Anhang: Originalarbeiten (A:1- A:9)

A:1

Subchronic treatment with lithium increases nerve growth factor content in distinct brain regions of adult rats

Subchronic treatment with lithium increases nerve growth factor content in distinct brain regions of adult rats

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Running Title: Lithium and brain NGF content

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Abstract

There is compelling evidence that withdrawal of neurotrophins can lead to impaired neuronal function and even apoptotic death of neurons. Recent experimental evidence suggests that antidepressant drugs and electroconvulsive treatment might work by enhancing CNS levels of neurotrophins. In addition, Lithium (LI) has been shown to exert robust neuroprotective effects apart from its well known mood-stabilizing effects in humans. In this study we investigated the effects of subchronic (14 days') treatment with various doses of LI on the NGF content of several regions of the adult rat brain. LI treatment, which resulted in prophylactic LI serum concentrations (0.72 ± 0.08 mMol/L), induced a significant ($p < 0.05$) increase in NGF concentrations in the frontal cortex (+23.2%), hippocampus (+72%), amygdala (+74%) and limbic forebrain (+46.7%) compared to untreated controls, whereas no effects on NGF concentrations were observed in the striatum, the hypothalamus or the midbrain, even using various LI doses. Moreover, no significant change in NGF concentrations in the frontal cortex was observed after acute (1 days) treatment with LI. Our findings lend support to the notion that an enhancement of NGF production may be specifically involved in the mechanisms of action of antibipolar treatments.

Key words: antibipolar drugs, affective disorder, depression, neurotrophins, neuroprotection, plasticity, stress

Introduction

Manic-depressive disorder affects one to two percent of the population and is characterized by episodic mood swings in the form of depressive and manic episodes[1]. Lithium (LI) has been demonstrated to be the most effective drug in acute and prophylactic treatment of bipolar affective disorder lithium[2]. Although a number of different biochemical and neurophysiological effects of lithium have been described, the exact molecular mechanism(s) and particular target regions accounting for its mood-stabilizing effect remain unknown.

Neurotrophins form a small group of dimeric proteins with similar biochemical characteristics. In mammals five different neurotrophins have been identified to date: the prototypical nerve growth factor (NGF)[3,4], brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3, -4 and –5[5-7]. There is strong evidence supporting the importance of the family of NGF-related neurotrophins in maintaining the function of mature neurons in the CNS[8-10] as neurotrophins are known to be critical for both growth and survival of neurons during development in vivo and in vitro[3,5,6]. Withdrawal of neurotrophins can lead to impaired neuronal function and even apoptotic death of adult neurons[9]. Recent studies have elucidated the mechanisms that underlie neurotrophic inhibition of cell death[11], including the mechanisms of regulation of both Bcl-2 and Bad, with one of the targets of this pathway being cAMP-response element binding protein CREB[11,12].

The NT hypothesis proposes that repetitive neuronal activity enhances the expression, secretion and/or actions of NTs at the synapse to modify synaptic transmission and connectivity and thus provides a connection between neuronal activity and synaptic plasticity[13-15]. Recent experimental evidence suggests that antidepressant drugs and electroconvulsive treatment (ECT) might work by enhancing the expression of neurotrophins in distinct regions of the CNS[15-16]. It has been proposed that BDNF, in particular, contributes to hippocampal mossy fiber that sprouts after repeated administration of electroconvulsive seizures[16], leads to the recovery of hallmark behavioral deficits in depression-model animals[17] and that its mRNA is increased by long-term antidepressant treatment[18]. Although the issue of neuroprotection and neurotrophins is now recognized as an important new lead in the quest for a deeper understanding of mood disorders and the mechanisms of action of antidepressants and mood stabilisers[15,19], to our knowledge there is as yet no data on the changes in brain concentrations of NGF following treatment with LI. In this study we looked at the effects of different doses of LI on the NGF content of several regions of the rat CNS of rats after 14 days' administration.

Materials and Methods

Materials

Chemicals of analytical grade were purchased from Merck (Darmstadt, Germany). Special reagents were obtained from Sigma Chemical Company (Deisenhofen, Germany). Anti-Mouse-NGF-Antibodies (clone 27/21) and anti-mouse-β-NGF (clone 27/21)-β-galactosidase-conjugate were acquired from Chemicon (USA, formerly Boehringer Mannheim) and mouse-NGF was gifted from Prof. Dr. Rohrer, Max-Planck-Institute for Brain Research, Frankfurt/Main, Germany.

Animals

Fifty-six adult male Sprague Dawley rats weighing about 250 g were used for the experiments. The animals were housed under standard conditions on a 12h light, 12 h dark cycle and had free access to laboratory chow and tap water. An adjustment period of at least 1 week was allowed before the pharmacological studies started. A second bottle of fluid containing 0.9% NaCl was installed in the cages of rats receiving LI. Untreated rats were included in each experiment, serving as corresponding controls.

Group 1 consisted of 32 rats which were fed on either a LI diet of 0.3% (n=16) or a control diet (n=16) and were decapitated at 4 a.m. or 8 p.m. [for details see table 1]. We intentionally decapitated the rats during their active phase, as performing the experiment during the daytime (i.e., during the rats' resting phase) would have involved a stress factor such as sleep deprivation. Two groups of rats were sacrificed at 8 p.m. and 4 a.m., respectively, in order to investigate whether possible effects of LI on NGF concentrations are dependent on a circadian rhythm. The rats in this group received subchronic LI treatment for 14d. Rats receiving a 0.3% LI-containing diet exhibited a significant weight loss in comparison with the controls and some of the rats in this group had toxic serum concentrations of LI [see table 1]. We therefore also investigated the effects of a 0.15% LI diet on NGF content in a further group (group 2). In this group 6 rats were treated with a 0.15% LI diet for 14d and were compared to 6 controls. All the rats in this group were decapitated at 8 p.m. [see table 1].

We also investigated whether acute (24 h) LI treatment affects NGF concentrations in rat brain (group 3). For this purpose 6 rats received an intraperitoneal injection of 3 mmol LiCl

(dissolved in 1 ml of 0.9% NaCl) per kg body weight at 6 p.m. and were sacrificed together with the control group (n=6), which received the vehicle only, 24 h later [see table 1].
All rats were decapitated without anaesthesia. The hippocampus, striatum, frontal cortex, limbic forebrain, amygdala, septum, hypothalamus and midbrain were dissected according to Glowinski and Iversen[20] and all tissues were immediately frozen at -70°C.

Homogenization procedure and determination of NGF levels

Tissue samples were individually homogenized on ice in 5 to 6 vol. 0.25 mol/l sucrose, 10 mMol/L HEPES (pH 7.0) containing 10 mMol/L DTT, immediately frozen in a dry ice/acetone bath and stored at –80° until NGF measurement. Since the NGF content of brain tissues is several fold higher than generally reported and largely associated with sedimentable fractions[21], the homogenates were centrifuged at 10,000 x g for 10 min at 15° C. The remaining pellets were each dissolved in 750 µl NGF-homogenization buffer, treated with ultrasound for 3 min and processed for quantification of endogenous NGF as described in detail elsewhere[22,23,24]. The measured and recovery-corrected NGF contents were expressed in ng NGF per g protein in the resuspended NGF homogenate, which was quantified by Bio-Rad® protein assays[25]. Our results obtained in untreated brain tissues are highly consistent with those previously reported by Hoener and coworkers[21].

Statistical analysis

All data are presented as means ± SEM. As not all subgroups showed a normal distribution (Kolgomorov Smirnoff Index), nonparametric testing (Mann Whitney U test for unpaired samples) was used to detect significant differences between the different treatment groups (two concentrations of LI and the control groups).

Results

Group 1

The NGF concentrations of this group were measured at 8 p.m. in all brain regions. Administration of 0.3% LI over a 14-days' period significantly enhanced the NGF concentrations in the hippocampus (71.9 ± 3.5 in controls ng/g vs. 133.1 ± 14.4 ng/g in the LI treatment group; $p = 0.001$) and the frontal cortex (46.6 ± 5.6 ng/g in controls vs. 67.9 ± 6.7 ng/g in the LI treatment group; $p = 0.02$). The LI-associated NGF increase in the frontal cortex has been confirmed by a different set of experiments (32.8 ± 0.8 ng/g vs. 63.6 ± 15.5 ng/g; $n = 5$ and 6, respectively; $p=0.03$). Moreover, LI trended to increase NGF concentrations by about 50% in the limbic forebrain (61.8 ± 8.8 ng/g in controls vs. 109.0 ± 16.9 ng/g in LI-treated rats) and in the striatum (7.15 ± 1.05 ng/g in controls vs. 10.5 ± 1.4 ng/g in LI-treated rats), but these effects of LI failed to reach statistical significance ($p = 0.08$ and $p = 0.11$, respectively) . No effects of LI were observed in the hypothalamus (7.92 ± 0.65 ng/g in controls vs. 8.47 ± 0.94 ng/g in LI-treated rats; $p = 0.75$) and in the midbrain (4.62 ± 0.44 ng/g in controls vs. 5.53 ± 0.57 ng/g in LI-treated rats; $p = 0.34$).

The NGF concentrations were also measured at 4 a.m. in two brain areas of major interest, i.e. the hippocampus and frontal cortex. Administration of 0.3% LI also enhanced NGF concentrations in the morning in the frontal cortex (41.8 ± 4.4 ng/g in controls vs. 84.5 ± 7.8 ng/g in LI-treated rats; $p = 0.002$) and in the hippocampus (87.9 ± 6.3 ng/g in controls vs. 119.1 ± 10.0 ng/g in LI-treated rats; $p = 0.02$). Thus, the magnitude of the NGF-increasing effect of LI seemed to depend at least in the brain regions investigated on a circadian rhythm for still unknown reasons, whereas the corresponding control rats did not show such a circadian variability.

Group 2

Some of the rats in group 1, which received a 0.3% LI-containing diet exhibited a significant weight loss in comparison with the controls and some of the animals had toxic serum concentrations of LI [for details see table 1]. We therefore also investigated the effects of a 0.15% LI diet on the cerebral NGF content in group 2 [Fig. 1], resulting in therapeutic serum concentrations of LI (0.72 ± 0.08 mMol/L). The NGF concentrations in the hippocampus increased from 90.7 ± 7.6 ng/g to 153.9 ± 12.0 ng/g ($p = 0.007$). Significant, LI-related increases of NGF were also seen in the frontal cortex (32.8 ± 0.8 ng/g vs. 52.1 ± 5.0 ng/g; $p = 0.03$), in the limbic forebrain (61.8 ± 8.8 ng/g vs. 100.1 ± 6.6 ng/g; $p = 0.02$) and in the

amygdala (48.1 ± 4.1 vs. 83.8 ± 10.9 ng/g; p = 0.03). No significant effects of LI treatment on NGF concentrations were seen in the striatum (7.15 ± 1.05 ng/g vs. 8.98 ± 0.45 ng/g; p = 0.15), in the midbrain (4.62 ± 0.44 ng/g vs. 4.78 ± 0.41 ng/g; p = 0.75) and in the hypothalamus (7.92 ± 0.65 ng/g vs. 7.98 ± 0.75 ng/g; p = 0.87).

Group 3

No significant change in NGF concentrations in the frontal cortex was observed after acute treatment of rats with 3mmol LI per kg body weight (26.0 ± 2.8 ng/g in controls vs. 20.9 ± 1.3 ng/g in LI-treated rats; p = 0.25).

Discussion

We found that subchronic (i.e. 14d') treatment of adult rats with LI, which resulted in prophylactic LI serum concentrations [see table 1], increased NGF concentrations specifically in the frontal cortex, hippocampus, the amygdala and the limbic forebrain by maximally 74%. By contrast, subchronic LI treatment had no significant effect on NGF concentrations in the striatum, hypothalamus or midbrain. Acute (i.e. 1d) LI treatment had no effect on the NGF content in the frontal cortex. These findings are in line with both the time course of the onset of the treatment effects and the CNS regions which are thought to be relevant for the clinically seen effects of LI on mood stabilization, as in affective disorders.

Neuronal atrophy and loss in depression has been suggested by clinical and animal studies[26]. It has been demonstrated, especially in the hippocampus[27], but also in post-mortem studies of the cerebral cortex[28]. There is emerging evidence of brain changes, e.g. differences in three dimensional magnetic resonance imaging (MRI) volumes, which have been identified in the frontal cortex[29], caudate nucleus[30], hippocampus and the core nuclei of the amygdala[27,31]. Loss of glial cells which produce trophic factors or stress-induced inhibition of neurogenesis are potential mechanisms that could account for the structural findings accompanying depression[27]. Although it has not yet been demonstrated whether LI can contribute to a regeneration of structural damages presumed to be associated with depression, it seems conceivable that the observed LI-associated increase in NGF concentrations may be relevant for the neuroprotective and neurotrophic effects of LI observed in humans previously[32,33].

Adenosine A1 receptors have been shown to be upregulated after sleep deprivation[34], chronic treatment with the prophylactic drug carbamazepine[35] and ECT[36]. Stimulation of the A1 receptors has been found to induce synthesis and release of NGF in cultured astrocytes[37,38] and stimulation of A2a-receptors modulates microglial NGF expression[39]. We suggest that the mechanism underlying our observation of an NGF elevation following LI treatment could possibly be due to an A1 or A2a receptor mediated action.

There is increasing evidence that the NGF-related neurotrophin BDNF is involved in the pathophysiology and possibly the treatment of depression. Accordingly, infusion of BDNF has been shown to lead to recovery of the hallmark behavioral deficits in depression-model animals[17]. Moreover, physical activity[18] and treatment with various antidepressants[40] increase BDNF mRNA in different areas of the rodent brain.

In conclusion, we report that subchronic treatment with LI specifically enhances NGF concentrations in the hippocampus and frontal cortex, and also in two other areas relevant for

the modulations of emotions, such as the amygdala and limbic forebrain. Our results may therefore add further support to the hypothesis that both antidepressant and prophylactic drugs may stimulate the production and function of various neurotrophic factors in distinct brain areas relevant for the pathogenesis of depression.

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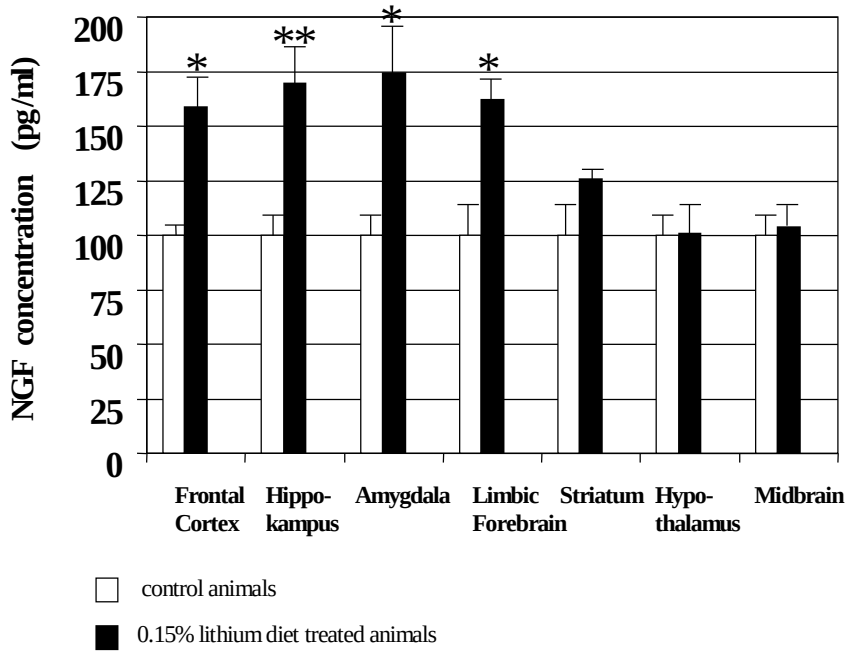
Figure Legend

Figure 1. Effects of a 0.15% LI diet given over 14 days on cerebral NGF concentrations in the frontal cortex (FC), hippocampus (Hip), amygdala (Amy), limbic forebrain (LF), striatum (Str), hypothalamus (Hyp), and midbrain (Mid) of adult rats (black columns). Rats (n=12) were treated as described in the Materials and Methods section, and data (being further detailed in the Results section) were presented as percentage of corresponding controls (open columns). * = $p<0.05$, ** = $p<0.01$ compared with controls.



Group	n	Drug/mode of application	Dose	Time or duration of LI administration	Time of decapitation	LI serum concen-tration (mMol/L)	Change in body weight during the treatment (percent)
1	8	control diet	-	14d	4 A.M.	-	+22.4 ± 2.3%
	8	control diet	-	14d	8 P.M.	-	+21.3 ± 3.5%
	8	LI diet	0.3%	14d	4 A.M.	1.30 ± 0.35	-20.2 ± 6.7%
	8	LI diet	0.3%	14d	8 P.M.	1.37 ± 0.31	-18.3 ± 6.5%
2	6	LI diet	0.15%	14d	8 P.M.	0.72 ± 0.08	+18.3 ± 3.4%
	6	control diet	-	14d	8 P.M.	-	+20.2 ± 4.5%
				Acute:			
3	6	LI IP	3.0 mMol/ L	10 P.M. (12h)	10 A.M.	0.44 ± 0.07	-
	6	control IP	0.9% NaCl	10 P.M. (12h)	10 A.M.	-	-

Table 1. Drug doses, duration of administration, LI serum concentrations, changes in body weight, and time of death in the different treatment groups. Adult rats (n=56) were treated as described in the Materials and Methods section; intraperitoneal (IP), lithium (LI).