BDNF protein levels are decreased in transformed lymphoblasts from lithium-responsive patients with bipolar disorder

Michael Tseng, MD, PhD; Martin Alda, MD; Li Xu, MSc; Xiujun Sun, MSc; Jun-Feng Wang, PhD; Paul Grof, MD, PhD; Gustavo Turecki, MD, PhD; Guy Rouleau, MD, PhD; L. Trevor Young, MD, PhD

Tseng, Xu, Wang, Grof, Young — Centre for Addiction and Mental Health, and Department of Psychiatry, University of Toronto, Toronto, Ont.; Alda — Department of Psychiatry, Dalhousie University, Halifax, NS; Turecki — Department of Psychiatry, McGill University; Rouleau — Department of Medicine, Université de Montréal, Montréal, Que.; Young — Department of Psychiatry, University of British Columbia, Vancouver, BC

Objective: Brain-derived neurotrophic factor (BDNF) is a key factor in neuroplasticity and has been implicated in the affective disorders; studies have demonstrated elevated BDNF in patients taking lithium and other mood stabilizers. The objective of our study was to analyze BDNF in lithium-responsive patients with bipolar disorder (BD) to further understand the role of BDNF in the pathophysiology of BD. **Methods:** Using enzyme-linked immunosorbent assay, we measured transformed B lymphocytes for BDNF protein. **Results:** BDNF levels were 36% lower in lymphoblasts from patients with BD (n = 12), compared with matched control participants (n = 13), and 55% lower when compared with their unaffected relatives (n = 14). Lithium significantly decreased BDNF levels in patients with BD and healthy control participants, although BDNF levels remained lower (33%) in the BD group posttreatment. **Conclusion:** Decreased BDNF may constitute part of the pathophysiologic process of BD in a lithium-responsive subgroup of individuals with this disease. A compensatory mechanism protecting the genetically predisposed unaffected relatives from phenotypic expression of BD is suggested.

Objectif : Le facteur neurotrophique dérivé du cerveau (FNDC) joue un rôle clé dans la neuroplasticité et est mis en cause dans des troubles de l'affectivité. Des études ont démontré des concentrations élevées de FNDC chez les patients qui prenaient du lithium et d'autres thymorégulateurs. Nous voulions analyser la concentration de FNDC chez les patients atteints d'un trouble bipolaire (TB) répondant au lithium afin de comprendre davantage le rôle du FNDC dans la pathophysiologie du TB. **Méthodes :** Nous avons mesuré par dosage immunoenzymatique les lymphocytes B transformés pour déterminer les protéines dans le FNDC. **Résultats :** Les concentrations de FNDC étaient plus faibles de 36 % dans les lymphoblastes provenant de patients atteints d'un TB (n = 12) que dans ceux des participants témoins jumelés (n = 13), et 55 % moins élevés que chez les membres de leur parenté non atteints (n = 14). Le lithium a réduit considérablement les concentrations de FNDC chez les patients atteints de TB et les participants témoins en bonne santé, même si elles sont demeurées plus faibles (33 %) chez les patients atteints de TB après le traitement. **Conclusion :** La diminution des concentrations de FNDC peut constituer un élément du processus pathophysiologique du TB chez un sous-groupe d'individus atteints de la maladie qui répondent au lithium. On pense qu'un mécanisme compensatoire protège contre l'expression phénotypique du TB les patients apparentés non touchés qui sont génétiquement prédisposés.

Introduction

Bipolar disorder (BD) is a highly heterogeneous disorder, with neuropathologic findings that are often variable and inconsistent.[1] Lithium is considered first-line treatment for BD,[2,3] and an increasing body of observations support the notion that lithium responsiveness in bipolar patients is a characteristic that defines a more homogeneous subpopulation of people

Correspondence to: Dr. L.T. Young, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver BC V6T 2A1; fax 604 822-0399; Trevor.Young@ubc.ca

Medical subject headings: bipolar disorder; brain-derived neurotrophic factor; enzyme-linked immunosorbent assay; lithium.

J Psychiatry Neurosci 2008;33(5):449–53.

Submitted Oct. 26, 2007; Revised Dec. 7, 2007; Accepted Dec. 31, 2007

with BD. The criteria for lithium responsiveness are quite stringent and are described elsewhere.[4,5] There are 4 advantages to considering this population as an entity in its own right: 1) lithium responders have distinct clinical features, including episodic course and absence of rapid cycling; 2) lithium responders have distinct family histories (more specific for mood disorders) and strong genetic loading; 3) lithium responders differ from responders to other mood stabilizers, with lower rates of comorbidity and fewer atypical features; 4) increasing knowledge of lithium's mechanisms of action can aid in the distinction of lithium-related endophenotypes.[6] Examining the unaffected relatives of a bipolar population may aid in the study of endophenotypes in BD because, although they possess a genetic complement similar to that of their relatives with BD and are therefore at risk for developing the illness, they do not express the disease phenotype.

Neurotrophic factors play a role in modulating cell survival and promoting neuronal plasticity. Therefore, deficient or altered expression of these factors may contribute to, or be a consequence of, the pathophysiology of mental illness. In particular, brain-derived neurotrophic factor (BDNF) has been implicated in the affective disorders. Numerous studies have investigated the val66met BDNF single nucleotide polymorphism in relation to BD, with mixed results.[7,8] Although studies of major depressive disorder have shown an increase in BDNF in the postmortem hippocampus in response to antidepressant therapy,[9] findings in regard to untreated BD have been mixed. In lymphoblasts from BD patients, BDNF messenger ribonucleic acid (mRNA) expression did not differ in comparison with control participants.[10] Recently, serum studies have shown a decrease in BDNF protein in BD patients with mania and depression but not in euthymic BD patients.[11] The purpose of this study was to measure basal and lithium-induced BDNF protein levels in cultured B lymphoblasts from lithium-responsive BD patients and nonpsychiatric, nonneurologic control participants and to examine basal BDNF protein levels in the unaffected family members of BD patients.

Methods

Study population

We studied a group of 12 lithium-responsive BD I patients, 14 of their unaffected family members and 13 nonpsychiatric, nonneurologic control participants. Table 1 summarizes the demographic data of this study sample. All participants were interviewed with the Schedule for Affective Disorders and Schizophrenia—Lifetime version[12] and then diagnosed according to Research Diagnostic Criteria.[13] All patients also met the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition,[14] for BD I without comorbid diagnoses. BD patients also had to meet the criteria for a full response to lithium prophylaxis, which are described elsewhere.[4,5] Briefly, all lithium-responsive patients had been maintained on lithium monotherapy for a minimum of 3 years, with an average plasma concentration of 0.6 mEq/L and no affective episodes during the course of treatment. They were, however, known to be at high risk for recurrence before the initiation of lithium. Healthy control participants were married-in members of probands' families; none had a family history of BD or major depression among first- or second-degree relatives. The study was approved by the Research Ethics Board at the Centre for Addiction and Mental Health in Toronto, Ontario, and the Capital District Health Authority in Halifax, Nova Scotia, and we obtained written informed consent regarding participation in the study from all participants.

Cell isolation, culture and drug treatment

Lymphoblastoid cell lines were generated from blood samples, as described previously.[15] Human Epstein–Barr virus–transformed B lymphocytes were cultured in Iscove's Modified Dulbecco's Medium (Invitrogen) containing 15% fetal calf serum with 100 units/mL penicillin, 100 µg/mL streptomycin, 2 µg/mL amphotericin B and 2 µg/mL sodium desoxycholate in 5% CO_2/95% air at 37°C in a humidified incubator. Cells from BD and control groups were treated with 1 mM lithium chloride (LiCl) or vehicle (dH_2O) every other day for 7 days, respectively.

Enzyme-linked immunosorbent assay (ELISA)

After treatment with 1 mM LiCl or dH_2O for 7 days, cultured B lymphocytes from BD, unaffected relatives and control participants were collected and homogenized in 100 mM Tris-HCl pH 7.0, with 2% bovine serum albumin, 1 M NaCl, 4 mM EDTA-Na_2, 2% Triton X-100, 0.1% Na-azide, aprotinin 0.2 µg/mL, pepstatin A 0.2 µg/mL, leupeptin 0.5 µg/mL and 0.01 mM phenylmethylsulphonyl fluoride. Homogenates were centrifuged (12000 × *g*, 4°C, 40 min), and the resulting supernatant was used to perform the ELISA according to the instructions of a BDNF Sandwich ELISA Kit (Chemicon). The BDNF concentration for each sample was determined by interpolating the sample's optical density at 450 nm into the linear range of a standard curve generated with serial concentrations of BDNF protein.

Data and statistical analyses

We tested between-group differences in the demographic characteristics of age and sex, using analyses of variance

Table 1: Demographic data for study participants

Variable	Group; mean (and SD) [range]*		
	Control *n* = 13	BD lithium-responsive *n* = 12	Unaffected relatives *n* = 14
Men/women	7/6	6/6	3/11
Age, yr	40.8 (9.8) [21–52]	42.9 (9.2) [24–55]	35.5 (13.4) [19–61]
Age at onset, yr	NA	24.0 (9.2)	NA
Episodes before Li	NA	8.7 (5.7)	NA
Li treatment, yr	NA	11.9 (6.4)	NA

BD = bipolar disorder; Li = lithium; NA = not applicable; SD = standard deviation.
*Unless otherwise indicated.

(ANOVA) and χ^2 tests, respectively. We used repeated-measures ANOVA to analyze paired data, including cells with and without lithium treatment, and we examined the remaining measures with 1-way ANOVA followed by Tukey post hoc tests to assess the difference between groups in BDNF expression. All results were expressed as the mean (and standard error of the mean [SEM]). Statistical significance was set at $p < 0.05$ for all analyses. All statistical analyses were carried out with BDMP version 8.1 for Windows.

Results

The BDNF levels were significantly lower (36%, $F_{1,23} = 6.21$, $p = 0.02$) in transformed lymphoblasts from lithium-responsive BD patients (mean 18.6, SEM 2.7 optical density [OD] units), compared with healthy control participants (mean 27.7, SEM 2.9 OD units) (Fig. 1). Of additional interest, BDNF levels in lymphoblasts from BD patients were 55% lower ($p = 0.003$) than in cells from unaffected relatives (mean 41.4, SEM 6.8 OD units), but they did not differ significantly between healthy control participants and unaffected relatives of BD patients. Further, lithium treatment significantly decreased BDNF levels in both treatment groups (12%, $F_{1,23} = 4.36$, $p = 0.05$). After lithium treatment, BDNF levels were still significantly lower (33%) in BD patients (mean 15.9, SEM 2.6 OD units), compared with control participants (mean 24.8, SEM 2.7 OD units). No age- or sex-related effects were observed in BDNF expression as examined by ANOVA and χ^2 tests, respectively (data not shown).

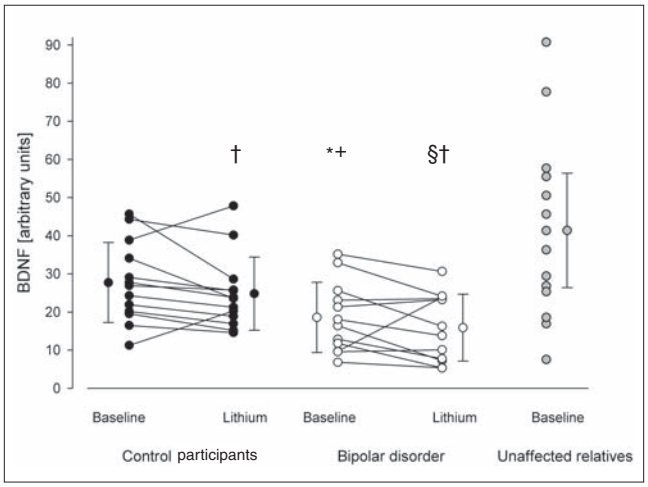

Fig. 1: Brain-derived neurotrophic factor (BDNF) protein levels in transformed lymphoblasts from nonpsychiatric control subjects and lithium-responsive bipolar disorder (BD) patients (with and without 1 mM lithium treatment for 7 days) and unaffected relatives. BDNF protein levels were 36% lower in subjects with BD when compared with nonpsychiatric control subjects (*$p = 0.02$), and this decrease remained (33%, §$p = 0.02$) after lithium treatment. Lithium treatment decreased BDNF levels in both BD and control populations (†$p = 0.05$). BDNF protein levels were 55% lower in BD patients when compared with their unaffected relatives (+$p = 0.003$). All measures are expressed as mean (and standard deviation).

Discussion

The present study reveals that basal BDNF protein levels are decreased in cultured lymphoblasts from lithium-responsive BD patients when compared with both their unaffected relatives and with healthy control participants. There was no significant difference in BDNF levels between unaffected relatives and healthy control participants. Treatment of cultured lymphoblasts with lithium decreased BDNF levels in all participants, but the difference between BD patients and healthy control participants remained.

BDNF is a member of the neurotrophin family, and plays an important role in neuroplasticity and neuroprotection. In BD patients, serum BDNF has been reported as low during manic and depressive episodes[11] and has been negatively correlated with severity of manic episodes.[16] Further, the BDNF val66met single nucleotide polymorphism has been associated with BD with mixed results[7]; a recent meta-analysis found a lack of association of this polymorphism with BD.[8] Our study revealed a decrease in BDNF protein levels in the transformed lymphoblasts from lithium-responsive bipolar patients when compared with BDNF levels in cells from their unaffected relatives and from healthy control participants. In addition, we did not find significant differences between the latter 2 groups. Taken together, our data suggest that BDNF levels in lymphoblasts may be indicative of a pathophysiologic process within this disease entity and may also be a suitable biomarker for trait-related factors in BD. These results also suggest that lithium responsiveness may confer pathophysiologic changes on BD lymphoblasts because a previous lymphoblast study did not reveal BDNF mRNA expression differences between BD patients and healthy control participants.[10] This may provide a further rationale for future studies comparing lithium-responsive and lithium-unresponsive BD patients.

The study of endophenotypes in BD is useful because it permits the identification and examination of a more homogeneous clinical population. The use of a lithium-responsive population is advantageous in this regard. If BDNF protein levels are indeed an endophenotypic marker of BD, one might predict the expression of BDNF in unaffected relatives to be in the mid-range between that found in control participants and that found in BD patients, according to recently modified BD endophenotype criteria.[17] However, we found no significant differences in BDNF levels between transformed lymphoblasts of control participants and those of unaffected relatives, and paradoxically, we found higher BDNF levels in the relatives compared with their BD counterparts. One possible explanation for this discrepancy is a compensatory mechanism wherein unaffected relatives of lithium-responsive BD patients upregulate peripheral BDNF protein levels, ultimately protecting against expression of the bipolar phenotype in a genetically predisposed cohort. According to this hypothesis, one might expect BDNF levels to drop in unaffected relatives in whom BD is later diagnosed. Prospectively examining BDNF levels from transformed lymphoblasts in this subpopulation may improve our understanding of the role of BDNF in the emergence and progression of BD pathology. BDNF's role in neuroprotective signal transduction pathways makes it an

ideal candidate in such a compensatory mechanism, and exploring the role of related signal transduction factors such as the cAMP response element-binding protein or mitogen-activated protein kinases in unaffected relatives may inform our understanding of such a mechanism.

Treatment with antidepressants and mood stabilizers increases BDNF levels, as observed in human postmortem hippocampus,[9] in rat hippocampus and frontal cortex[18] and in several serum studies of individuals with depression.[19–22] In contrast, our study demonstrates a decrease in BDNF levels after lithium treatment of transformed lymphoblasts (Fig. 1) in both bipolar and healthy control populations. This study is the first to examine lymphoblast BDNF levels in response to lithium, a response that may not be directly comparable with the effects in brain tissue or serum. The decrease in BDNF may be a dose-dependent response to lithium, or it may be related to length of treatment. Dosage and duration of lithium treatment were chosen on the basis of established methods from previous studies.[23–25] However, lithium may also have a biphasic effect.[26] Further studies examining a wider range of dosages and treatment lengths may elaborate on BDNF response to lithium in lymphoblasts.

Limitations of this study include the lack of data on lithium-treated lymphoblasts from the unaffected relatives of lithium-responsive BD patients. Also, the transformation process of the lymphocytes may induce changes in BDNF expression, although in this experiment, any such effect should have been uniform across all experimental groups. Additionally, the relevance of BDNF levels in peripheral cells to BDNF expression and function in the central nervous system of BD patients is not entirely clear. In support of this approach, BDNF levels in serum and cortex have been positively correlated in rats,[27] and serum BDNF levels have recently been associated with expression of the neuronal integrity marker *N*-acetylaspartate in the human cerebral cortex.[28] Despite their peripheral source, transformed lymphoblasts are easily isolated and cultured and have the tremendous advantage of allowing the study of putative biomarkers in their native, living context, free of medication or hormonal influences. A prolonged study duration might have revealed longer-term lithium effects on BDNF levels. Finally, our findings might have been strengthened with a larger number of study participants, particularly in regard to increasing the power of statistical analyses applied to discern family-of-origin effects.

In conclusion, we demonstrate a decrease in BDNF levels in transformed lymphoblasts from lithium-responsive BD patients when compared with BDNF levels in cells from their unaffected relatives and healthy control participants, even when they are compared with healthy control participants after lithium treatment. This raises the possibility that measuring BDNF expression in transformed lymphoblasts could be a useful diagnostic marker for a predisposition to develop BD. Our results also suggest that, in unaffected relatives who are genetically predisposed to BD, a potential BDNF-related compensatory effect may be taking place and preventing emergence of a bipolar phenotype. Finally, we observed a decrease in BDNF levels after lithium treatment, suggesting that the mechanism of BDNF response to lithium in lymphoblasts may be different when compared with that in cortex or serum. A more detailed characterization of BDNF's role in the response to lithium therapy in both human and animal models is warranted.

Acknowledgements: This study was supported by grant #64410 from the Canadian Institutes of Health Research.

Competing interests: None declared.

Contributors: Drs. Alda, Wang, Turecki, Rouleau and Young designed the study. Drs. Tseng, Alda, Xu, Sun, Grof and Rouleau collected the data, which Drs. Alda, Xu and Young analyzed. Drs. Tseng and Young wrote and reviewed the article, which Drs. Alda, Xu, Sun, Wang, Grof, Turecki and Rouleau also reviewed. All authors gave final approval for publication.

References

1. Alda M. The phenotypic spectra of bipolar disorder. *Eur Neuropsychopharmacol* 2004;14(Suppl 2):S94-9.

2. American Psychiatric Association. Practice guideline for the treatment of patients with bipolar disorder (revision). *Am J Psychiatry* 2002;159(4 Suppl):1-50.

3. Yatham LN, Kennedy SH, O'Donovan C, et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) guidelines for the management of patients with bipolar disorder: consensus and controversies. *Bipolar Disord* 2005;7(Suppl 3):5-69.

4. Grof P, Alda M, Grof E, et al. Lithium response and genetics of affective disorders. *J Affect Disord* 1994;32:85-95.

5. Turecki G, Grof P, Cavazzoni P, et al. Evidence for a role of phospholipase C-gamma1 in the pathogenesis of bipolar disorder. *Mol Psychiatry* 1998;3:534-8.

6. Alda M, Grof P, Rouleau GA, et al. Investigating responders to lithium prophylaxis as a strategy for mapping susceptibility genes for bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;29:1038-45.

7. Post RM. Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. *J Psychiatr Res*. In press.

8. Kanazawa T, Glatt SJ, Kia-Keating B, et al. Meta-analysis reveals no association of the Val66Met polymorphism of brain-derived neurotrophic factor with either schizophrenia or bipolar disorder. *Psychiatr Genet* 2007;17:165-70.

9. Chen B, Dowlatshahi D, MacQueen G, et al. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry* 2001;50:260-5.

10. Karege F, Schwald M, Papadimitriou P, et al. The cAMP-dependent protein kinase A and brain-derived neurotrophic factor expression in lymphoblast cells of bipolar affective disorder. *J Affect Disord* 2004;79:187-92.

11. Cunha AB, Frey BN, Andreazza AC, et al. Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neurosci Lett* 2006;398:215-9.

12. Endicott J, Spitzer RL. A diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psychiatry* 1978;35:837-44.

13. Spitzer RL, Endicott J, Robins E. Research diagnostic criteria: rationale and reliability. *Arch Gen Psychiatry* 1978;35:773-82.

14. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington: The Association; 1994.

15. Alda M, Keller D, Grof E, et al. Is lithium response related to G(s)alpha levels in transformed lymphoblasts from subjects with bipolar disorder? *J Affect Disord* 2001;65:117-22.

16. Machado-Vieira R, Dietrich MO, Leke R, et al. Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. *Biol Psychiatry* 2007;61:142-4.

17. Hasler G, Drevets WC, Gould TD, et al. Toward constructing an endophenotype strategy for bipolar disorders. *Biol Psychiatry* 2006; 60:93-105.

18. Einat H, et al. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J Neurosci* 2003;23:7311-6.

19. Aydemir O, Deveci A, Taneli F. The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminary study. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;29:261-5.

20. Gonul AS, Akdeniz F, Taneli F, et al. Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *Eur Arch Psychiatry Clin Neurosci* 2005;255:381-6.

21. Huang T, Lee C, Liu Y. Serum brain-derived neurotrophic factor levels in patients with major depression: effects of antidepressants. *J Psychiatr Res* 2008;42:521-5.

22. Piccinni A, Marazziti D, Catena M, et al. Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments. *J Affect Disord* 2008;105:279-83.

23. Young LT, Woods CM. Mood stabilizers have differential effects on endogenous ADP ribosylation in C6 glioma cells. *Eur J Pharmacol* 1996;309:215-8.

24. Ozaki N, Chuang DM. Lithium increases transcription factor binding to AP-1 and cyclic AMP-responsive element in cultured neurons and rat brain. *J Neurochem* 1997;69:2336-44.

25. Kato T, Shioiri T, Inubushi T, et al. Brain lithium concentrations measured with lithium-7 magnetic resonance spectroscopy in patients with affective disorders: relationship to erythrocyte and serum concentrations. *Biol Psychiatry* 1993;33:147-52.

26. Manji HK, Lenox RH. Long-term action of lithium: a role for transcriptional and posttranscriptional factors regulated by protein kinase C. *Synapse* 1994;16:11-28.

27. Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett* 2002;328:261-4.

28. Lang UE, Hellweg R, Seifert F, et al. Correlation between serum brain-derived neurotrophic factor level and an in vivo marker of cortical integrity. *Biol Psychiatry* 2007;62:530-5.