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Chronic Administration of Mood Stabilizers Upregulates BDNF and Bcl-2 Expression Levels in Rat Frontal Cortex

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Abstract

Brain-derived neurotrophic factor (BDNF) and B-cell lymphoma-2 (Bcl-2) proteins are neuroprotective factors involved in neuronal signaling, survival and plasticity. Both can be regulated by cyclic AMP response element binding (CREB) protein. Decreased levels of BDNF and Bcl-2 are implicated in the pathogenesis of bipolar disorder. The present study investigated whether chronically administered mood stabilizers to rats would increase BDNF and/or Bcl-2 levels in brain. Real time RT-PCR, sandwich ELISA and Western blotting were used to measure BDNF and Bcl-2 mRNA and protein levels in the frontal cortex of rats chronically administered carbamazepine (CBZ) or lamotrigine (LTG) to produce plasma concentrations therapeutically relevant to bipolar disorder. Chronic CBZ and LTG significantly increased BDNF and Bcl-2 mRNA and protein levels in the frontal cortex. A common mechanism of action of mood stabilizers in the treatment of bipolar disorder may involve neuroprotection mediated by upregulation of brain BDNF and Bcl-2 expression.

Keywords

BDNF; Bcl-2; lithium; valproate; carbamazepine; lamotrigine; brain; bipolar Disorder; rat

Introduction

Brain-derived neurotrophic factor (BDNF) and B-cell lymphoma-2 (Bcl-2) expression are regulated mainly by cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) [1], and play important roles in cell survival, cell plasticity, and the growth and differentiation of new neurons and synapses [2]. BDNF is expressed in the human hippocampus and surrounding neocortex throughout development, maturation, and aging, and is involved in early growth and maintenance of neurons throughout life [3]. Bcl-2 acts as an anti-apoptotic cell survival protein [4,5] and exerts neurotrophic activity in the central nervous system [5,6]. Decreased protein levels of BDNF and Bcl-2 have been implicated in the pathogenesis of bipolar disorder (BD) [7,8].

Neuroprotection may be important in treating BD in so far as *in vivo* imaging and postmortem studies have revealed significant brain atrophy in BD patients, with a decrease in cortical thickness [9], as well as reduced numbers and/or sizes of glia and neurons in discrete brain areas [10,11]. These changes were attributed to reduced neuronal survival factors [11].

The mood stabilizers, carbamazepine (CBZ) and lamotrigine (LTG), have been approved by the FDA as monotherapy for BD. However, mechanisms underlying their therapeutic actions

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are not agreed upon. CBZ is preferred for treating the mania of BD, whereas LTG is preferred for the depressive phase and rapid cycling [12].

One common mechanism of each of the two drugs may involve correcting aberrant signaling pathways in specific areas of the brain and preventing brain cell loss and atrophy by their effects on BDNF and Bcl-2, among other factors. In this regard, two approved mood stabilizers, lithium (Li^+) and valproate (VPA), have been reported to increase brain levels of BDNF and Bcl-2 when given chronically to rats [13-15].

To determine if the effect of Li^+ and VPA represented a general effect of mood stabilizers, we decided to examine brain BDNF and Bcl-2 mRNA and protein levels in rats chronically administered CBZ and LTG at doses therapeutically relevant to BD. We performed our measurements on the rat frontal cortex because this region has been implicated in the pathogenesis of BD, and because we previously studied the effects of mood stabilizers in this region of rat brain [11,16, and 17].

Methods and Materials

Animals

The study was conducted following the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 80-23) and was approved by the *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development Animal Care and Use Committee. Adult male CDF-344 rats (200-250 g) that were purchased from Charles River Laboratories (Wilmington, MA) received CBZ [$n = 8$, 25 mg/kg body weight dissolved in 0.9% saline/DMSO 50/50 (vol/vol) for 30 days, daily intraperitoneal (IP) injection, Sigma Chemical Co., St Louis, MO], or LTG [$n = 8$, 10 mg/kg body weight for 42 days, oral administration, Sigma]. We used different modes of administration because these doses by these modes have been shown to produce therapeutically relevant plasma concentrations of CBZ ($53.6 \pm 5.2 \mu\text{M}$) [18] and LTG [19]. Control rats ($n = 8$, 8 respectively) received the appropriate vehicle. Animals were anesthetized by carbon dioxide (CO_2) inhalation and killed by decapitation. The brain was rapidly excised and the frontal cortex was dissected and frozen in 2-methylbutane at -50°C , then stored at -80°C until studied.

Preparation of Cytosolic Fractions

Cytosolic extracts were prepared from the frontal cortex of drug-treated and control rats as previously described [20]. Protein concentrations were determined using the Bio-Rad protein reagent (Bio-Rad, Hercules, CA).

Total RNA isolation and real time PCR

Total RNA was isolated from the frontal cortex using an RNeasy mini kit (Qiagen, Valencia, CA) for brain and lipid tissue. Complementary DNA (cDNA) was prepared from total RNA using a high capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). mRNA levels of BDNF and Bcl-2 were measured by quantitative real time RT-PCR, using an ABI PRISM 7000 sequence detection system (Applied Biosystems). Specific primers and probes for BDNF and Bcl-2 were purchased from TaqMan^R gene expression assays (Applied Biosystems), and consisted of a 20X mix of unlabeled PCR primers and Taqman minor groove binder (MGB) probe (FAM dye-labeled). The fold-change in gene expression was determined by the $\Delta\Delta\text{C}_\text{T}$ method [22]. Data were expressed as the relative level of the target gene (BDNF and Bcl-2) in the chronic CBZ or LTG administered animals normalized to the endogenous control (β -globulin) and relative to the control (calibrator), as previously described. All experiments were carried out twice in triplicate with 5 independent samples and data were expressed as relative expression.

Sandwich ELISA Method

Levels of BDNF in the cytosolic extracts were assayed using the *ChemiKine* BDNF Sandwich ELISA Kit (Chemicon, Temecula, CA), according to the manufacturer's instructions. Briefly, the cytosolic extracts and BDNF standards were incubated for 2 h, followed by washing with the appropriate buffer. Plates were incubated with anti-BDNF polyclonal antibody at room temperature for 2 h, washed, and then incubated with anti-IgG antibody conjugated to horseradish peroxidase for 1 h at room temperature. Finally, the plates were incubated in peroxidase substrate and tetramethylbenzidine solution to produce a color reaction. The reaction was stopped with addition of 1 M HCl. Absorbance was measured at 450 nm using a microplate reader. Values are expressed in percent of control.

Western Blot Analysis

Cytosolic extracts (65 µg) were separated on a 4-20% SDS-polyacrylamide gel (Bio-Rad) and transferred to a nitrocellulose membrane. Blots were incubated overnight with primary antibody (1:1000) for Bcl-2 (Cell Signaling, Danvers, MA) in TBS buffer containing 5% nonfat dried milk and 0.1% Tween-20, followed by horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000) (Bio-Rad). Blots were visualized and quantified after correcting for β -actin as described by [21].

Statistical Analysis

Data are expressed as percent of control, and means are compared using a two-tailed, unpaired t-test (mean $\pm$ SEM) with $p < 0.05$.

Results

Carbamazepine and lamotrigine upregulate BDNF mRNA and protein levels in rat frontal cortex

Frontal cortex BDNF mRNA levels were significantly increased in rats chronically administered CBZ (2 fold, $n = 5$, $p < 0.001$) and LTG (1.8 fold, $n = 5$, $p < 0.001$), compared to controls ($n = 5,5$) (Fig. 1). Chronic administration of CBZ also significantly increased mean BDNF protein in the frontal cortex (123%, $n = 5$, $p < 0.001$) (Fig. 1), as did administration of LTG (28%, $n = 8$, $p < 0.001$) (Fig. 1).

Carbamazepine and lamotrigine upregulate Bcl-2 mRNA and protein levels in rat frontal cortex

Bcl-2 mRNA levels were significantly increased by chronic CBZ (2 fold, $n = 5$, $p < 0.001$) and LTG (1.9 fold, $n = 5$, $p < 0.001$) in rat frontal cortex region (Fig. 2). Consistent with these increases, chronic CBZ (90%, $n = 7$, $p < 0.05$) and LTG (49%, $n = 7$, $p < 0.05$) significantly increased mean Bcl-2 protein levels in the rat frontal cortex compared to the control level ($n = 6$) (Fig. 2).

Discussion

The present study demonstrates that chronic administration of CBZ and LTG to rats, so as to produce plasma concentrations therapeutically relevant to BD, increased mean mRNA and protein levels of BDNF and of Bcl-2 in the frontal cortex, compared to levels in vehicle-treated rats. Chronic Li^+ and VPA also have been reported to upregulate BDNF and Bcl-2 expression in rat brain [15,23-25]. Our studies extend these observations. Together, the data support the hypothesis that BDNF and Bcl-2 are common targets of mood stabilizers used in the treatment of BD. This common action may contribute to their therapeutic efficacy in the disease, particularly because BDNF and Bcl-2 protein levels have been reported to be reduced in

postmortem brain from BD patients [7,8]. Their decrease in postmortem brain may be directly related to neurodegeneration, atrophy and cell loss [9,10,11].

BDNF transcription is regulated mainly by CREB [1]. CREB must be phosphorylated to pCREB in order to transcribe CREB-regulated genes, including BDNF [5]. Both chronic Li⁺ and VPA have been shown to increase pCREB in rat brain [26]. Chronic CBZ also may do this, thereby upregulating the BDNF and Bcl-2 mRNA levels in rat frontal cortex, since an earlier *in vitro* study showed that CBZ facilitates activation of CREB in human neuroblastoma SH-SY5Y cells [27]. Increased BDNF may help to prevent the reported decreases in neuronal and glial numbers in BD [8], which have been attributed to decreased levels of BDNF and other neurotrophic factors [8,28].

The cause for decreased BDNF in the BD brain is not clear. Studies indicate altered transcription of the BDNF gene, possibly due to reduced CREB phosphorylation [8,29] or to polymorphisms of the gene itself [30,31]. Rats deprived of dietary essential n-3 polyunsaturated fatty acids for 15 weeks exhibit BD-like symptoms [32], and have a reduced brain concentration of docosahexaenoic acid (22:6n-3) and increased brain metabolism of arachidonic acid (20:4n-6) (increased expression of arachidonic acid-selective cytosolic phospholipase A₂ (cPLA₂), and cyclooxygenase (COX)-2) [32,33]. Further, levels of pCREB and of BDNF mRNA and protein are reduced in the n-3 polyunsaturated fatty acid deprived rats [21], and exposure to high levels of arachidonic acid decreases BDNF protein in spinal cord neurons *in vitro* [34]. In contrast, chronic administration of Li⁺ or CBZ to rats downregulates cPLA₂ expression, and chronic Li⁺, VPA, CBZ or LTG downregulates brain COX-2 activity and PGE₂ concentration [35]. VPA and LTG also downregulate COX-2 mRNA [35]. Thus, BDNF gene transcription may be related to brain arachidonic acid and/or docosahexaenoic acid metabolism [33].

Chronic administration of mood stabilizers to rats decreases arachidonic acid signaling in rat brain [35], and there is some evidence that this effect could increase brain expression of BDNF and Bcl-2 [34,43-46]. Increased arachidonic acid signaling may interfere with transcription of neuronal survival factors, while decreased arachidonic acid signaling by mood stabilizers may have an opposite effect on BDNF and other neuronal survival factors.

There is no consensus on how transcription of Bcl-2 in the brain is upregulated by chronically administered mood stabilizers. Studies suggest that BDNF can regulate the Bcl-2 level through activation of the mitogen activated protein kinase cascade and downstream phosphorylation of CREB [40]. Bcl-2 is repressed by the activator protein (AP)-2 transcription factor, which can induce apoptosis [41]. Chronically administered CBZ decreased AP-2 DNA-binding activity and its AP-2 α subunit level in rat frontal cortex [42], which may allow for the increase in Bcl-2 protein. Further studies are needed to understand the transcriptional regulation of Bcl-2 by chronic administration of mood stabilizers.

Genetic manipulation of the BDNF/MAPK pathway has been shown to regulate affective-like behaviors in rodents, as seen by the increased aggression and increased locomotion in open field testing [14]. Furthermore, heterozygous and homozygous BDNF knockout mice have a normal life span but show increased scores on tests of aggression and depression [37,38]. Infusion of BDNF into the midbrain or hippocampus of these mice reverses their abnormal behavior, producing behavioral effects similar to those produced by antidepressants [39]. Taken together, these studies support the notion that BDNF is vital not only for neuroprotection but also for normal behavior.

In conclusion, chronic administration of CBZ and LTG to rats upregulates BDNF and Bcl-2 mRNA and protein levels in the frontal cortex. This common effect, comparable to that reported

for VPA and Li⁺, suggests that mood stabilizers effective in BD universally work to correct the reductions in the levels of BDNF and Bcl-2 that have been reported in the BD brain.

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Abbreviations

AP-2, activator protein-2; BDNF, brain derived neurotrophic factor; Bcl-2, B-cell lymphoma-2; BD, bipolar disorder; CBZ, carbamazepine; CREB, cAMP response element binding protein; LTG, lamotrigine; pCREB, phosphorylated CREB.

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Figure 1.

Mean BDNF mRNA and protein levels in frontal cortex from control and mood stabilizer-treated rats. BDNF mRNA and protein levels were measured using real time RT-PCR and an ELISA. Data are expressed as percent of control and are compared using a two-tailed, unpaired *t*-test (mean ± SEM, **p* < 0.05, ***p* < 0.005).

Figure 2.

Representative mRNA and protein levels of Bcl-2 in frontal cortex from rats chronically administered saline or mood-stabilizers. mRNA levels of Bcl-2 were measured using real time RT-PCR. Bar graphs are ratios of optical densities of Bcl-2 to β -actin. Data are expressed as percent of control, and compared using an unpaired *t*-test, mean $\pm$ SEM (**p* < 0.05, ***p* < 0.01).