Changes in CREB Phosphorylation and BDNF Plasma Levels during Psychotherapy of Depression


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Key Words
Depression · CREB phosphorylation · Brain-derived neurotrophic factor · Interpersonal psychotherapy · Response

Abstract

Background: The cyclic adenosine monophosphate response element-binding proteins (CREB) and their interaction with brain-derived neurotrophic factor (BDNF) are essential elements in signal transduction pathways important for cellular resilience and neuroplasticity. They play a decisive role in the concept of altered neuroplasticity in major depression. We have previously demonstrated that the increase in phosphorylated CREB (pCREB) in T lymphocytes is significantly associated with clinical improvement in patients treated with antidepressants. In the present study, we focused on patients treated only with psychotherapy to exclude direct pharmacological actions. In addition to pCREB, we also measured the BDNF plasma levels. Methods: pCREB in T lymphocytes was determined by Western blot; the BDNF plasma levels with solid-phase ELISA. Psychopathology was evaluated with the Hamilton Rating Scale for Depression (HAMD). Thirty patients meeting DSM-IV criteria for major depressive episodes (MDE) were recruited into this 6-week study. They received interpersonal psychotherapy (IPT) twice weekly. Results: After 6 weeks of IPT, 17 patients responded (reduction of ≥50% of baseline HAMD); after 1 week of treatment pCREB increased significantly compared to the nonresponder group. Measurement of the BDNF plasma levels revealed no differences between the responder and nonresponder groups. Furthermore, the correlations between BDNF plasma levels and pCREB were not significant. Conclusions: The early increase in pCREB is related to treatment response and does not depend on pharmacological interventions or BDNF plasma levels. For the first time, cellular biological markers could be associated with response to psychotherapy.

Introduction

It has been hypothesized that the impairment of neuroplasticity and cellular resilience underlies the pathophysiology of depression and successful treatment may depend on neurotrophic effects [1, 2]. The main evidence for the development of the neurotrophic hypothesis came...
from preclinical studies, demonstrating on a cellular level that antidepressants, mood stabilizers and electroconvulsive therapy exert positive actions on neuroplasticity by positively influencing parameters such as cellular resilience, neurogenesis or synaptogenesis. Chronic antidepressant treatment upregulates the expression of brain-derived neurotrophic factor (BDNF), important for neuronal survival and plasticity in the CNS, and its tyrosine kinase receptor B (TrkB) by increasing the phosphorylation of cyclic adenosine monophosphate response element-binding protein (CREB) and, therefore, CREB-mediated gene expression [3, 4]. The widely acknowledged rise in serotonin or norepinephrine at the synapse that is induced by antidepressants is thought to activate cascades which eventually increase BDNF and TrkB expression by phosphorylating and thereby activating the transcription factor CREB. This would lead to neuronal sprouting, increased neurogenesis and synaptic plasticity [5]. The proof of this hypothesis in clinical studies has not been possible, since there is no access to neurons in humans. Our previous research successfully established T lymphocytes as a peripheral cellular model to investigate the activation of proplastic signal transduction cascades [6]. We have demonstrated in patients treated for major depressive episodes that the changes in CREB phosphorylation are associated with response to treatment [7]. Because some responders were treated exclusively with psychotherapy, it seems unlikely that the rise in phosphorylated CREB (pCREB) is due only to pharmacological action on the T lymphocytes. Rather, the pCREB increase is a cellular response to neurobiological parameters associated with clinical response, such as BDNF. BDNF is also expressed in thrombocytes, monocytes and lymphocytes [8–11]. It circulates freely between blood and brain compartments [12], presumably acting within the CNS. Some studies have reported that the serum levels of BDNF are negatively correlated with the severity of depression and that decreased levels normalize after successful treatment with antidepressants [13–15]. Since T lymphocytes bear the BDNF receptor TrkB [8, 16], alterations of the serum levels of BDNF in depressed patients might be one factor that contributes to the changes in pCREB. The present study tries to examine the following hypotheses: (1) the rise in pCREB is also associated with treatment response in patients free of medication, and (2) this increase occurs early in the course of treatment. Furthermore, by testing the plasma levels of BDNF we explore whether peripheral levels of BDNF contribute to changes in CREB phosphorylation during psychotherapy.

Patients and Methods

Patients and Study Design

This analysis is 1 of 3 separate pilot studies investigating different biological markers for the treatment response of interpersonal psychotherapy (IPT). These studies all included the same patients but used different research criteria and research schedules. The included patients met the DSM-IV criteria for MDE on the basis of an interview using the Structured Clinical Interview for DSM-IV. Individuals with severe MDE with or without psychotic features were excluded from the study. The patient recruitment covered the period 2001–2003 after consecutively screening every in- and outpatient who entered our department. The inpatients remained hospitalized throughout the observation period. The patients had to be free of any psychopharmacological treatment at least 1 week prior to inclusion. Psychiatric comorbidities that were excluded were substance-related disorders, psychotic disorders, dementia or other cognitive disorders, obsessive-compulsive disorders (axis I) and borderline personality disorder (axis II). Furthermore, the patients had to be free of symptoms and any history of acute or chronic infections, autoimmune diseases and medication that might have influenced the immune function. After inclusion, visits took place at baseline (T0), as well as on days 7 (T1), 14 (T2), 21 (T3) and 42 (T4). Each visit included a rating for the severity of depressive symptoms using the 21-item Hamilton Rating Scale for Depression (HAMD). Furthermore, the first 4 visits comprised the withdrawal of 10 ml of blood for the quantification of CREB phosphorylation (see below). Plasma samples for BDNF measurement were collected at baseline (T0), as well as on days 7 (T1) and 21 (T3). The treatment started after the baseline visit. The original sample consisted of 32 depressed patients (20 female, 12 male) with a score of $\geq 16$ on the 21-item HAMD (mean = 23.9). The age ranged from 20 to 64 years (mean = 39.5). All subjects were able to give written informed consent. Nine patients did not complete psychotherapy. Five of these discontinued the treatment very early after a maximum of 2 visits (T0, T1). One patient withdrew after completing all visits. Two subjects achieved long-lasting clinical remission within a maximum of 6 sessions, so they decided to stop IPT and were classified as responders to psychotherapy. One patient’s symptomatology dramatically changed for the worse during the first 6 sessions of IPT and adjunctive medication became necessary. In the end this patient discontinued IPT and was classified as a nonresponder to psychotherapy. In total there were 27 individuals for the assessment of response with 3 observations carried forward. They were treated exclusively with IPT [17], i.e. a manual-based, time-limited, disorder-specific approach that addresses interpersonal issues. The efficacy of IPT has been proven in several studies [18, 19]. On average the patients received IPT twice weekly. Overall IPT ran from 12 to 16 sessions and was conducted by 6 different therapists in our department. The assessors were independent of the therapists. Regular supervision by an examined IPT supervisor (C.H.), on average at least every fourth session during the course of the psychotherapy, ensured adherence. Treatment response was defined as a reduction of at least 50% of the baseline HAMD on day 42 (T4). The study was approved by the ethics committee of the Faculty of Medicine at Christian-Albrechts University.
For each sample, peripheral blood mononuclear cells (PBMC) were isolated from 10 ml of heparinized whole blood by density-gradient centrifugation (Ficoll solution, Pharmacia, Germany). For further isolation of the T lymphocyte fraction (from PBMC), we applied negative selection by plastic adherence of the mononuclear fraction, and to some extent, the B lymphocyte fraction. Therefore, PBMC were incubated for 1.5 h in tissue culture dishes (Falcon, Germany) in complete RPMI 1640 medium (GibcoBRL, Life Technologies, Karlsruhe, Germany) containing 10% fetal calf serum (FCS). Incubation took place at 37°C in a 5% CO₂/95% air-humidified atmosphere. After this incubation, the nonadherent cells (i.e., the T lymphocyte fraction) were removed and washed twice with phosphate-buffered saline (PBS). This procedure yields an enrichment of up to 89% T lymphocytes. In order to minimize variation in this procedure, all conditions were kept stable for all of the patients; in particular, only 1 batch of FCS was used. Purified T lymphocytes were adjusted to 1 × 10⁶ cells per sample and then centrifuged. The dry pellets were resuspended and lysed by adding a buffer containing 6.25 mM of Tris base, 2% sodium deoxycholate, 10% glycerol, 50 mM of DL-dithiothreitol and 0.1% bromphenol blue. These were then sonicated for 2–10 s to shear the DNA and reduce the sample viscosity. The lysates were stored at −80°C until use. Each patient’s samples (T0, T1, T2 and T3) were blotted, probed and quantified in the same assay. The protein concentration in each sample was determined by an assay that reacts to the presence of ampholytes, detergents and reductants (2-D Quant, Amersham, Freiburg, Germany). Adjusted relative to the protein concentration, the lysates of each sample were separated on 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Western blots were probed with rabbit anti-pCREB and anti-CREB (total) antibodies (New England Biolabs, Frankfurt, Germany). Bound antibodies were detected with goat anti-rabbit horseradish-peroxidase-conjugated IgG antibody obtained from Dianova (Hamburg, Germany). An enhanced chemiluminescence system (Biorad, Munich, Germany) was used for detection. The expression of pCREB (and CREB) was determined by densitometry and imaging software (Quantity One, Biorad). Due to general methodical issues, the interassay variability in Western blots is high. Therefore, the data were transformed to percentages, defining the baseline optical density (OD) (T0) as 100%.

**BDNF Stimulation of T Lymphocytes**

PBMC were isolated from heparinized blood of healthy volunteers via density-gradient centrifugation (Ficoll solution, Pharmacia) and washed twice with PBS. Cells were transferred to tissue culture flasks (Falcon) and stored for 24 h in RPMI 1640 (GibcoBRL) with 10% FCS (Biochrom, Berlin) at 37°C in a 5% CO₂/95% air-humidified atmosphere. In this step, mononuclear cells (and to some extent B lymphocytes) became adherent to the plastic surface. After incubation, nonadherent cells were removed and washed twice with PBS. Cells were then resuspended in RPMI 1640 without FCS. For each sample 1.5 × 10⁶ cells were transferred to a reaction tube (1.5 ml, Sarstedt) and then stimulated for 1 h with different concentrations of BDNF (1, 2, 4 ng/ml). Lyophilized recombinant human BDNF (R & D Systems) was reconstituted in RPMI 1640 without FCS. For SDS-PAGE and Western blot procedures, see above.

**Measurement of BDNF Plasma Levels**

Plasma was collected with EDTA as an anticoagulant and aprotinin as a broad-spectrum protease inhibitor. Blood was centrifuged for 15 min at 1,000 g at 2–8°C within 30 min of collection. Centrifugation of the separated plasma at 13,000 g was repeated for 10 min at 2–8°C for complete platelet removal. The samples were stored at −80°C until evaluation. The BDNF plasma levels were measured with solid-phase ELISA (R & D Systems). The assay procedure was performed following the manufacturer’s instructions. Plasma samples were collected at baseline (T0), as well as on days 7 (T1) and 21 (T3).

**Statistics**

Testing data for normality revealed an approximate gaussian distribution. With respect to CREB phosphorylation, variances differed significantly between the groups (p < 0.01). We therefore applied Welch’s modified t test in order to analyze the differences between the groups at T1, T2 and T3. We used the unpaired, 2-tailed Student’s t test for statistical evaluation of possible between-group differences in BDNF plasma levels, age, baseline HAMD (HAM-D T0), number of previous episodes and duration of current episodes. We corrected for multiple comparisons in accordance with the Bonferroni procedure. Distribution of gender and first versus recurrent episodes were assessed by Fisher’s exact test. For correlation calculations we applied Pearson’s test. Statistical analysis was performed using GraphPad Prism version 3.00 (GraphPad Software, San Diego, Calif., USA).

**Results**

After 6 weeks of treatment 17 patients showed a reduction of at least 50% of the baseline HAMD and were therefore defined as responders and the others as nonresponders. The mean duration of the episodes was significantly higher in the nonresponder group (48.9 compared to 13.6 weeks). With respect to age, gender, HAMD at baseline, in- or outpatient status and the number of previous depressive episodes, the groups did not differ significantly (table 1). With regard to CREB phosphorylation at weeks 1–3, the responder group showed a rise compared to baseline (table 2), whereas pCREB in the nonresponder group increased above baseline only at T2 (week 2). However, the standard deviations were high and differences between the groups were significant only at T1 (week 1). Using median split we furthermore grouped the responders with respect to the time of response. Early responders (n = 10) met response criteria within the first 3 weeks of treatment, whereas late responders (n = 7) did so within weeks 4, 5 or 6. Early responders showed the largest increase in pCREB at T1 and late responders at T2, but this difference did not reach a level of significance (data not shown).
Measurement of the BDNF plasma levels revealed no differences between the responder and nonresponder groups (table 3). Pearson correlations between BDNF plasma levels and pCREB on days 7 (T1) and 21 (T2) were not significant (T1: $r = -0.205$, $p = 0.372$; T2: $r = 0.146$, $p = 0.529$). Baseline values could not be compared due to the transformation of baseline CREB to 100% (see Patients and Methods). Furthermore, age, gender, HAMD, in- or outpatient status, number of previous depressive episodes or psychopharmacological treatment prior to IPT were not significantly correlated with the BDNF levels. The quantity of BDNF applied in the in vitro experiments was adapted to the concentration range of BDNF in the patient’s plasma (mean of all BDNF samples = 2.9 ± 2 ng/ml). The stimulation of freshly isolated T lymphocytes with 1, 2 or 4 ng/ml of BDNF increased pCREB within 1 h (fig. 1: representative blot from 3 independent experiments).

**Discussion**

The present data are in line with our previous study demonstrating that a rise in pCREB in lymphocytes is associated with clinical improvement [7]. We were now able to show this for patients treated exclusively with psychotherapy. The present results suggest that the pCREB in-
crease reflects a cellular response to biological parameters that are in some way associated with clinical response. This is supported by the fact that an early response tends to be related to an early rise in pCREB.

Remarkably, the pCREB increase occurred very early in the course of psychotherapy. Patients often feel stressed when addressing their problems during the early stages of therapy. Thus, the neuroendocrinological stress response could cause the early pCREB increase. On the other hand, the establishment of a successful therapeutic relationship at an early stage in psychotherapy might also have an impact on pCREB. This could explain the association between a biological process preceding clinical improvement and the outcome of psychotherapy.

BDNF has been implicated in the pathophysiology of affective disorders and several studies have found alterations of BDNF levels in depressive patients. However, some studies have failed to replicate these findings. In line with the latter we found no association between the BDNF levels and the severity of depression. This might be explained by the fact that the mean HAMD score in our study was lower than in investigations reporting negative correlations. In contrast to studies demonstrating that pharmacological treatment increases the BDNF levels, we found no impact of psychotherapy on BDNF. It has been suggested that alterations in BDNF serum concentrations are due to specific interactions of antidepressants and platelets and are not primarily related to biological changes that accompany clinical improvement.

We regard our results as further evidence for this hypothesis. We have demonstrated that BDNF is a potent inductor of pCREB in T lymphocytes, but BDNF does not contribute to the rise in CREB phosphorylation in the course of treatment with IPT.

In conclusion, our results suggest that, concordant with our hypothesis, the increase in pCREB occurs early in the course of psychotherapy. Furthermore, this rise is associated with treatment response and does not depend on pharmacological interventions. Remarkably, these changes apply to proplastic signal transduction cascades, albeit in lymphocytic cells. It is tempting to speculate that this variation indicates a systemic adaptation that is important for the treatment to succeed. The limitations to our study include a small sample size and large standard deviations of the results. This might reduce the general validity of our findings.

It has been demonstrated recently that the combination of psychotherapy and antidepressants is superior to medication alone. Moreover, cognitive behavioral therapy shows a significantly lower relapse rate in patients who discontinue maintenance pharmacotherapy for recurrent depression and it is useful in treating patients with comorbid depression in somatic disorders. However, for bipolar disorders it is a topic of debate for whom adjunctive psychological therapy should be provided. Future research might elucidate the interaction of pharmacological and psychotherapy by using biological parameters likely to be predictive of treatment response. This may lead to the identification of the patients requiring combination treatment in affective disorders.

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References


