Neuropsychopharmacology (2005) 30, 1181–1186

www.neuropsychopharmacology.org

Plasma Concentrations of Neuroactive Steroids before and after Electroconvulsive Therapy in Major Depression

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There is evidence that both cerebrospinal fluid (CSF) and plasma concentrations of 3α-reduced neuroactive steroids are decreased in major depressive disorder. Successful antidepressant pharmacotherapy, for example, with selective serotonin reuptake inhibitors (SSRIs), over several weeks is accompanied by an increase in CSF and plasma concentrations of these neuroactive steroids. However, no such increase has been observed during nonpharmacological treatments such as partial sleep deprivation or repetitive transcranial magnetic stimulation. In order to investigate whether concentration changes in neuroactive steroids are an important component of clinically effective antidepressant treatment, we examined plasma concentrations of the neuroactive steroids 3α,5α-tetrahydroprogesterone, 3α,5β-tetrahydroprogesterone, 3β,5α-tetrahydroprogesterone, and their precursors progesterone, 5α-dihydroprogesterone, and 5β-dihydroprogesterone in 31 pharmacotherapy-resistant depressed in-patients before and after unilateral electroconvulsive therapy (ECT) as a monotherapy over 4 weeks. Samples were quantified for neuroactive steroids by means of a highly sensitive and specific combined gas chromatography/mass spectrometry analysis. In all, 51.6% of the patients were treatment responders. There was no influence of ECT on the plasma concentrations of any neuroactive steroid studied. Moreover, neuroactive steroid levels did not differ between treatment responders and nonresponders. Our study shows that changes in neuroactive steroid plasma levels are not a mandatory factor for successful antidepressant treatment by ECT. Thus, the previously observed changes in plasma concentrations of neuroactive steroids following treatment with antidepressants such as SSRIs more likely reflect distinct pharmacological properties of these compounds rather than clinical improvement.

Neuropsychopharmacology (2005) **30,** 1181–1186, advance online publication, 9 February 2005; doi:10.1038/sj.npp.1300684

Keywords: neuroactive steroids; neurosteroids; electroconvulsive therapy; major depression; antidepressant therapy; treatment response

INTRODUCTION

3α-Reduced metabolites of progesterone such as 3α,5α-tetrahydroprogesterone (3α,5α-THP, 5α-pregnan-3α-ol-20-one, allopregnanolone) are potent positive allosteric modulators of the γ-aminobutyric acid A ($GABA_A$) receptor complex (Paul and Purdy, 1992; Rupprecht, 2003; Rupprecht and Holsboer, 1999). In contrast, 3β,5α-tetrahydroprogesterone (3β,5α-THP, 5α-pregnan-3β-ol-20-one, isopregnanolone), a stereoisomer of 3α,5α-THP, may act as a functional antagonist for GABA-agonistic steroids (Maitra and Reynolds, 1998; Prince and Simmonds, 1992; Rupprecht, 2003). *In vivo*, 3α-reduced neuroactive steroids have been shown to exert anxiolytic and antidepressant properties (Crawley *et al*, 1986; Khisti *et al*, 2000).

Neuroactive steroids interacting with the $GABA_A$ benzodiazepine receptor complex have been suggested to be involved in the pathophysiology of major depression. The concentrations of 3α-reduced neuroactive steroids are lower in the cerebrospinal fluid (CSF) and plasma of depressed patients in comparison to healthy controls (George *et al*, 1994; Rupprecht, 2003; Rupprecht and Holsboer, 1999; Uzunova *et al*, 1998). Moreover, it has been shown that selective serotonin reuptake inhibitors (SSRIs) enhance the formation of 3α-reduced neuroactive steroids in experimental animals (Uzunov *et al*, 1996), at the molecular level (Griffin and Mellon, 1999), and in depressed patients (Romeo *et al*, 1998; Uzunova *et al*, 1998).

To investigate whether changes in the concentrations of neuroactive steroids are an important component of clinically effective antidepressant treatment, or whether they are related to distinct pharmacological effects of

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Received 7 July 2004; revised 7 December 2004; accepted 15 December 2004
Online publication: 5 January 2005 at http://www.acnp.org/citations/NPP0105050403 14/default.pdf

antidepressant drugs, two nonpharmacological biological antidepressant treatments have been investigated so far. Partial sleep deprivation (PSD) as an established short-acting antidepressant therapy (Wu and Bunney, 1990) did not affect the concentrations of neuroactive steroids either in responders or in nonresponders (Schule *et al*, 2003). Moreover, plasma concentrations of a variety of neuroactive steroids including 3α-reduced pregnane steroids were not altered in spite of a significant clinical improvement after daily treatment with repetitive transcranial magnetic stimulation (rTMS) over a 2-week period (Padberg *et al*, 2002). Thereby, it has been suggested that the previously reported changes in neuroactive steroid concentrations following antidepressant pharmacotherapy (Romeo *et al*, 1998; Uzunova *et al*, 1998) more likely reflect specific pharmacological properties of the antidepressant drugs (Griffin and Mellon, 1999) than successful treatment and clinical improvement in general (Padberg *et al*, 2002).

However, the effects of PSD or rTMS may either be too weak or too short-lasting to be accompanied by changes in neuroactive steroids since the SSRI-induced changes have been observed after 20–50 days (Romeo *et al*, 1998) and after 8–10 weeks (Uzunova *et al*, 1998) of treatment. Electroconvulsive therapy (ECT) is still considered to be the most effective biological treatment of depression, especially in treatment-resistant major depression (ECT-review group, 2003). It usually is administered during 2–3 treatment sessions per week over a 4-week period.

In the current study, we therefore investigated the medium-term effects of ECT as a monotherapy on plasma concentrations of the neuroactive steroids 3α,5α-THP, 3α,5β-THP, 3β,5α-THP, as well as their precursors progesterone, 5α-dihydroxyprogesterone (5α-DHP, 5α-pregnan-3, 20-dione), and 5β-dihydroxyprogesterone (5β-DHP, 5β-pregnan-3, 20-dione) in major depressive patients within a 4-week treatment cycle.

PATIENTS AND METHODS

Patients and Clinical Assessments

A total of 31 pharmacotherapy-resistant depressed in-patients (mean age ± standard deviation: 50.5 ± 14.5 years, 16 women, 15 men) were included in an open-label study protocol after written informed consent had been obtained. The mean age of the female patients was 53.4 ± 15.9 years. A total of seven women (four responders, three nonresponders to ECT) were premenopausal and had regular menstrual cycles.

Pharmacotherapy resistance was defined as nonresponse to at least two antidepressant treatment trials using sufficient dosing over a time period of at least 6 weeks. Before starting the treatment, patients gave their written informed consent for ECT procedures and anesthesia separately. The study was conducted in accordance with the Declaration of Helsinki and it was approved by the local ethical committee.

The inclusion criteria were a major depressive disorder according to DSM-IV (American Psychiatric Association, 2000) criteria and a score of at least 18 on the 21-item Hamilton Depression Rating Scale (HAM-D21) (Hamilton, 1960). HAM-D21 ratings were obtained by trained raters in weekly intervals during the study period. The patients were drug-free for at least 1 week prior to inclusion. Major medical disorders, addiction or other comorbid psychiatric diagnoses, pregnancy, use of oral contraceptives or hormone replacement therapy led to exclusion from the study. None of the patients had been pretreated with fluoxetine or depot neuroleptics. The patients remained completely medication-free during the entire study.

ECT Treatments

Right unilateral ECT according the d́Elia method (d'Elia, 1970; d'Elia and Raotma, 1975) was performed using the Thymatron System-IV™ device, which provides stimulus delivery in the form of constant current (900 mA) bi-directional pulse wave stimulation. The first electrode was placed temporally 1 cm above the center-point of a line between the outer eyelid angle and the external acoustic meatus. The second electrode was placed at 12–13 cm distance from the first electrode 2–3 cm lateral of the vertex. Pulse width was 0.5 ms, and the frequency was 20–80 Hz (45.8 ± 14.6 Hz). The length of a stimulus train was 0.15–8 s (6.2 ± 1.7 s). Stimulus intensity was determined using the age-based dosing method (Abrams, 2002b). Due to an increasing seizure threshold with increasing age, older patients received higher stimulus intensity during the first ECT. During the first stimulation the minimal stimulus for all patients up to the age of 30 was an applied charge of 151 mC (device adjustment: 30%). It was increased linearly according to the age of the patient, for example, 40%/202 mC for 40-year-old patients, 50%/252 mc for 50-year-old patients and 60%/302 mC for patients at the age of 60 or older. Restimulation including dosage elevation in 10% steps was recommended during the subsequent treatments in case of short EEG (<30 s) or EMG (<25 s) seizure activity, or a too low postictal suppression index (<80%). The mean applied charge was 270.6 ± 107.9 mC, representing a stimulus intensity of 53.7 ± 21.4%. The mean postictal suppression index was 89.8 ± 7.8%. Seizure monitoring included two-lead EEG-, single-lead EMG and ECG monitoring. The EEG electrodes were left and right frontopolar (FP_1 and FP_2) and over the ipsilateral mastoids. EMG was recorded at a 5–10 cm distance over the flexor carpi ulnaris muscle. The mean seizure durations were 31 ± 14 s (EEG) and 17 ± 9 s (EMG), respectively.

Anesthesia

The administered anesthetic agents were thiopental (in 70.6% of treatments, mean dosage ± SD: 399 ± 84 mg, 5.1 ± 1.1 mg/kg), propofol (19.8%, 187 ± 52 mg, 2.1 ± 0.6 mg/kg), methohexital (9.1%, 143 ± 32 mg, 2.3 ± 0.8 mg/kg), and etomidate (0.5%, 20 mg, 0.3 ± 0.0 mg/kg). For muscle relaxation, succinylcholine or pyridostigmine together with atracurium for premedication were used.

Quantification of Neuroactive Steroids

Plasma samples were taken exactly at 0800 h to prevent an influence of circadian fluctuations (Corpechot *et al*, 1997). We quantified progesterone, 3α,5α-THP, 3α,5β-THP, 3β,5α-THP, 5α-DHP, and 5β-DHP on the day before the first ECT treatment (baseline) and 1 day after the last ECT treatment

(week 4) by means of a highly sensitive and specific combined gas chromatography/mass spectrometry analysis (GC/MS) after extraction with ethyl acetate as described previously (Padberg *et al*, 2002; Romeo *et al*, 1994, 1998; Schule *et al*, 2003; Strohle *et al*, 2002). A Finningham Trace GC/MS equipped with a capillary column was used to analyze the derivatized steroids in the negative ion chemical ionization mode. The detection limit was approximately 10 fmol.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows (Release 11.5.1, SPSS Inc., Chicago, IL 60606, USA). The one-sample Kolmogorov–Smirnov test was used to test about the normal distribution of HAMD-21 scores and levels of neuroactive steroids. Homogeneity between ECT responders and nonresponders in demographic variables was analyzed by the χ^2-test for contingency or two-sided Fisher's exact test with respect to qualitative variables (gender) or by two-tailed *t*-tests for independent samples with regard to quantitative variables (age, HAM-D21 sum scores, ECT treatment variables). ECT responders were defined by a HAM-D21 reduction from baseline to week 4 of at least 50%, remission was defined as HAM-D21 score $\leqslant 10$ at week 4. To evaluate significant time effects and the influence of gender on HAM-D21 scores, analysis of variance (ANOVA) for repeated measurements with time as a within-subjects factor, gender as a between-subjects factor, and age as a covariate was performed. For statistical comparisons of mean steroid concentrations before (baseline) and after ECT (week 4), and between responders and nonresponders, multivariate analysis of covariance (MANCOVA) with a repeated-measures design was performed. Thereby 'treatment' (ECT) and 'response' were considered as within-subjects and between-subjects factors with two levels, respectively, and, age' and 'gender' as covariates. MANCOVA with a repeated-measures design was also carried out to test the effects of 'treatment' and 'gender' on the steroids without differentiating between responders and nonresponders. Here, only 'age' was considered as a covariate.

RESULTS

Demographical and clinical variables are given in Table 1. A total of 51.6% of depressed patients (16 out of 31) responded to ECT, 25.8% (eight out of 31) remitted after treatment. Clinical and endocrinological variables were normally distributed. No significant differences between responders and nonresponders concerning age and clinical variables at baseline could be detected (Table 1, Figure 1). Among treatment responders, there were significantly more female patients than male patients (Fisher's exact test, two-sided: $p = 0.012$ or χ^2 test: $\chi^2 = 7.24$, $p = 0.007$). Female responders were older than nonresponders; however, this did not reach statistical significance ($T_{(15,17)} = -1.6$, $p = 0.13$, NS).

Clinical ECT treatment variables showed significant longer treatment series in nonresponders (40.6 ± 20.1 *vs* 29.7 ± 7.8 days, $T_{(435,268)} = 7.4$, $p < 0.0001$). Ictal electrophysiological measures and ECT treatment variables revealed several differences between the responder- and the nonresponder groups, which are shown in Table 2. Since there was a significant difference in gender distribution between ECT responders and nonresponders, 'gender' was used as a covariate in the ANOVA. Both at baseline and after ECT, the concentrations of neuroactive steroids were comparable between men and women (MANCOVA, Wilks' multivariate tests of significance; effect of 'gender': $F_{(6,31)} = 0.197$, $p = 0.97$, NS). By considering 'ECT treatment' and 'response' as influencing factors and 'gender' and/or 'age' as covariates, analysis of covariance did not reveal any significant effects of 'treatment' ($F_{(6,31)} = 0.649$, $p = 0.69$, NS), nor of 'treatment by response' ($F_{(6,31)} = 0.799$, $p = 0.58$,

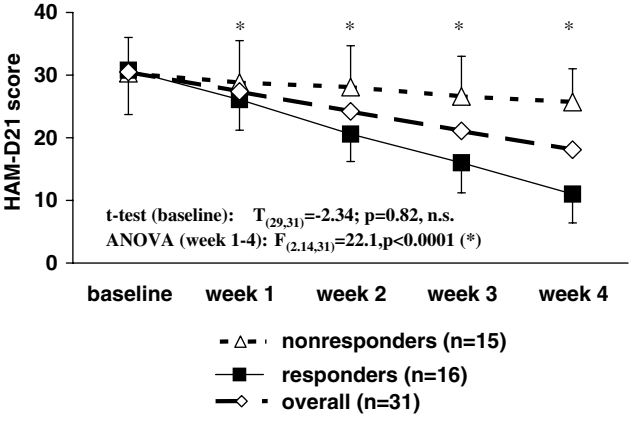

Figure 1 No significant differences in HAM-D21 scores at baseline. Significant better amelioration of depressive symptoms in responders from week 1 to week 4 in comparison to nonresponders.

Table 1 Demographical and Clinical Variables of 31 Patients

	Total	Responders	Nonresponders	*t*-test, χ^2-test, ANOVA
n	31	16	15	
Age (mean ± SD)	50.5 ± 14.5	48.0 ± 12.8	52.9 ± 16.0	$T_{(29,31)} = 0.31$;
Range	23–76	23–76	31—74	$p = 0.98$, NS
Gender (male/female)	15/16	4/12	11/4	$\chi^2 = 7.24$, $p = 0.007$
Diagnoses (ICD-10) (unipolar/bipolar)	29/2	15/1	14/1	$\chi^2 = 0.002$, $p = 0.96$, NS
HAM-D21 score reduction	39.1 ± 29.2%	62.8 ± 15.2%	13.8 ± 16.0%	week 1–4: $F_{(2.14,31)} = 22.1$, $p < 0.0001$

For analyses of quantitative variables, *t*-tests (age) and ANOVA (HAMD-scores) were used; for analyses of qualitative variables (diagnoses), the χ^2-test was used.

Table 2 Ictal Electrophysiological Variables during ECT Treatment Series in 16 Treatment Responders and 15 Nonresponders

	Total	Responders	Nonresponders	*t*-test
Number of ECT treatments	12.6±3.8	11.5±2.3	14.0±4.8	$T_{(30,31)} = 1.82$; $p = 0.08$, NS
Duration of ECT series (days)	35.0±16.0	29.7±7.8	40.6±20.1	$T_{(433,31)} = 7.37$; $p < 0.0001$
Duration of convulsions—EEG (seconds)	30.5±13.5	32.9±14.3	28.0±12.2	$T_{(433,31)} = -3.22$; $p = 0.001$
Duration of convulsions—EMG (seconds)	16.9±9.1	18.8±9.8	14.5±7.6	$T_{(280,31)} = -4.17$; $p < 0.0001$
Applied charge (mC)	270±108	286±111	254±101	$T_{(433,31)} = -3.21$; $p = 0.001$
Postictal suppression index (%)	89.8±7.8	90.9±7.5	88.5±7.9	$T_{(433,31)} = -2.67$; $p = 0.008$
Seizure energy index	579±639	643±653	511±618	$T_{(433,31)} = -1.90$; $p = 0.058$, NS
Seizure concordance index	71.6±18.4	74.1±17.4	68.0±19.2	$T_{(433,31)} = -2.88$; $p = 0.004$

Results of *t*-tests for quantitative ictal ECT measures.

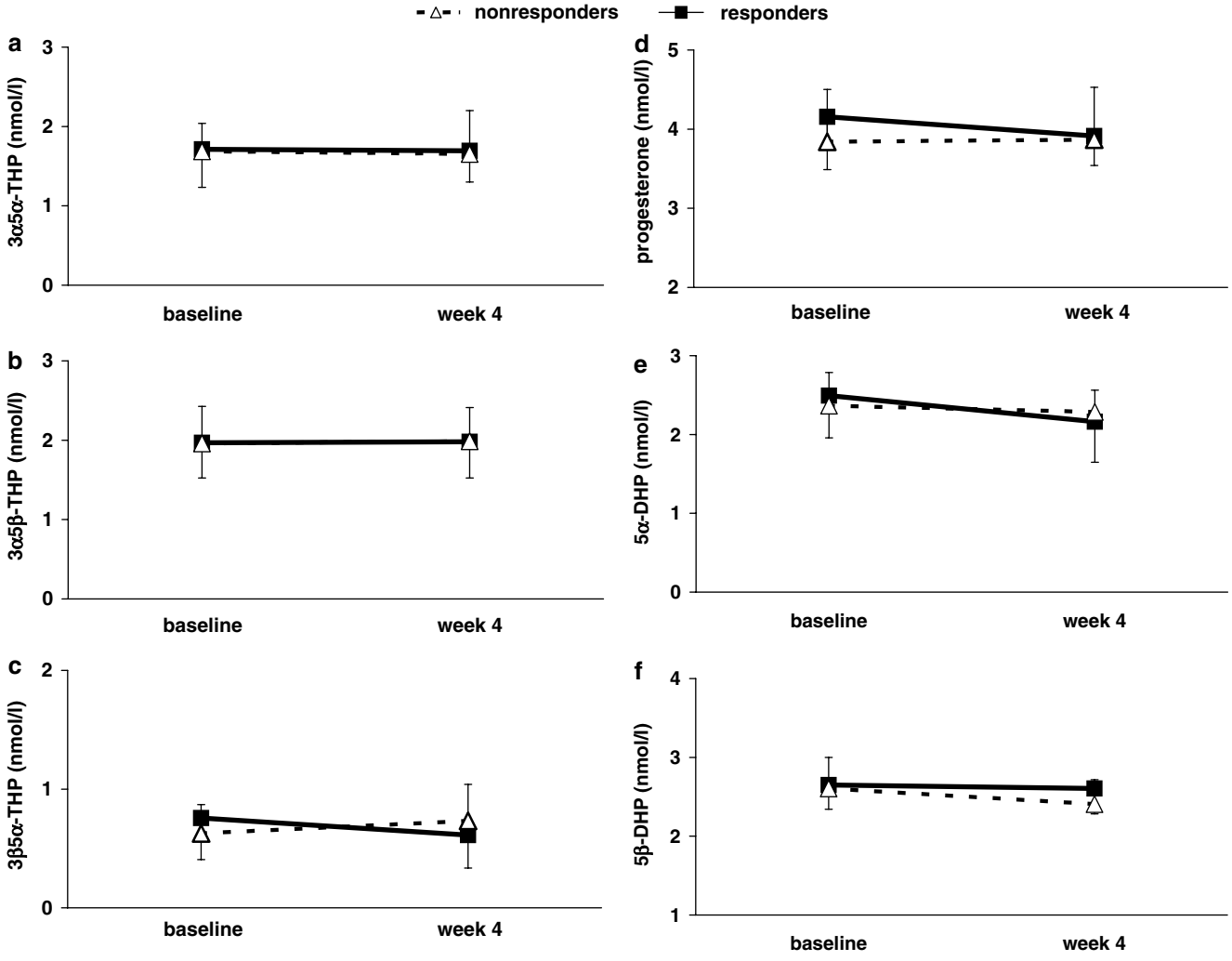

Figure 2 No significant influence of ECT on neuroactive steroid plasma concentrations in pharmacotherapy-resistant major depression. No significant differences between treatment responders and nonresponders.

NS) interaction on the concentrations of neuroactive steroids (Figure 2). Moreover, there was no effect of 'ECT treatment' ($F_{(24,31)} = 1.187$, $p = 0.35$, NS) and 'gender' ($F_{(24,31)} = 0.538$, $p = 0.21$, NS) without differentiating between responders and nonresponders on the neuroactive steroids studied. The remitter analysis revealed comparable results.

DISCUSSION

Unilateral ECT treatment in pharmacotherapy-resistant depression caused a significant amelioration of depressive symptoms and therapeutic response in 51.6% of the patients. About half of these patients achieved the remission criteria. In 48.4% of the patients further therapeutic

approaches, such as bilateral ECT or a second series using high-dose ECT in combination with pharmacotherapy, were necessary. Our data are in contrast to response rates of about 80–90% in patient populations not fulfilling the criteria of therapy resistance (Prudic *et al*, 1990, 1996). Due to the relatively high doses of anesthetic agents (Abrams, 2002a) used by our anesthesiologists, higher seizure thresholds seem to be possible. Nevertheless, ictal EEG assessments showed adequate seizure parameters and our data are consistent with response rates of about 50–60% in pharmacotherapy-resistant depression (Sackeim *et al*, 2000).

We could not detect any changes in plasma concentrations of the neuroactive steroids investigated after ECT treatment. This is in contrast to antidepressant pharmacotherapy (Romeo *et al*, 1998; Uzunova *et al*, 1998), where a normalization of altered neuroactive steroid levels both in the CSF (Uzunova *et al*, 1998) and plasma has been described (Romeo *et al*, 1998). Moreover, in two other investigations of nonpharmacological antidepressant treatment interventions, sleep deprivation (Schule *et al*, 2003) and rTMS (Padberg *et al*, 2002) failed to show any significant effect on plasma concentrations of neuroactive steroids. However, these two treatment strategies may either exert antidepressant effects that are too weak or the duration of treatment may have been too short to influence neuroactive steroid concentrations in a similar way as pharmacotherapies which develop their full antidepressant activity action usually within 4–6 weeks. Up to now, ECT is the most effective somatic therapy, especially for severe treatment-resistant major depressive disorder (ECT-review group, 2003). Overall, a 20% better improvement in comparison to tricyclic antidepressants (TCA) and a 45% better improvement in comparison to monoaminooxidase inhibitors (MAOI) (Janicak *et al*, 1985), as well as a better improvement in comparison to the SSRI paroxetine (Folkerts *et al*, 1997), have been described. In addition, a more rapid improvement in comparison to pharmacotherapeutic approaches has been demonstrated (ECT-review group, 2003; Prudic *et al*, 1996; Sackeim *et al*, 1993). The lack of effects of all the three nonpharmacological treatment procedures in contrast to the previously reported changes in neuroactive steroid concentrations following antidepressant pharmacotherapy (Romeo *et al*, 1998; Uzunova *et al*, 1998) suggests that the latter rather reflect specific pharmacological effects of antidepressant drugs such as interference with neurosteroidogenic enzymes (Griffin and Mellon, 1999). Moreover, our data show that clinically effective antidepressant treatment is not necessarily accompanied by changes in plasma neuroactive steroid concentrations. However, whether alterations in neuroactive steroid levels may nevertheless contribute to antidepressant or anxiolytic effects of antidepressants or whether neuroactive steroids may exert antidepressant effects themselves cannot be clarified by the present study.

As the concentrations of neuroactive steroids were not assessed immediately after each ECT session, the eventual acute effects of ECT on neuroactive steroids cannot be ruled out. However, such assessments would have been hampered by the effects of anesthesia. Moreover, a single acute ECT treatment session is not sufficient to achieve stable antidepressant effects. Differential effects of anesthetic agents on neuroactive steroid levels in treatment responders and nonresponders appear to be rather unlikely, because no significant differences in anesthetic procedures in both groups could be found.

Only a small proportion of the investigated women were premenopausal and these were equally distributed among the responder and nonresponder group. Therefore, a major influence of the menstrual cycle on neuroactive steroid concentrations seems to be not very likely in our sample. Nevertheless, because the measurement of neuroactive steroids was repeated after 4 weeks, the menstrual cycle could be a potential confounding variable in case of irregular or prolonged menstrual cycles, which were not systematically recorded in our seven premenopausal female patients. Moreover, our sample size was too small to conduct analyses of neuroactive steroid concentrations in dependency of the menstrual cycle.

Up to now, the mechanisms of action underlying the antidepressant efficacy of ECT remain still unclear. Our data do not support the hypothesis that neuroactive steroids determine the long-term beneficial effects of ECT in depressive disorders, although an effect of ECT on brain concentrations of neuroactive steroids cannot be excluded. Thus, other effects including a potential impact of ECT on the hypothalamic pituitary adrenal (HPA) system, such as an acute stimulatory effect (Florkowski *et al*, 1996) and a long-term reduction of HPA axis overdrive (Grunhaus *et al*, 1987), or effects on neurotrophic factors (Altar, 1999; Smith *et al*, 1997), are more likely contributing factors for the clinical efficacy of ECT.

ACKNOWLEDGEMENTS

We thank Mrs M Ertl, Mrs A Johnson, and Mr K Neuner for patient nursing and laboratory assistance. This work was supported by a Tandem-project of the Max-Planck Society. Parts of this study were carried out in the framework of the doctoral thesis of Mrs Theresa Zeller, which have been submitted to the Faculty of Medicine, University of Munich.

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