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Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes

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Abstract

Genetic and pharmacological studies have suggested that brain-derived neurotrophic factor (BDNF) may be associated with the pathophysiology of bipolar disorder (BD). The present study investigated serum BDNF levels in manic, depressed, euthymic BD patients and in matched healthy controls, using an enzyme-linked immunosorbent assay (sandwich-ELISA). Serum BDNF levels were decreased in manic ($p = 0.019$) and depressed ($p = 0.027$) BD patients, as compared with euthymic patients and controls. Serum BDNF levels were negatively correlated with the severity of manic ($r = -0.37$, $p = 0.005$) and depressive ($r = -0.30$, $p = 0.033$) symptoms. These findings further support the hypothesis that the BDNF signaling system may play a role in the pathophysiology of BD.

Keywords: Bipolar disorder; Brain-derived neurotrophic factor; Depression; Mania; Mood stabilizers; Pathophysiology

Bipolar disorder (BD) is a prevalent, highly disabling illness, characterized by the presence of manic and depressive symptoms [1]. Although genetic and familial studies strongly suggest that a neurobiological basis may underlie the pathophysiology of BD [13], its exact etiology is poorly understood. Gene studies have consistently demonstrated that genetic heritability increases the predisposition to BD [3]. In addition, several family based association studies have reported that polymorphisms in the brain-derived neurotrophic factor (BDNF) gene may be involved in the pathophysiology of BD [7,14,17,23]. In particular, one single nucleotide polymorphism, caused by the substitution of valine to methionine at codon 66 (val66met), has been associated with poorer neuropsychologial performance [20] and better response to lithium prophylaxis [21]. Pharmacological studies have demonstrated that the mood stabilizers lithium and valproate modulate intracellular signaling pathways associated with neuronal plasticity and survival [2]. Further, it has been demonstrated that chronic administration of lithium and valproate increased BDNF expression in rat brain [6] and that the induction of the BDNF/TrkB pathway underlies some neuroprotective effects of lithium [9]. Previous studies have reported that serum BDNF is reduced in major depression (unipolar) disorder [10,22] and in drug-naïve and lithium-treated manic BD patients [16]. The present study aims to investigate serum BDNF levels in BD patients during mania, depression and euthymia, as compared to healthy controls.

The present study was approved by the local ethics committee (Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil) and all subjects provided written informed consent before entering in the study. Thirty-two euthymic, 21 depressed, and 32 manic patients were recruited from the Bipolar Disorders Program—Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil, and the Inpatient Psychiatric Unit—Hospital Universitário de Santa Maria, Santa Maria, Brazil. Diagnoses were carried out using the Structured Clinical Interview for DSM-IV-Axis I (SCID-I) [5]. In this study, only BD type-I patients were

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Table 1
Characteristics of bipolar disorder patients and healthy controls

	Control group	Bipolar patients			*p*-value
		Euthymic	Manic	Depressed	
Gender (female)	65.6%	62.5%	43.8%	71.4%	0.162*
Mean age (S.D.), years	40.69 (12.12)	40.28 (11.99)	40.13 (12.6)	40.71 (9.25)	0.997**
Mean years of schooling (S.D.)	8.69 (3.64)	9.94 (4.80)	7.69 (3.65)	7.53 (4.77)	0.117**
Mean number of medications (S.D.)	–	2.41 (0.95)	3.41 (1.39)	2.86 (1.23)	0.016**
Mean age of first mood episode (S.D.)	–	23.13 (11.38)	27.19 (10.87)	21.24 (13.89)	0.320**
Mean years of illness (S.D.)	–	17.34 (11.88)	12.78 (9.62)	19.50 (14.17)	0.209**
Mean HDRS score (S.D.)	–	4.28 (4.16)	5.16 (3.39)	22.81 (4.36)	0.001**
Mean YMRS score (S.D.)	–	3.16 (5.44)	34.47 (7.06)	5.10 (3.19)	0.001**
Presence of psychotic symptoms	–	12.5%	68.8%	28.6%	0.001*
Mean serum BDNF (S.D.) pg/μL Protein	0.20 (0.07)	0.19 (0.08)	0.14 (0.04)	0.15 (0.13)	0.019[a] 0.027[b]

HDRS, hamilton depression rating scale; YMRS, young mania rating scale; BDNF, brain-derived neurotrophic factor.
* Chi-square test.
** One-way ANOVA test.
[a] Manic vs. euthymic/control.
[b] Depressed vs. euthymic/control.

enrolled. Manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS) [25] and the Hamilton Depression Rating Scale (HDRS) [8], respectively. Patients were considered euthymic if they scored <7 on both YMRS and HDRS scales. Manic and depressed patients fulfilled criteria for current manic or depressive episode, respectively, according to SCID-I. The control group consisted of 32 healthy volunteers matched by age, gender and education, who manifested interest in participating in the study. Psychiatric assessment was carried out using SCID-I, non-patient version. Control subjects were non-smokers, were not on medication, and had no history of major psychiatric disorders, dementia, mental retardation, cancer or tumor in their first-degree relatives.

Five milliliters of blood were withdrawn from each subject by venipuncture into a free-anticoagulant vacuum tube. The blood was immediately centrifuged at 3000 × *g* for 5 min, and serum was kept frozen at −80 °C until assayed. BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Chemicon, USA). Briefly, microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluents and standard curve ranged from 7.8 to 500 pg of BNDF. Plates were then washed four times with wash buffer, added monoclonal anti-BNDF rabbit antibody (diluted 1:1000 with sample diluents), and incubated for 3 h at room temperature. After washing, a second incubation with anti-rabbit antibody peroxidase conjugated (diluted 1:1000) for 1 h at room temperature was carried out. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF was determined (absorbance set in 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

The outcome measures of the four groups were compared using the one-way ANOVA test for heterogeneity. The individual differences were assessed using a Tukey test if the ANOVA was significant. Pearson's correlation coefficient was performed to examine the relationship between YMRS and HDRS with serum BDNF levels. *p*-values <.05 were considered significant.

The characteristics of BD patients and controls are summarized in Tables 1 and 2. BD patients and controls did not differ in terms of gender, age or years of schooling. BD patients were similar in age of first mood episode and length of illness. As expected, manic patients had significantly higher rates of manic symptoms than depressed and euthymic patients ($p = 0.001$) and depressed patients had significantly higher rates of depressive symptoms than manic and euthymic patients ($p = 0.001$). In addition, manic patients had significantly more psychotic symptoms (presence of delusions or hallucinations) than depressed or euthymic BD subjects ($p = 0.001$). Serum BDNF levels were lower in BD patients during both manic ($p = 0.019$) and depressive ($p = 0.027$) episodes, as compared with euthymic patients and healthy controls (Fig. 1, Table 1). This finding suggests that the effects of pharmacological treatment on BDNF levels may be associated with mood stabilization. In addition, serum BDNF levels were negatively correlated with YMRS ($r = -0.37$, $CI_{95\%}$

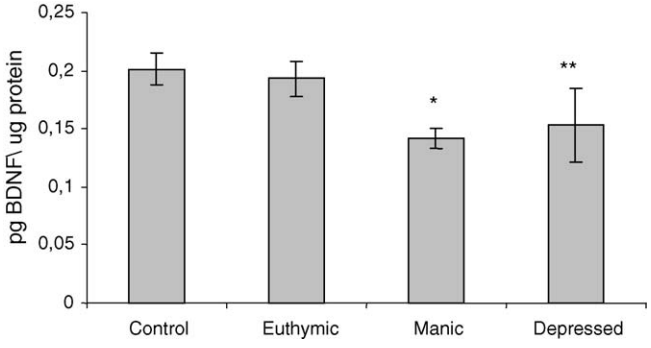

Fig. 1. Serum BDNF levels in BD patients and healthy controls. Legend: one-way ANOVA; Tukey posttest; $p = 0.019$; $p = 0.027$.

Table 2
Current medications of bipolar disorder patients

Mood	Patient	Medication
Euthymia	1	Lithium
	2	Valproate, chlorpromazine and fluoxetine
	3	Lithium, carbamazepine and risperidone
	4	Lithium, clozapine, risperidone and sertraline
	5	Lithium, levomepromazine and biperiden
	6	Lithium, carbamazepine and sulpiride
	7	Lithium and risperidone
	8	Lithium, chlorpromazine and fluoxetine
	9	Carbamazepine
	10	Lithium, risperidone and paroxetine
	11	Lithium
	12	Lithium and valproate
	13	Valproate
	14	Lithium, valproate and pimozide
	15	Lithium and carbamazepine
	16	Lithium and valproate
	17	Lithium and clonazepam
	18	Valproate and carbamazepine
	19	Lithium, valproate, risperidone and amytripitiline
	20	Lithium
	21	Lithium and chlorpromazine
	22	Lithium, sulpiride and sertraline
	23	Valproate
	24	Lithium, valproate and sulpride
	25	Lithium and valproate
	26	Lithium, valproate and chlorpromazine
	27	Lithium, valproate, haloperidol and bupropion
	28	Lithium and clonazepam
	29	Valproate and olanzapine
	30	Lithium, haloperidol and biperiden
	31	Lithium, risperidone and clonazepam
	32	Valproate and bupropion
Mania	33	Lithium and chlorpromazine
	34	Lithium, chlorpromazine, haloperidol and biperiden
	35	Lithium, chlorpromazine, haloperidol and biperiden
	36	Lithium, chlorpromazine, diazepam, haloperidol and biperiden
	37	Lithium, haloperidol, diazepam and biperiden
	38	Lithium, haloperidol, diazepam and biperiden
	39	Valproate, haloperidol, diazepam and biperiden
	40	Lithium
	41	Lithium, haloperidol, diazepam and biperiden
	42	Haloperidol and biperiden
	43	Lithium, chlorpromazine, haloperidol, diazepam and biperiden
	44	Lithium, haloperidol, levomepromazine, diazepam, and biperiden
	45	Lithium, valproate, haloperidol, diazepam and biperiden
	46	Lithium, haloperidol, diazepam and biperiden
	47	Lithium, haloperidol, levomepromazine, diazepam, and biperiden
	48	Lithium, chlorpromazine and biperiden
	49	Lithium, carbamazepine, haloperidol and biperiden
	50	Lithium and diazepam
	51	Lithium and diazepam
	52	Carbamazepine, haloperidol, chlorpromazine diazepam and biperiden
	53	Lithium
	54	Lithium and diazepam
	55	Lithium and diazepam
	56	Valproate, haloperidol and diazepam
	57	Lithium
	58	Lithium, haloperidol, diazepam and biperiden
	59	Valproate, haloperidol, diazepam and biperiden
	60	Lithium and diazepam
	61	Lithium, valproate, chlorpromazine, diazepam and biperiden
	62	Lithium, carbamazepine, haloperidol and biperiden
	63	Lithium, haloperidol, diazepam and biperiden
	64	Lithium and diazepam
Depressed	65	Lithium
	66	Lithium, carbamazepine and haloperidol
	67	Valproate and chlomipramine
	68	Lithium, carbamazepine and haloperidol
	69	Lithium, clonazepam and imipramine
	70	Valproate and haloperidol
	71	Lithium, carbamazepine, clozapine, and clonazepam
	72	Lithium, carbamazepine, lamotrigine and clonazepam
	73	Valproate and carbamazepine
	74	Lithium, valproate, levomepromazine and diazepam
	75	Carbamazepine and chlorpromazine
	76	Lithium and imipramine
	77	Valproate, carbamazepine, chlorpromazine and clonazepam
	78	Lithium, carbamazepine, tioridazine and clonazepam
	79	Valproate and thioridazine
	80	Lithium and clonazepam
	81	Lithium, carbamazepine, risperidone, clonazepam, and biperiden
	82	Valproate, risperidone and clonazepam
	83	Valproate and risperidone
	84	Lithium and chlorpromazine
	85	Valproate and chlorpromazine

[−138.21; −25.55], $p = 0.005$) and HDRS ($r = -0.30$, $CI_{95\%}$ [−81.27; −3.60], $p = 0.033$) scores. The presence of psychotic symptoms did not correlate with serum BDNF levels ($p > 0.05$; one-way ANOVA).

To the best of our knowledge, this is the first study that assessed serum BDNF levels in BD patients during manic, depressive and euthymic phases. We were able to demonstrate that serum BDNF is decreased in BD patients during manic and depressive episodes. However, euthymic BD patients had similar serum BDNF to healthy controls. These findings suggest that changes of BDNF levels may be state-related rather than trait-related. Our results are in accordance with previous studies suggesting that changes in BDNF signaling may be associated with the pathophysiology of BD and pharmacological response [13,21]. Furthermore, we found that serum BDNF had a significant negative correlation with the severity of manic and depressive symptoms. It cannot be ruled out that the use of medication may alter the levels of BDNF. However, it is unlikely that medication would account for the correlation between the

levels of BDNF and the severity of manic and depressive symptoms. Our results are in accordance with a recent report that BDNF levels were reduced during the manic episode of patients with BD, and the level of serum BDNF was negatively correlated with the severity of the manic symptoms [16]. Using a peripheral cell model, Karege et al. [12] recently reported that downregulation of PKA signaling decreased BDNF expression in lymphoblasts of BD patients. In addition, three independent studies reported that serum BDNF levels were reduced in unipolar depressive patients and were negatively correlated with the severity of depressive symptoms [10,22]. Studies designed to compare serum BDNF levels between bipolar and unipolar patients during depressive episodes are needed to further clarify this matter.

It is well known that BDNF exerts an important role in the modulation of cognitive processes such as learning and memory. It has been reported that BDNF modulates basal synaptic transmission and long-term potentiation in rat hippocampus [19]. Egan et al. [4] elegantly demonstrated that BDNF val66met polymorphism is associated with impaired episodic memory, abnormal overactivation of caudal hippocampus and decreased hippocampal *N*-acetyl-aspartate (a marker of neuronal integrity) in human brain. Moreover, it has been recently found that BDNF val66met polymorphism is associated with decreased hippocampal volume in humans [24]. Indeed, several studies have consistently demonstrated that cognitive impairment is a frequent finding in BD patients [18].

There are some limitations in the present study. Firstly, we measured BDNF levels in serum. Although the specific cellular sources of plasma BDNF are still unknown, it has been reported that platelets, vascular endothelial cells and neurons may contribute to the circulating BDNF content [15]. In addition, it has been demonstrated that BDNF can cross the brain–blood barrier, and there is a high positive correlation ($r = 0.81$) between serum and cortical BDNF levels [11]. Therefore, it has been suggested that the changes of plasma BDNF levels may partly reflect the changes of brain BDNF secretion [15]. Secondly, we have studied medicated BD subjects. Since it is well established that psychotropic medications may change BDNF level, we cannot rule out the effects of medications on the present findings. Studies comparing BDNF levels in medicated vs. unmedicated BD patients, and also before and after pharmacological treatment are warranted to control for this potential confounder.

In conclusion, we found that serum BDNF levels were decreased in BD patients during manic and depressive episodes, as compared with euthymic BD patients and healthy controls. We were also able to demonstrate that serum BDNF levels were negatively correlated with the severity of manic and depressive symptoms. Our findings further support the hypothesis of BDNF involvement in the pathophysiology of BD.

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