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BRIEF COMMUNICATION

Decreased plasma neurotrophin-4/5 levels in bipolar disorder patients in mania

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Objective: To evaluate two poorly explored neurotrophins (NT), NT-3 and NT-4/5, in bipolar disorder (BD).
Methods: Forty patients with type I BD (18 in remission and 22 in mania) and 25 healthy controls matched for age, gender, and educational attainment were enrolled in this study. All subjects were assessed by the Mini-International Neuropsychiatric Interview; the Young Mania Rating Scale and the Hamilton Depression Rating Scale were used to evaluate severity of symptoms in BD patients. Plasma levels of NT-3 and NT-4/5 were measured by enzyme-linked immunosorbent assay (ELISA).
Results: BD patients in mania presented decreased NT-4/5 plasma levels in comparison with controls ($p < 0.05$). There were no significant differences in NT-3 plasma levels between BD patients and controls.
Conclusion: These findings corroborate the view that neurotrophin dysfunction is associated with mood states in patients with BD.

Keywords: Neurotrophin; mania; depression; bipolar disorder; biomarker

Introduction

Neurotrophins constitute a family of proteins responsible for orchestrating complex processes in the central nervous system (CNS), such as cellular proliferation, differentiation, growth, migration, modulation of neuronal excitability, and synaptic transmission. As a result, neurotrophins have been implicated in the pathophysiology of a wide variety of neurodegenerative and psychiatric disorders, and have also been regarded as potential therapeutic targets. The neurotrophin family comprises brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5).

The pathophysiology of bipolar disorder (BD) is complex, and neurotrophin dysfunctions seem to play a pivotal role in the neurobiology of the disease.[1] BDNF is the most abundant neurotrophin in the CNS, particularly in the amygdala, hippocampus, and prefrontal cortex, brain areas directly involved in emotional regulation and several aspects of cognition (including attention, memory, and executive functioning).[2] Several studies and meta-analyses have reported decreased BDNF levels in patients with BD during acute mood episodes in comparison with healthy controls.[3,4] Our group demonstrated that BD patients in mania exhibit lower plasma NGF levels in comparison with controls and BD patients in euthymia.[5] The biological effects of the neurotrophins are mediated through the tropomyosin-related kinase (Trk) family of receptor tyrosine kinases (TrkA, TrkB, and TrkC) and the p75 neurotrophin receptor, a member of the tumor necrosis factor receptor superfamily. Despite recent reports of neurotrophic signaling dysfunction in BD, little attention has been paid to the study of NT-3 and NT-4/5, neurotrophins that modulate basal synaptic transmission and long-term potentiation in the hippocampus.

The main aim of the present study was to evaluate plasma levels of NT-3 and NT-4/5 in patients with BD during manic episodes and compare these levels with those of healthy controls. As a secondary aim, the levels of these neurotrophins were correlated with clinical parameters.

Methods

The present study included 40 medicated patients with type 1 BD and 25 controls matched for age, gender, and educational attainment. Patients were recruited at Hospital Governador Israel Pinheiro, Belo Horizonte, state of Minas Gerais, Brazil. The diagnosis of BD was independently confirmed by two psychiatrists using the Mini-International Neuropsychiatric Interview

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(MINI-Plus).[6] All patients were assessed with the Young Mania Rating Scale (YMRS)[7] and the Hamilton Depression Rating Scale (HDRS).[8] YMRS and HDRS were administered to evaluate the severity of manic and depressive symptoms respectively. Mania was defined according to the psychiatric evaluation. BD patients with a YMRS score $>$ 13 were defined as being in mania. Remission was defined by YMRS score $<$ 7 and HDRS score $<$ 7 points for at least 8 consecutive weeks. The control group was recruited from the local population. Controls were required to not have any psychiatric disorder, family history of psychiatric disorder, or cognitive deficits; the MINI-Plus interview was used to to exclude psychiatric disorders. The study was approved by the local ethics committees. All participants provided written informed consent.

Peripheral blood samples (10 mL) were drawn at 8-10 a.m. from each subject by venipuncture into a heparin-containing vacuum tube at the moment of clinical interview. The blood was immediately centrifuged twice at 3,000 g for 10 min, and plasma samples were kept frozen at -70°C until assayed. Plasma NT-3 and NT-4/5 levels were measured using enzyme-linked immunosorbent assay (ELISA) kits for NT-3 and NT-4/5 (DuoSet, R&D Systems, Minneapolis, MN, USA), in accordance with manufacturer instructions. Concentrations were expressed as pg/mL.

All variables were tested for normality of distribution by means of the Kolmogorov-Smirnov test. Descriptive statistics were used to report socio-demographic and clinical features of the sample. Comparisons between dichotomous variables were assessed with the chi-square or Fisher's exact test as appropriate. Between-group differences (patients vs. controls) were assessed with the Mann-Whitney *U* test. Differences among the three groups (patients in mania vs. patients in remission vs. controls) were compared with the Kruskal-Wallis test. Multiple comparisons among levels were checked with Dunn's post-hoc test. Spearman's correlation analysis was performed for NT-3 and NT-4/5 levels, age, disease duration, and YMRS and HDRS scores. All statistical tests were two-tailed and performed at a significance level of $p < 0.05$. Statistical analyses were performed using SPSS version 17.0.

Results

Eighteen BD patients were in remission (14 female; age [mean ± SD] 49.78±8.92 years) and 22 BD patients were in mania (15 female; age [mean ± SD] 48.04±14.22 years). The mean ± SD length of illness was 26.61±11.85 years in BD patients in remission and 19.0±13.43 years in BD patients in mania ($p = 0.09$, Mann-Whitney *U* test). The mean YMRS and HDRS scores in mania were 26.0 and 4.77, respectively. Four of the 18 patients in remission had nicotine dependence, as did nine of the 22 patients in mania ($p = 0.31$). In the control group, 19 out of 25 controls were female and mean age was 49.16±7.36 years. No control subjects had nicotine dependence. There were no significant differences between BD patients in remission, BD patients in mania, or controls regarding age, gender, or educational attainment.

Plasma NT-3 levels were similar in BD patients in mania and in remission and in controls (median [interquartile range]: 52.79 [30.97-190.12] pg/mL for BD patients in mania; 41.76 [28.19-70.46] pg/mL for BD patients in remission; 44.52 [69.69-396.14] pg/mL in controls; $p > 0.05$, Kruskal-Wallis test) (Figure 1A).

BD patients in mania exhibited decreased NT-4/5 plasma levels in comparison with controls (median [interquartile range]: 89.88 [21.10-158.66] pg/mL for BD patients in mania vs. 57.74 [56.12-214.53] pg/mL in controls; $p < 0.05$, Dunn's post-hoc test). There were no significant differences between BD patients in mania vs. BD patients in remission (median [interquartile range] for BD patients in remission, 37.09 [34.27-69.60] pg/mL; $p > 0.05$, Dunn's post-hoc test) or BD in remission vs. controls ($p > 0.05$, Dunn's post-hoc test) (Figure 1B).

Plasma levels of NT-4/5 and NT-3 were not associated with the presence of psychiatric or clinical comorbidities,

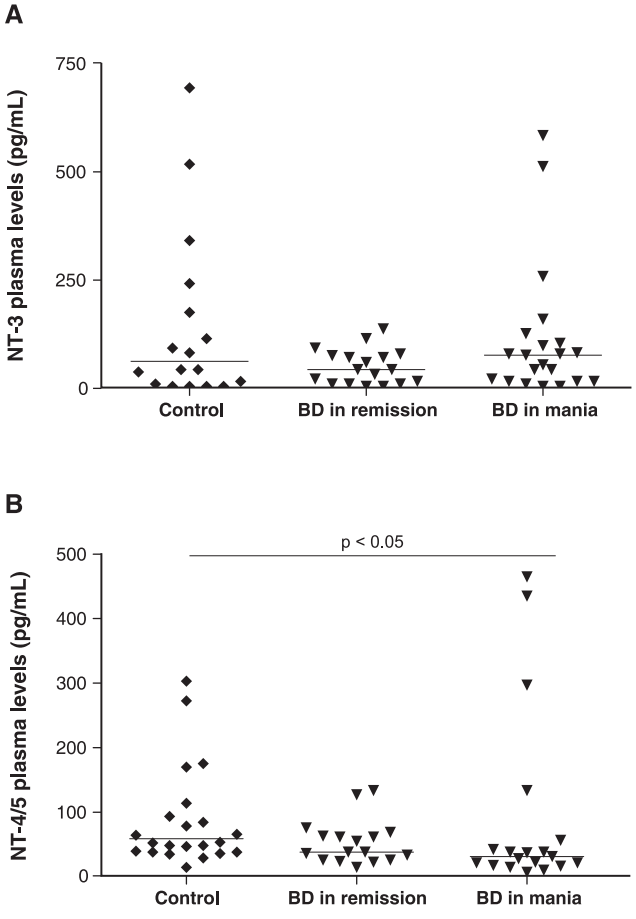

Figure 1 NT-3 (A) and NT-4/5 (B) plasma levels in controls, patients with BD in mania, and patients with BD in remission. Bars represent median values. BD = bipolar disorder; NT = neurotrophin. $p < 0.05$, Kruskal-Wallis test with post-hoc Dunn's test.

substance dependence, nicotine dependence, or use of any mood stabilizer (i.e., atypical antipsychotics, lithium, or anticonvulsants). NT-3 and NT-4/5 plasma levels did not correlate with age, disease duration, educational attainment, or HDRS and YMRS scores.

Discussion

In the present sample, BD patients in mania had decreased circulating levels of NT-4/5 in comparison with healthy subjects and BD patients in remission. To the best of our knowledge, this is the first study to report lower plasma levels of NT-4/5 in patients with BD.

Previous studies evaluating NT-4/5 levels have reported discordant results. Walz et al.[9] demonstrated increased NT-4/5 serum levels in BD, regardless of mood state, compared with controls. Another study found that mRNA NT-4/5 expression in total blood of BD patients in depression was not significantly different from that of controls.[10] The reasons for such discordant results are unclear.

NT-4/5 and BDNF exert their specific biological activities through the same receptors (TrkB and p75), but NT-4/5 seems to be more potent than BDNF in terms of influencing neurite outgrowth.[11] In this line, and given the consistent finding of decreased circulating BDNF levels in BD patients during acute mood episodes,[3,4] decreased plasma levels of NT-4/5 in BD patients in mania were to be expected.

Regarding NT-3 plasma levels, our result is consistent with a previous study that evaluated mRNA expression of NT-3 in peripheral blood cells from BD patients and did not find any difference in comparison with controls.[10] Other studies demonstrated increased NT-3 serum levels in BD patients in acute episodes.[12-14] These discordant results might be due to distinct inclusion criteria (exclusively type 1 BD in the present study vs. type 1 and 2 BD in previous studies) and due to methodological differences, such as serum vs. plasma measurements.

It is difficult to draw a definitive conclusion regarding the neurotrophin profile of patients with BD. Previous studies have reported decreased BDNF levels in acute mood episodes[3,4] and decreased NGF levels in mania,[5] and the present study found decreased NT-4/5 levels in mania. It thus seems reasonable to assume that all neurotrophins are decreased during acute mood episodes (particularly manic episodes) in BD, which is in line with evidence that the related signaling pathways are altered in BD.[15]

Longitudinal studies controlling for methodological issues and confounding factors are necessary to confirm this assumption, as there are several conflicting reports. Furthermore, it is still uncertain whether plasma or serum levels reflect the distribution of neurotrophins in the CNS. Notably, no previous study has evaluated NT-3 or NT-4/5 levels in the CNS of BD patients.

This study has strengths and limitations that must be considered when interpreting its results. The diagnostic interviews of both patients and controls were performed using the same protocol, overcoming a limitation of previous studies. In addition, the exclusion of patients with other medical conditions, such as inflammatory diseases, can be regarded as strength of the study. The lack of strict control for confounding factors, such as body mass index, medication use, and number of cigarettes, must be considered limitations, as must the small sample size. Furthermore, the question of whether these neurotrophin levels represent primary-causal or secondary-reactive changes remains unaddressed.

In conclusion, our findings reinforce the view that neurotrophin dysfunction is present in BD, especially during acute mood episodes. These findings support a role for NT-4/5 as a potential therapeutic target in BD.

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Disclosure

The authors report no conflicts of interest.

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