 4.2, *SD* = 5.1) and had not been hospitalized during the previous six months. None of the patients fulfilled the clinical criteria for a major depression according to DSM-IV. Gastrointestinal complaints after CMI were rated on a 3-item scale (0: no complaints at all; 1: slight to moderate nausea; 2: nausea longer than 15 min or vomiting). All comparison subjects had been medication-free (including alcohol) for at least the previous 2 weeks. Six patients had been medication-free. The remaining subjects had discontinued their medication 1 day before the examination. A longer medication-free period was not possible because of ethical reasons, avoiding an increase of the relapse risk. After complete description of the study to the subjects, written informed consent was obtained.

CLOMIPRAMINE INFUSION PROCEDURE

After overnight fasting, three to four subjects from each family were transferred to the hospital. The experiment started between 9 and 10 a.m. After physical examination and a psychiatric rating including HAMD and clinical global impression on severity, a venous puncture was performed. Subjects rested comfortably for 150 min. Baseline blood samples were taken: 30, 15 min, and immediately before starting CMI. Further blood samples were taken 10, 30, 50, 70, 90, and 120 min after it. CMI (12.5 mg clomipramine/100 mL isotonic NaCl solution) was done within 15 min.

COLLECTION AND ASSAY TECHNIQUES

Blood sample preparation, prolactin, and cortisol determination. Blood was drawn into polypropylene tubes filled with glass pearls and immediately placed on ice. Samples were centrifuged at 3,000g for 10 min and the supernatant was stored at −70°C until examination. Serum prolactin concentration was determined with a double antibody radioimmunoassay (detection limit 0.2 ng or 40 μIU/mL; intraassay and interassay coefficients of variation 4.5 and 6.1%, respectively). Serum cortisol was measured using the enzyme immunoassay technique (detection limit 6.5 nmol/

TABLE 2. Demographic characteristics, HAMD score and medication status of subjects

Family	Type	Gender	Age	Diagnosis (DSM-IV)	Relationship	HAMD-score	Medication
A-1	P	F	38	Bipolar I		5	Lithium
A-2	C FH−	M	42	—	Husband of A-1		—
B-1	P	F	45	Bipolar I		1	Amitriptyline, alprazolam
B-2	C FH+	F	20	—	Daughter of B-1		—
C-1	P	M	37	Bipolar I		13	Lithium, diazepam
C-2	P	M	29	Bipolar I	Brother of C-1	6	Haloperidol, clozapine
C-3	C FH+	F	44	—	Sister of C-1		—
C-4	C FH+	F	34	—	Sister of C-1		—
C-5	C FH+	M	31	—	Brother of C-1		—
D-1	P	F	63	Bipolar I		0	Sertraline
D-2	P	F	38	Bipolar I	Daughter of D-1	0	Lithium, haloperidol
D-3	C FH−	M	65	—	Husband of D-1		—
D-4	C FH−	M	45	—	Husband of D-3		—
E-1	P	M	64	Bipolar I		0	Haloperidol
E-2	P	M	35	Bipolar I	Son of E-1	10	Venlafaxine, sulpirid, diazepam
F-1	P	M	67	Bipolar I		1	Fluoxetine, valproate
F-2	C FH+	M	34	—	Son of F-1		—
G-1	P	F	62	Bipolar I		2	—
G-2	P	F	54	Bipolar I	Sister of G-1	6	Lithium, valproate, sertraline, imipramine
H-1	P	M	42	Recurrent depression		6	Fluoxetine, lorazepam
H-2	P	F	38	Recurrent depression	Sister of H-1	6	Lithium, paroxetine, clomipramine, lorazepam
H-3	C FH−	F	32	—	Wife of H-1		—
H-4	P	F	61	Bipolar I		19	—
H-5	C FH+	M	32	—	Son of H-4		—
H-6	P	M	38	Recurrent depression	Son of H-4	0	—
H-7	P	M	29	Bipolar II	Son of H-4	0	—
H-8	C FH−	F	29	—	Wife of H-7		—
H-9	P	F	43	Bipolar I	Niece of H-4	0	—
I-1	P	F	22	Recurrent depression		0	—
I-2	C	M	36	—	Husband of I-1		—
J-1	P	M	47	Bipolar I		3	Lithium, valproate
K-1	P	M	36	Recurrent depression	Husband of H-2, FH+ (father, brother)	7	Fluphenazine decanoate, imipramine, fluoxetine, flurazepam

P, patient; C, comparison subjects; FH+ and FH−, positive or negative family history regarding affection of any relatives with affective psychosis; DSM-IV, Diagnostic and Statistical Manual for Mental Disorders; HAMD, Hamilton Depression Rating Scale.

L, intraassay and interassay coefficients of variation below 7.4%). All measurements were carried out with commercially available kits from Biochem Immunosystems (Germany).

STATISTICAL ANALYSES

First repeated measurement analyses of variance (ANOVAs) were carried out as an exploratory analysis strategy. ANOVAs were applied with "time" as the within-subject factor (six differences between baseline and post-infusion concentrations) and "group" as between-subjects factor (patients with a history of affective disorders vs. non-affected relatives). For each subject the maximum post-infusion change of the serum prolactin concentration was calculated as a difference compared to the pre-infusion baseline value (Δ max_{prol}). Blunted prolactin response to serotonergic challenge including CMI is a robust finding in the literature. Therefore, we predicted as our main hypothesis that patients with a major affective disorder had a significantly lower Δ max_{prol} compared with their non-affected relatives. In this study, the 20 patients were recruited from 11 families. Furthermore, some family members were related across groups. This produced a non-independence condition on the data, which requires a statistical analysis that makes it possible to cope with this problem to examine group differences of Δ max_{prol}. The general "Mixed" procedure was used for this purpose. This procedure fits a variety of mixed linear models to data, which are a generalization of the standard linear model permitting the data to exhibit correlation and non-constant variability. For testing group differences of Δ max_{prol}, we fitted a model with "family" as a random effect, "group" as a fixed effect, and different variances for the groups and applied an approximate *F*-test with a general Satterthwaite approximation for

the denominator degrees of freedom. Supplementary analyses of Δ max_{prol} were done after separating the patients according to their medication status (unmedicated vs. medicated) using the Kruskal–-Wallis test for determination of group differences and the Mann–Whitney *U*-test for comparison of patient subgroups with healthy subjects. All tests were analogously analyzed for cortisol data. For all tests α was set as $P = .05$. All *P*-values are two-tailed. Statistical analysis was carried out using the statistical software package SAS.[38]

RESULTS

Patients and comparison group did not differ significantly with respect to age (mean = 44.3, *SD* = 13.1 years vs. mean = 37.1, *SD* = 11.2; $P = .14$), gender (10 males, 10 females vs. seven males, five females; $P = 0.48$), baseline prolactin (mean = 167.9, *SD* = 62.2 vs. mean = 201.8, *SD* = 116.7 μIU/mL; $P = 0.80$) and cortisol concentrations (mean = 111.8, *SD* = 49.4 vs. *mean* = 101.6, *SD* = 50.5 ng/mL; $P = 0.59$). The incidence of gastrointestinal complaints rated on the 3-item scale were higher in the patients (mean = 1.08, *SD* = .79 and mean = .15, *SD* = .49 points; $P = 0.002$). One healthy subject and one patient dropped out prematurely after 90 and 70 min, respectively, because of nausea with vomiting, but were included in the statistical analysis. In the exploratory ANOVA, there was a significant group effect ($P < 0.001$) and a significant group by time interaction ($P = 0.005$), indicating that the course of the prolactin concentration curves was different in both groups (Fig. 2). Apart from that there was a gender effect ($P < .001$). Because of this finding, we repeated the ANOVA for both genders separately. For the male subsample ($N = 17$), no group by time interaction could be found but a group difference still existed ($P = .01$). In the female subsample ($N = 15$), the group effect ($P = .003$) and the group by time interaction ($P = .016$) could not be found either, furthermore, a time effect ($P = .001$) popped up for that subgroup. The peak increase of serum prolactin Δ max_{prol} was significantly lower in the patients than in the comparison group for the whole sample (mean = 37.5, *SD* = 65.1 μIU/mL vs. mean = 239, *SD* = 256 μIU/mL; $P = 0.002$). Because of the gender effect in the exploratory ANOVA, this analysis was done twice, the second time for both genders separately. Even if the sample is split by sex, a significant group difference could be shown (male: mean = 2.8, *SD* = 35.2 vs. mean = 129.1, *SD* = 135.7; $P = .012$; female: mean = 72.1, *SD* = 70.5 vs. mean = 405.5, *SD* = 325.9; $P = .007$). Regarding the medication status (Fig. 2), there were significant group differences ($P = .006$). There were both differences between comparison subjects and unmedicated patients ($P = .049$) and between comparison group and medicated patients ($P = .003$). The exploratory ANOVA revealed a significant group effect on cortisol values ($P = .017$) and a significant group by time interaction ($P = .013$). Here gender effects could be found and therefore no separate analyses have been conducted. There was also a significantly higher peak change of serum cortisol Δ max_{cort} in the comparison group than in patients (mean = 92.0, *SD* = 67.5 nmol/L vs. mean = 47.8, *SD* = 58 nmol/L; $P = .046$). There were significant group differences with respect to the medication status ($P = .005$). However, comparison subjects differed only from medicated ($P = .011$), but not from unmedicated patients ($P = .85$).

DISCUSSION

This study demonstrates a blunted prolactin and cortisol response after CMI in remitted patients with hereditary affective disorder compared with healthy related as well as unrelated members from the same families. These effects exist even in respect to gender. Prolactin response was blunted in medicated as well as in unmedicated patients and therefore cannot be only an effect of medication. We were not able to verify this for blunted cortisol response. These results confirm the findings of Thompson et al.[39], who postulated that bipolar patients suffered from different cognitive deficits during a state which is statistically or otherwise normal; a state which is neither elated or depressed. In this study, it could be shown that there are not only cognitive residual symptoms but also changed neuroendocrine reactions. In our study, this blunted prolactin and cortisol reactions can be seen as a biological marker for affective disorders. As the etiology of affective disorder in a randomly ascertained sample may be very heterogeneous, with respect to biological as well as environmental and cultural factors, this study's major advantage is the recruitment of patients and comparison subjects from large families, probably sharing the same genetic predisposition for major affective, mainly bipolar disorder, within a geographically restricted region. There is an increased frequency of affective disorders in families who have an index patient with bipolar disorder. Patients and comparison subjects had, furthermore, comparable socioeconomic status and were investigated simultaneously within 1 week. One problem resulting from this special situation is the dependence between the participants. We dealt with this problem with a special statistical method called "Mixed model", which takes into consideration this special characteristic of the data. And despite this heterogeneity of disorders and the possible dependence of the patients and comparison groups regarding a genetic predisposition, which might impair group discrimination, we still found a significant difference between patients and healthy relatives. As this study investigated highly selected patients with increased risk for affective disorders, a substantial subgroup was on a psychotropic medication for relapse prevention. By including patients on a variety of medications, the ability to disentangle a medication effect from a disease effect might be compromised. One could argue that discontinuation of medication for 1 day before CMI was too short to exclude long-lasting

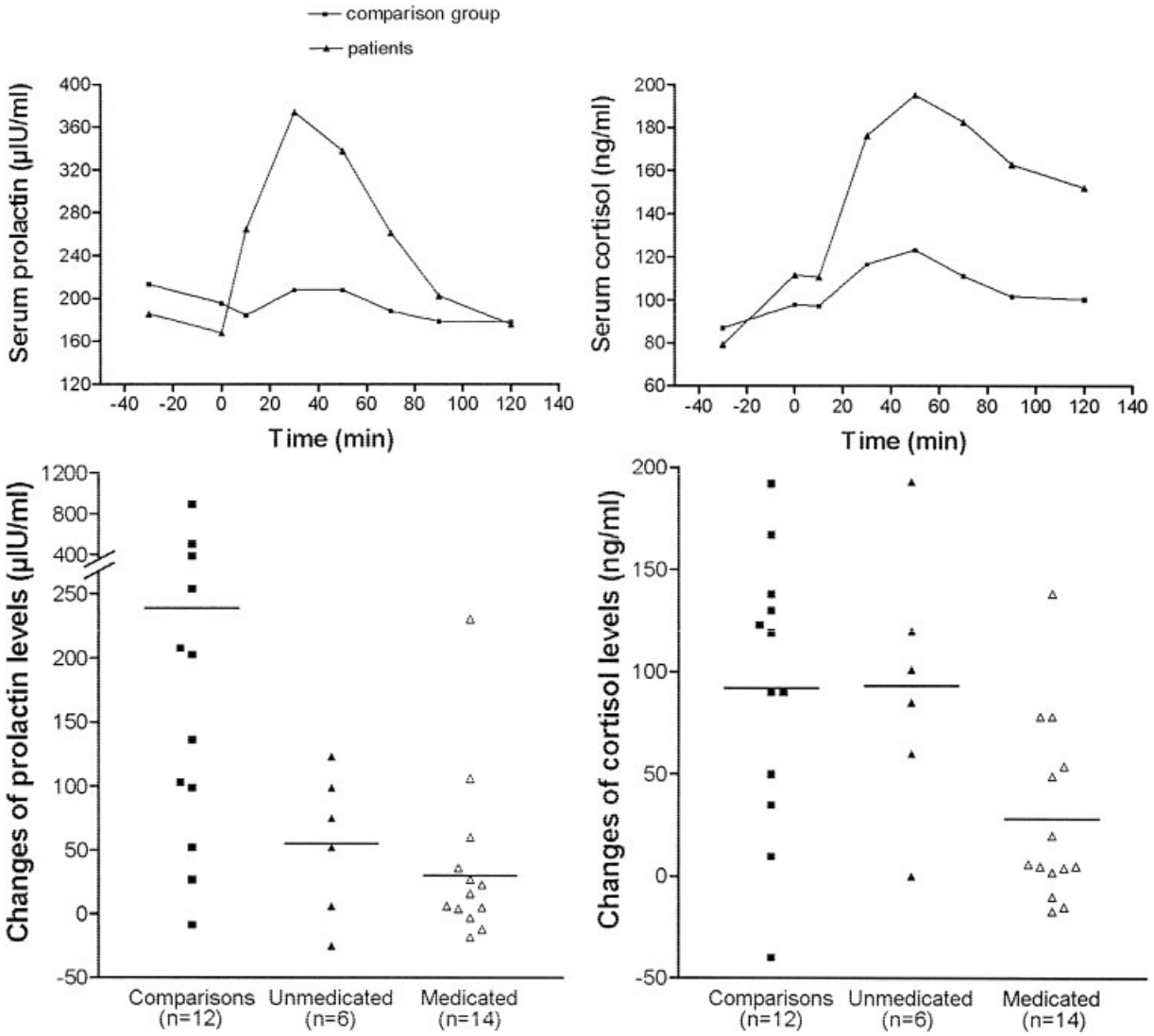

Figure 2. Serum hormone concentrations (top) and maximal individual changes (bottom) of prolactin (left) and cortisol (right) serum levels in the comparison group and patients before and after clomipramine infusion.

effects. For prolactin, the unmedicated patients ($n = 6$) did not differ from either the comparison group or medicated patients ($n = 14$). However, for cortisol values we were not able to find this effect. On one hand, maybe there is a real effect of medication; on the other, it could be a consequence of a lack of power because of the small subsamples.

Findings of other investigators demonstrate that antidepressants *restore* an initially blunted prolactin response to serotonergic challenge in major depression [e.g. [18,31,40]]. Prolactin responses to D-fenfluramine were enhanced after fluoxetine or amitriptyline, irrespective of the therapeutic outcome.[32] A longitudinal study demonstrated a significantly lower prolactin response before antidepressant treatment, but no difference between two posttreatment experiments with or without ongoing antidepressant medication.[31] Another longitudinal study found blunted prolactin responses in patients with major depression during their illness and their recovery as well.[41] Similarly, an increased cortisol response to the 5-HT precursor 5-hydroxytryptophan was found in depressed patients treated with fluoxetine.[42] Increased prolactin responses to D-fenfluramine were also found during treatment with tricyclic antidepressants or a specific noradrenergic reuptake inhibitor.[7,40] However, one study in depressed patients with normal prolactin response found no influence of a 8-week treatment with fluoxetine.[43] In summary, the majority of these studies suggest that treatment with most antidepressants should enhance the prolactin response to serotonergic challenge in major depression and therefore cannot explain blunting of prolactin response in our medicated patients. This leads to the broader conclusion that blunting of prolactin response in our patients is presumably a *trait-like* finding, present also

in unmedicated and non-depressed individuals with lifetime history and genetic predisposition for major affective disorder. Our results confirm the findings of a study[34] that similarly reported a blunted prolactin response after oral fenfluramine challenge in unmedicated remitted depressive patients compared with carefully matched healthy persons. However, our major finding stands in contrast to other reports, which interpreted a blunted prolactin response to serotonergic challenge as a *pure state* marker, present only during the depressive episode [e.g. [31]]. *One possible explanation for this discrepancy might be that both—state and trait elements—are playing their parts.* Blunting of prolactin response might reflect *state-dependent* neurobiochemical changes during major depression and partly a *trait-like*, persistently reduced serotonergic sensitivity. However, our comparison group consisted in part of first-degree relatives from the same families with multigenerational cases of major affective disorder, therefore having a high genetic risk and probably having comparable trait characteristics regarding personality and impulsive behavior to the patients. Interestingly, there was a differential effect on the regulation of prolactin and cortisol release in medicated and unmedicated patients: prolactin response was blunted in the patient group irrespective of the medication status, whereas only the medicated subgroup also had a blunted cortisol response. This might be due to pharmacological blockade of 5-$HT_{2A/C}$ receptors, which is known to attenuate elevated cortisol responses after serotonergic challenge.[36] Blunted cortisol response has previously been described in major depression[44] and correlates with poor response to antidepressants.[45] Thus, as an alternative explanation for our findings, the subjects with ongoing antidepressant medication might represent a subgroup with unfavorable prognosis. This interpretation is further supported by studies suggesting that pharmacological treatment with selective noradrenergic reuptake inhibitors[45] or serotonin reuptake inhibitors[46] restores an initially blunted cortisol response in major depression. Moreover, cortisol response to 5-HTP is also enhanced by clomipramine, which is known to be an antagonist with respect to 5-$HT_{2A/C}$ receptors.[47] Therefore, antidepressant medication is unlikely to be responsible for a blunted cortisol response.

In summary, data from this and other studies suggest that the prolactin and cortisol response to a serotonergic challenge might depend on multiple factors. Our study demonstrates a blunted prolactin and cortisol response in remitted patients from families with hereditary major affective disorder. As other groups demonstrated a serotonergic subsensitivity after D-fenfluramine or 5-hydroxytryptophan also in patients with bipolar mania[48] or bipolar depressives,[49] we suggest that our finding might be a *trait-like* characteristic of the patients investigated here.

Further research in these families should elucidate the underlying mechanism, especially regarding metabolism or availability of tryptophan,[50,51] cerebral serotonin receptor densities,[26] or regional cerebral blood flow[52] and genetic factors.[53]

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