

Effects of lithium and valproate on serum and hippocampal neurotrophin-3 levels in an animal model of mania

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Abstract

It has been demonstrated that lithium (Li) and valproate (VPT), first line mood stabilizers, increase BDNF content in rat hippocampus and frontal cortex, which suggests that the regulation of neurotrophic factors might be associated with their pharmacological effects. In sight of the scarcity of studies with other neurotrophins, and the possible relevance of multiple neurotrophic signaling systems in bipolar disorder we investigated the effects of Li and VPT on NT-3 levels in rat serum and hippocampus, using an animal model of mania induced by amphetamine (AMPH). In the reversal model, adult male Wistar rats received AMPH or saline for 14 days, and between the 8th and 14th days, animals were treated with Li, VPT or saline. In the prevention model, rats were pretreated with Li, VPT or saline, and between the 8th and 14th days, the animals received AMPH or saline. Li increased serum and hippocampal NT-3 levels in all conditions, whereas VPT increased hippocampal NT-3 in the prevention model only. Li reversed AMPH changes in NT-3 in the reversal model, and VPT prevented AMPH changes in NT-3 in the prevention model. These results suggest that both Li and VPT modulate serum and central (hippocampal) NT-3 levels, and further support that the regulation of neurotrophic signaling systems may be related to the mechanisms of action of mood stabilizers.

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1. Introduction

Bipolar disorder (BD) is a common, severe, and chronic illness associated with higher rates of suicide and general medical illnesses (Belmaker, 2004). BD is reported to occur within families and can be treated with mood stabilizing agents, such as lithium (Li), valproate (VPT) and carbamazepine (CBZ). However, the pathophysiology of BD

remains largely unknown and one of the difficulties in the approach to its pathophysiology is the paucity of animal modeling for BD. Part of this problem relies on the fact that BD has a complex clinical presentation, where depressive phases alternates with periods of mania and euthymia. Nevertheless, the unique hallmark of BD is acute mania (Belmaker, 2004). With this in mind, we have used a validated animal model of mania by means of a chronic amphetamine challenge (Frey et al., 2006a,b,c). AMPH was chosen as a model of mania based upon the fact that it is able to induce manic symptoms in both healthy humans (Strakowski and Sax, 1998) and BD subjects (Anand et al., 2000).

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There is increasing evidence suggesting an involvement of neurotrophic factors in BD (Hashimoto et al., 2004; Cunha et al., 2006; Rosa et al., 2006). More specifically, we have showed that serum BDNF is decreased (Cunha et al., 2006) while serum GDNF (Rosa et al., 2006) and serum NT-3 (Walz et al., 2007) are increased during manic and depressive episodes in BD patients, and that serum BDNF was negatively correlated with the severity of manic and depressive symptoms (Cunha et al., 2006). Furthermore, it has been demonstrated that Li and VPT, first line mood stabilizers, increase BDNF content in rat hippocampus and frontal cortex (Einat et al., 2003). Interestingly, these neurotrophic factors seem to be altered during acute mood episodes but not in periods of remission (euthymia) (Cunha et al., 2006; Rosa et al., 2006; Walz et al., 2007). In order to deepen the understanding of the role of neurotrophins in the amphetamine model of mania, this study was designed to investigate the effects of Li and VPT on serum and hippocampal NT-3 levels.

2. Materials and methods

In the present study, we have extended the investigation of the effects of Li and VPT on neurotrophic factors by measuring NT-3 levels in hippocampal and serum samples that were kept frozen in -80°C from one of our previous experiments (Frey et al., 2006a). The detailed description of the experiments has been published elsewhere (Frey et al., 2006a); therefore, here we summarize the treatment regimens and describe the subsequent steps performed for the present investigation.

2.1. Animals

The experiments were performed in male Wistar rats (age: 3–4 months; weight: 220–310 g), obtained from our breeding colony. Rats were housed five to a cage, on a 12-h light/dark cycle (lights on between 7:00 a.m. and 7 p.m.), and food and water were available *ad libitum*. All experimental procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

2.2. Reversal treatment

The first model was designed in order to reproduce the management of a manic episode (reversal treatment). Animals received one daily IP injection of either D-amphetamine (AMPH–Sigma, St Louis, USA) 2 mg/kg or saline for 14 days (45 animals per group). Between the 8th and the 14th day, saline and AMPH animals were divided in three experimental groups (15 animals per group): Li treatment, VPT treatment, or saline (SAL) treatment. Li-treated animals received Li 47.5 mg/kg IP twice a day, and VPT-treated animals received VPT 200 mg/kg IP twice a day.

All injection volumes were 1 mL/kg with saline as vehicle. The decapitation blood was collected without anticoagulants, after that we centrifuged the blood in 3000 rpm for 15 min. The hippocampus was dissected, rapidly frozen, and stored at -80°C until assayed.

2.3. Prevention treatment

The second model was designed to mimic the maintenance treatment used to prevent future episodes (prevention treatment). Animals received either Li 47.5 mg/kg IP twice a day, VPT 200 mg/kg IP twice a day or saline for 14 days (30 animals per group). Between the 8th and the 14th day, Li, VPT and saline-treated animals were divided in two experimental groups (15 animals per group): each group received one daily IP injection of either AMPH 2 mg/kg or saline. The decapitation blood was collected without anticoagulants, after that we centrifuged the blood in 3000 rpm for 15 min. The hippocampus was dissected, rapidly frozen, and stored at -80°C until assayed.

2.4. Biochemical measures

NT-3 levels in serum and hippocampal samples were measured with sandwich-ELISA ($N = 5$ animals per group), using a commercial kit according to the manufacturer's instructions (Chemicon, USA). Briefly, brain slices were homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM (EGTA). Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent for brain slices and 1:6 for serum and standard curve ranged from 7.8 to 500 pg of NT-3. Then, plates were washed four times with sample diluents and was added a monoclonal anti-NT-3 rabbit antibody diluted 1:1000 in sample diluent which was incubated for 2 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 performed. After addition of streptavidin-enzyme, substrate and stop solution the amount of NT-3 was determined by absorbance at 450 nm. The standard curve demonstrated a direct relationship between optical density (OD) and NT-3 concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

2.5. Statistical analysis

All data are presented as mean \pm SEM. Differences between experimental groups were determined by one-way ANOVA followed by Dunnett post hoc test if applicable. P values less than 0.05 were considered to indicate statistical significance. Correlation between serum and hippocampal NT-3 levels was determined using Pearson coefficient correlation.

3. Results

In the reversal treatment (Fig. 1a and b), AMPH and Li administration increased serum ($F = 16.45$; $df = 5,29$; $p = 0.001$) and hippocampal ($F = 21.77$; $df = 5,29$; $p = 0.021$) NT-3 levels in saline-pretreated rats. However, when Li was administered to AMPH-pretreated rats, serum NT-3 was increased ($p = 0.011$), while hippocampal NT-3 levels were not different from controls ($p > 0.05$). When VPT was administered to AMPH-pretreated animals hippocampal NT-3 levels remained increased ($p = 0.005$), while serum NT-3 levels were similar to the control group ($p > 0.05$).

In the prevention treatment (Fig. 2a and b) Li and AMPH increased serum ($F = 5.01$; $df = 5,29$; $p = 0.002$) and hippocampal ($F = 9.12$; $df = 5,29$; $p = 0.041$) NT-3 levels in saline-treated rats, while VPT increased hippocampal but not serum NT-3 levels in saline-treated rats. When AMPH was administered to Li-pretreated rats, hippocampal NT-3 levels remained increased ($p = 0.011$), while serum NT-3 levels were similar to the control group ($p > 0.05$). When AMPH was administered to VPT-pre-

treated rats, both serum ($p < 0.001$) and hippocampal ($p = 0.023$) NT-3 levels were increased.

No correlation between serum and hippocampal NT-3 levels was observed ($p > 0.05$).

4. Discussion

The present study showed that Li and VPT differentially regulate serum and central (hippocampal) levels of NT-3. These responses were observed both before (prevention treatment) and after the use of AMPH (reversal treatment). When given alone Li increased serum and hippocampal NT-3 in all conditions, whereas VPT increased hippocampal NT-3 levels in the prevention model only. This result suggests that a longer period of exposure to VPT is needed to enhance central NT-3, at least in the hippocampus. The reason why a longer period of treatment with VPT is needed to increase hippocampal NT-3 levels is unknown, but it is possibly due to VPT and Li's distinct effects on intracellular signaling systems that are known to regulate the expression of neurotrophic factors. More specifically, it has been demonstrated that Li and CBZ, but not VPT,

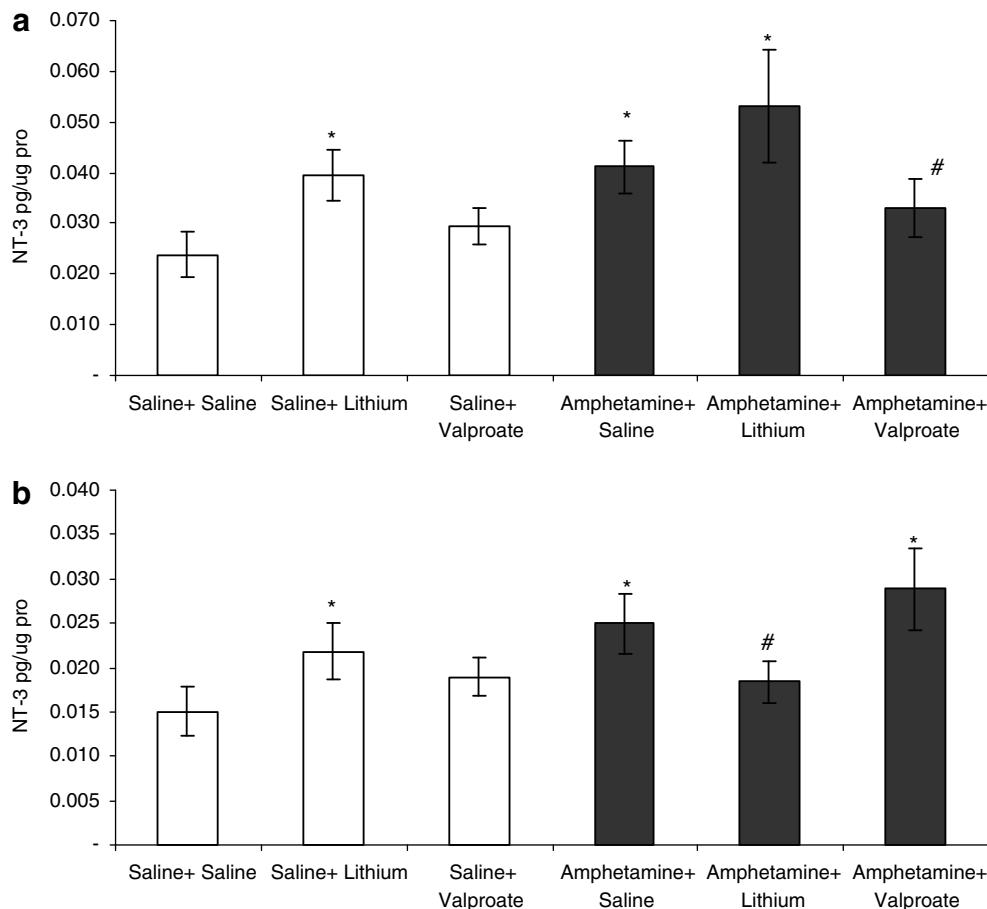


Fig. 1. Serum (a) and hippocampal (b) NT-3 levels in reversal treatment ($N = 5$ animals per treatment group). Results are presented as mean \pm SEM. White bars represent saline (days 1–7) + saline or mood stabilizers (days 8–14), and black bars represent amphetamine (days 1–7) + saline or mood stabilizers (days 8–14). *Statistical differences as compared to saline + saline (control group). #Statistical differences as compared to amphetamine + saline group.

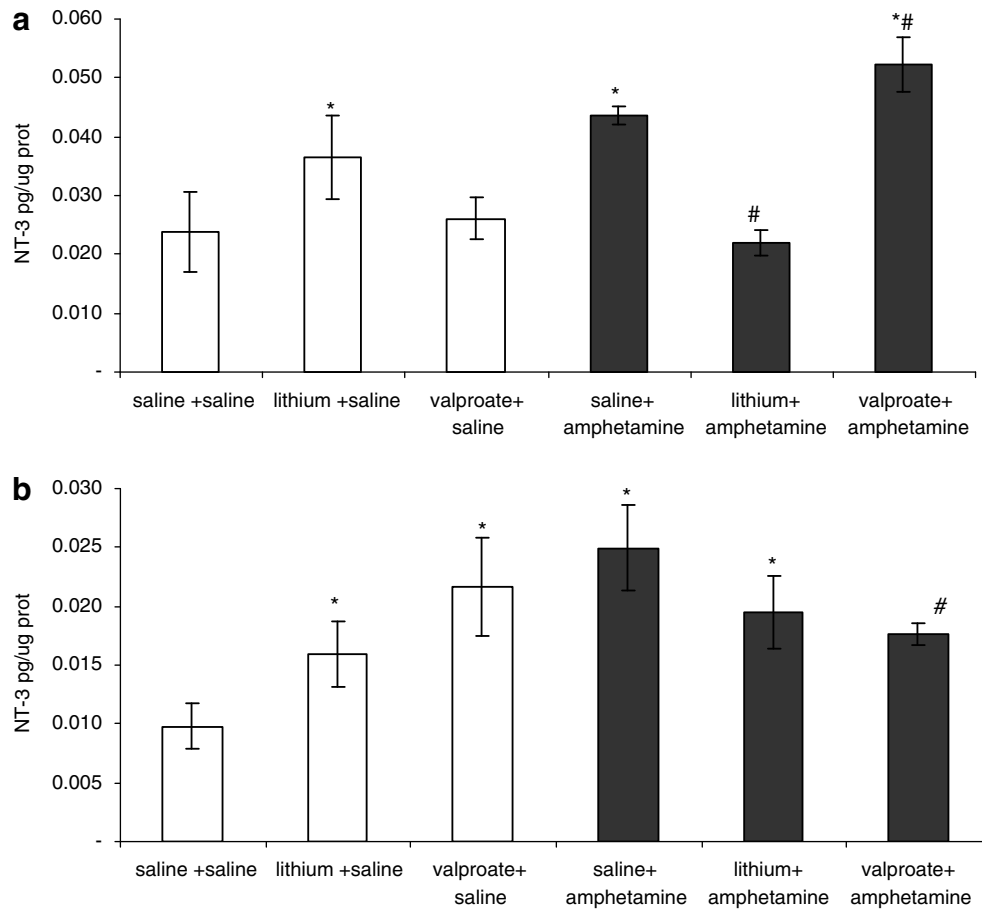


Fig. 2. Serum (a) and hippocampal (b) NT-3 levels in prevention treatment ($N = 5$ animals per treatment group). Results are presented as mean \pm SEM. White bars represent mood stabilizers or saline (days 1–7) + saline (days 8–14), and black bars represent saline or mood stabilizers (days 1–7) + amphetamine (days 8–14). *Statistical differences as compared to saline + saline (control group). #Statistical differences as compared to saline + amphetamine group.

facilitated BDNF-induced phosphorylation of extracellular signaling-regulated kinase (ERK) 1/2 and cyclic AMP response element binding (CREB) in glycogen synthase kinase 3- β -overexpressing SH-SY5Y cells (Mai et al., 2002). Whereas the administration of VPT to differentiated SH-SY5Y cells for 1 h did not affect ERK 1/2 phosphorylation (Mai et al., 2002), the administration of VPT to undifferentiated SH-SY5Y cells for 24 h was shown to increase ERK 1/2 phosphorylation (Yuan et al., 2001). In addition, Einat et al. (2003) found that 28 days of treatment with Li, but not VPT, increased phospho-ERK 42 and ribosomal protein S6 kinase-1 expression in rat hippocampus in vivo. In regard to the hippocampal NT-3 measures, our results showed that Li reversed AMPH changes in NT-3 in the reversal model, while VPT had no effect. On the other hand, VPT prevented AMPH changes in NT-3 in the prevention model, while the effect of Li points to the same direction albeit the effect was not statistically significant. These findings suggest that Li and VPT might have distinct neurotrophic effects when used in the treatment of acute mania or in prevention therapy, and again may indicate that longer periods of exposure to

VPT are needed to modulate the hippocampal expression of NT-3.

Recent studies have suggested that peripheral levels of neurotrophic factors oscillate in an orchestrated fashion during the course of acute mood episodes and euthymic phases in BD subjects. More specifically, serum BDNF is reduced in bipolar depression and mania (Cunha et al., 2006; Machado-Vieira et al., 2007), while serum GDNF (Rosa et al., 2006) and NT-3 (Walz et al., 2007) are increased during mania and bipolar depression. It is noteworthy that neurotrophic factors seem to be altered only during the course of acute mood states, while stabilized (euthymic) patients have neurotrophic factors in the same levels as controls (Cunha et al., 2006; Rosa et al., 2006; Walz et al., 2007). In our studies in humans, although the vast majority of the BD patients were using mood stabilizers, most of them were not on monotherapy (Cunha et al., 2006; Rosa et al., 2006; Walz et al., 2007). Therefore, we could not determine whether the results were related to a specific class of medication. It has been reported that NT-3 is temporally and spatially expressed in the place of BDNF in some neuronal populations to compensate for

the loss of BDNF (Agerman and Ernfors, 2003). For instance, Minichiello and Klein (1996) found that the survival of hippocampal and cerebellar granule cells are altered only when the signaling of both BDNF and NT-3 are blunted. Further, NT-3 infusion increases BDNF mRNA expression in the cerebral cortex (Schutte et al., 2000), and produces BDNF-like effects inducing cortical tyrosine kinase B phosphorylation (Bothwell, 1995). Therefore, we are tempted to speculate that this increment of serum NT-3 levels observed in the present study may occur in tandem with the lowering in BDNF serum levels described previously (Cunha et al., 2006). Such an increase in NT-3 could act as a compensatory physiological response to the strain imposed by mood episodes. This is in line with previous findings showing that NT-3 and BDNF infusion in the dentate gyrus attenuated the correlates of depression in learned helplessness and forced swim test paradigms (Shirayama et al., 2002). Although some human and animal studies support the assumption that BDNF and NT-3 might have mutual compensatory effects, this assumption is only partially supported when we compare the present study with our previous BDNF findings (Frey et al., 2006a). In the prevention model, the administration of AMPH decreased BDNF and increased NT-3 levels. However, in the reversal model AMPH increased NT-3 but did not change BDNF levels. Conversely, co-administration of Li and AMPH increased NT-3 and BDNF in both models. Future studies addressing this particular question in different brain regions and using distinct stress models would help to clarify this issue.

Neurotrophic factors contribute to growth, development, and plasticity of selective neuronal populations in the central nervous system acting via their high-affinity receptors in specific nerves cells to influence survival and gene expression. NT-3 couples to the same signal transduction pathways as BDNF through their respective receptors, and decreased expression of these factors could lead to alterations in the structure and function of subpopulations of hippocampal neurons, depending on the complements of receptors that are expressed in each cell type (Duman and Monteggia, 2006). In addition, it has been reported that NT-3 modulates basal synaptic transmission and long-term potentiation in rat hippocampus (Kato et al., 2003). Despite recent reports that multiple neurotrophic signaling systems may be abnormal in BD (Takebayashi et al., 2006; Cunha et al., 2006) and major (unipolar) depression (Gonul et al., 2005), and that the regulation of neurotrophic factors is thought to be part of the therapeutic effects of mood stabilizers (Coyle and Duman, 2003), little attention has been paid to the study of NT-3 on neuropsychiatric disorders. While some studies have shown a down-regulation of NT-3 mRNA in cerebral ischemia, seizures, and brain trauma (Gall, 1993; Lindvall et al., 1992; Hicks et al., 1997), clearly more research on this field is warranted.

We found no correlation between serum and hippocampal NT-3 levels in the present model. However, because we

investigated only one specific brain region, and given the fact that NT-3 crosses the brain-blood barrier (Poduslo and Curran, 1996) a whole brain homogenate assay would be desirable to better determine the correlation between brain and blood NT-3 levels. Further studies are necessary to clarify this important question. In conclusion, we demonstrated that both Li and VPT modulate serum and central (hippocampal) NT-3 levels. The extent to which these findings in an animal model can be translated to the clinical setting remains to be determined. Nevertheless, the present study further supports the notion that the regulation of neurotrophic signaling systems might be associated with the therapeutic effects of mood stabilizers.

Conflicts of interest

The authors declare no conflicts of interest.

Contributors

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