Lithium treatment alters brain concentrations of nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in a rat model of depression

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Abstract

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are proteins involved in neuronal survival and plasticity of dopaminergic, cholinergic and serotonergic neurons in the central nervous system. Since decreased size and impaired function of some neuronal populations may be relevant in depression it has been hypothesized that these molecules may have a functional role in the pathophysiology as well as treatment of depression. Using an animal model of depression, the Flinders Sensitive Line (FSL) rats and their controls, the Flinders Resistant Line (FRL), we investigated the effects of chronic lithium treatment on brain NGF, BDNF and GDNF. Lithium was administered as food supplementation during 6 wk. NGF, BDNF and GDNF measurements were performed by enzyme-linked immunosorbent assay (ELISA). Lithium altered the brain concentrations of neurotrophic factors in the hippocampus, frontal cortex, occipital cortex and striatum. Moreover, the changes were different in the two rat strains. Our data support the notion that neurotrophic factors play a role in depression and in the mechanism of the action of lithium.

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Introduction

Recent studies indicate that neurotrophic factors might be important molecules for understanding the mechanisms involved in depressive disorders. The finding that depressed patients have reduced hippocampal volume as well as reduced prefrontal cortex volume (Bremner et al., 2000) has led to the hypothesis that neuronal loss or decreased neurogenesis occur in brains of subjects with a long history of depression (Duman et al., 1997).

In line with the hypothesis of neurotrophic deficits in depression, other authors have hypothesized that antidepressant treatments might exert their beneficial action by regulating synthesis and/or release of neurotrophic factors (Duman et al., 1999). This hypothesis also seems to be supported by the findings that brain-derived neurotrophic factor (BDNF) injected into the brain has an antidepressant-like effect (Suciak et al., 1997) and that antidepressant drugs and electroconvulsive treatment (ECT) enhance central nervous system (CNS) expression of neurotrophic factors and their receptors (Duman and Vaidya, 1998; Krystal and Weiner, 1999; Nibuya et al., 1995; Thoenen, 1995; Zetterström et al., 1998). More recently, another treatment modality (lithium), has been shown to induce an increase in the expression of BDNF in the hippocampus and cortex of rats (Fukumoto et al., 2001).

In a previous study we have demonstrated that Flinders Sensitive Line (FSL) rats, an animal model of depression (Overstreet, 1993), had altered levels of BDNF and nerve growth factor (NGF), in the frontal cortex, occipital cortex, and hypothalamus, compared to their controls, the Flinders Resistant Line (FRL). The results also showed that CNS distribution of these neurotrophic factors is differently expressed in male and female brains (Angelucci et al., 2000a). To
further characterize the role of neurotrophic factors in depression, in this study we used the FSL model of depression and investigated the effect of chronic lithium treatment (probably the most effective mood stabilizer) on brain regional concentrations of neurotrophic factors. Moreover, we extended our NGF and BDNF studies to also include glial cell line-derived neurotrophic factor (GDNF), a more recently identified and characterized neurotrophic factor for dopaminergic neurons (Bohn et al., 2000).

Materials and methods

Animals

Adult male FSL and FRL rats, approx. 70 d old, from breeding colonies maintained at the Karolinska Institute were used. The animals were housed five per cage (Makrolon IV) under controlled conditions of temperature (22 ± 1°C), relative humidity (45–55%) and daylight cycle (12:12 h, lights on at 06:00 hours). Rat chow (R36 Lactamin, Stockholm, Sweden) and tap water were available ad libitum. All animal experimentation was conducted in accordance with the Karolinska Institute’s Guidelines for the Care and Use of Laboratory Animals. The experiments were approved by the Stockholm Ethical Committee for the Protection of Animals.

Lithium and vehicle treatments

FSL and FRL animals received either lithium or vehicle (7 animals/2 strains/2 conditions = 28 animals). Food pellets were supplemented with either lithium (3 g LiSO₄/kg fodder), or vehicle for a 42-d period. All lithium-exposed rats had free access to a bottle containing 0.45% NaCl to prevent lithium toxicity (Ellis and Lenox, 1990). Under these conditions the mean serum lithium concentration will be approx. 0.5 mequiv./l (Mathé et al., 1994) and within the range of therapeutic dose levels (0.5-1.0 mequiv./l) in humans (Gelenberg et al., 1985). Ther.

Tissue preparation

At the end of the treatments (24 h after 6 wk of lithium treatment), the animals were sacrificed. The brains were quickly removed and dissected on ice into hypothalamus, hippocampus, striatum, frontal cortex and occipital cortex, according to the method of Glowinski and Iversen (1965) and immediately used for further processing. For extraction of neurotrophic factors the brain tissues were homogenized with ultrasonication in extraction buffer as previously described (Angelucci et al., 2000a,b). The homogenates were centrifuged at 10000 g for 20 min, the supernatants were collected, diluted 1:1 with extraction buffer containing 0.1% Triton X-100 and processed for quantification of NGF, BDNF and GDNF by sensitive and specific two-site enzyme immunoassays.

NGF measurement by enzyme-linked immunosorbent assay (ELISA)

As previously described (Angelucci et al., 2000a), 96-well immunoplates (NUNC, Denmark) were coated with 50 µl/well of 0.4 µg/ml monoclonal anti-mouse-NGF antibody 27/21 (Boehringer, Germany). After an overnight incubation at 4°C the samples were added to the coated wells (50 µl each). Then the antigen was incubated with 50 µl/well of the monoclonal antibody 27/21 conjugated with β-d-galactosidase for 2 h at 37°C (enzyme activity 0.5 mU/well) and with Chlorophenol Red-β-d-galactopyranoside (Boehringer, Germany) for another 2 h at 37 °C. The colorimetric reaction product was measured at 570 nm using a microplate reader (Dynatech MR 5000, Germany). NGF concentrations were determined from the regression line for the purified mouse NGF standard (ranging from 1.56 to 1000 pg/ml). The sensitivity was 3 pg/ml. Data are presented as pg/g wet weight and all assays were performed in triplicate (Weskamp and Otten, 1986).

BDNF measurement by ELISA

Quantification of endogenous BDNF was performed by a two-site enzyme immunoassay kit (Promega, USA). As for the NGF assay, 96-well immunoplates (NUNC) were coated with 100 µl/well of monoclonal anti-mouse BDNF antibody and incubated overnight at 4°C. Then the samples were added to the coated wells (100 µl each) for 2 h at room temperature with shaking. After 2 h, the antigen was incubated with an anti-human BDNF antibody for 2 h at room temperature with shaking and then incubated with an anti-IgY HRP for 1 h at room temperature. The plates were then incubated with a TMB/peroxidase substrate solution for 15 min and 1 m phosphoric acid (100 µl/well) was added to the wells. The colorimetric reaction product was measured at 450 nm. BDNF concentrations were determined from the regression line for the BDNF standard ranging from 7.8 to 500 pg/ml purified mouse BDNF. Consistent with previous results (Aloe et al., 1999; Angelucci et al., 2000b) the sensitivity of the assay was 15 pg/ml of BDNF and the cross-reactivity with other related neurotrophic factors, e.g. NGF, NT-3 and NT-4 was less than 3%. Data
are presented as pg/g wet weight and all assays were performed in triplicate.

**GDNF measurement by ELISA**

Quantification of GDNF was performed by a two-site enzyme immunoassay kit (Promega, USA). This ELISA method differs only marginally from that used for evaluation of BDNF. Ninety-six-well immunoplates (NUNC) were coated with 100 μl/well of monoclonal anti-GDNF antibody. After overnight incubation at 4°C, the samples were incubated in the coated wells (100 μl each) with shaking for 6 h at room temperature. The plates were then washed and the antigen incubated overnight with a polyclonal anti-human GDNF antibody at 4°C. The plates were washed again with buffer and then incubated with an anti-chicken IgY HRP conjugate for 2 h at room temperature with shaking. From that point on, the procedure did not differ from that used for BDNF. The colorimetric reaction product was measured at 450 nm. GDNF concentrations were also determined from the regression line for the GDNF standard (ranging from 15.6 to 1000 pg/ml purified human GDNF). The sensitivity of the assay was 30 pg/ml and the cross-reactivity with other related neurotrophic factors, e.g. TGFβ1, rhTGFα and NGF, was less than 5%. GDNF concentration is expressed as pg/g wet weight and all assays were performed in triplicate.

**Statistical analysis**

Two-way analysis of variance (ANOVA) was used with treatment (lithium) and strains (FSL and FRL) as variables. When significant differences were obtained, post-hoc comparisons within logical sets of means were performed using the Newman–Keuls test.

**Results**

**Baseline NGF, BDNF and GDNF brain concentrations in FSL and FRL rats**

NGF, BDNF and GDNF concentrations in each brain region examined (Figures 1–3) were similar to those reported in our and other previous studies (Angelucci et al., 2000a, 2002a; Nawa et al., 1995).

**Effects of lithium on NGF, BDNF and GDNF**

**NGF**

In the hippocampus (Figure 1) an effect of the treatment (p < 0.05) and an interaction between treatment and strain (p < 0.001) were observed; lithium increased

**BDNF**

Lithium had no effect in the hippocampus (Figure 2). In the frontal cortex (Figure 2) an interaction between treatment and strain (p < 0.001) was observed; lithium decreased BDNF concentration in the FSL rats compared to FSL vehicle-treated (p < 0.05 post hoc) and FRL lithium-treated rats (p < 0.05 post hoc). Lithium had no effect in the frontal cortex and occipital cortex (Figure 1). In the striatum (Figure 1), an effect of the treatment (p < 0.05) was observed; lithium decreased NGF concentration in FRL rats (p < 0.05 post hoc).

**GDNF**

In the hippocampus (Figure 3) an effect of the treatment (p < 0.05) and an interaction between treatment and strain (p < 0.05) were observed; lithium decreased
The results demonstrate that chronic lithium treatment significantly alters the constitutive brain concentrations of NGF, BDNF and GDNF. In addition, the brain levels of these neurotrophic factors are differentially affected in FSL, compared to their controls (FRL rats). This observation is consistent with the hypothesis that altered synthesis and release of neurotrophic factors play a role in depression (Altar, 1999).

Chronic lithium diet altered the levels of neurotrophic factors in some of the brain regions examined. It has been previously demonstrated that lithium alters the brain levels of several neuropeptides, including neuropeptide Y (NPY), neurokinin A (NKA), substance P (SP), and calcitonin gene-related peptide (CGRP) (Mathe´ et al., 1994, 1990). Since NPY, NKA, SP and CGRP are, in part, regulated by neurotrophic factors the possibility arises that the action of lithium on neuropeptides is mediated, inter alia, by neurotrophic factors. Indeed, in a recent paper (Fukumoto et al., 2001) it was reported that lithium and valproate treatments for 14 and 28 d, respectively increased the concentration of BDNF in the hippocampus, frontal cortex and temporal cortex of Wistar rats. Here we demonstrated that lithium also alters the concentration of NGF and GDNF; NGF was increased in the hippocampus of FSL rats while GDNF was decreased in the hippocampus of FRL rats. Despite numerous studies, neither the aetiology/pathophysiology of bipolar disorder nor the therapeutic mechanism of lithium is well understood. The results presented here seem to indicate that the increase in NGF concentrations may be relevant for the neuroprotective and neurotrophic action of lithium previously observed in human (Manji et al., 2000a). Lithium increases the levels of neuroprotective proteins, such as Bcl-2 (Manji et al., 2000b), and downregulates the proapoptotic gene Bax (Chen and Chuang, 1999) in the rat brain. As an increased synthesis of NGF in the hippocampus seems to be neuroprotective/antiapoptotic, it is conceivable that lithium exerts its neurotrophic/neuroprotective action through NGF and other neurotrophins (Hellweg et al., 2002). After lithium treatment, NGF increased only in the hippocampus of FSL but not FRL rats. Likewise, using the same model of depression we found that electroconvulsive stimuli (ECS) increased NGF only in the hippocampus of FSL rats (Angelucci et al., 2002b). Whether a reduced NGF synthesis is relevant to the pathophysiology of depression is not known.
However, these results suggest that ECS as well as lithium treatment can reverse the atrophy of hippocampal neurons via regulation of neurotrophic factors. Such an assumption is in line with findings that acute and chronic ECS increase NGF mRNA in limbic brain regions (Follesa et al., 1994). Whether lithium also affects NGF mRNA brain levels is not known and will be the focus of further studies.

It is of interest that an increase in the production of neurotrophic factors might be associated with increased neurogenesis induced by stimuli such as environmental enrichment (Ickes et al., 2000) and dietary restriction (Lee et al., 2002). Since antidepressant treatments increase neurogenesis in the adult rat hippocampus (Malberg et al., 2000), it is conceivable that increased levels of neurotrophic factors might promote neurogenesis by increasing the proliferation of progenitor cells and/or by inducing their differentiation into neurons. Indeed, such a role for BDNF has been proposed by Fukumoto et al. (2001). Our data in the occipital cortex are in line with that study, as lithium increased BDNF in our control strain, the FRL rats. We found no differences in the baseline striatal BDNF levels between the FLS and FRL rats and lithium decreased BDNF in both strains. Moreover, lithium also decreased BDNF in the frontal cortex. This apparent discrepancy between the above-cited study (Fukumoto et al., 2001) and our results could be due to the fact that healthy Wistar rats were used in that study, while we used ‘depressed’ FSL rats. Moreover, Fukumoto et al. (2001) reported an increase in BDNF after 14 d, but not at the end of the treatment, i.e. 28 d, whereas our treatment time was longer, i.e. 42 d. Although the significance of a decrease in BDNF is not clear, it is consistent with the notion that a dysfunction in medial prefrontal cortex areas may be implicated in phenomenon of anhedonia and perhaps pathogenesis of depressive symptoms (Drevets, 2000).

In our study on FRL rats, lithium increased GDNF concentrations in the frontal cortex and occipital cortex, but decreased it in the hippocampus. In the only other study that we could identify (Fukumoto et al., 2001), no significant effects of lithium on GDNF were observed. The reasons for this discrepancy could be the same as mentioned earlier. Moreover, it is possible that GDNF changes are the result of subsequent adaptation phenomena and not a primary cellular response to lithium.

These effects of lithium on BDNF in the striatum and frontal cortex and on GDNF in the hippocampus appear to be inconsistent with the robust neuroprotective role of this mood stabilizer. However, time of treatment could be important for the observed effects on neurotrophic factors. For example, lithium administered at a different time and dose produces different effects on glutamate and GABA levels (Antonelli et al., 2000), which in turn regulate the production of neurotrophic factors in the frontal cortex and hippocampus (Zafra et al., 1991). In addition, chronic lithium treatment (28 d) in rats diminishes neostriatal dopaminergic activity (Carli et al., 1994), which is also regulated by BDNF and GDNF (Yurek and Fletcher-Turner, 2002). Another possibility is that the decrease in BDNF and GDNF levels is a consequence of a decreased synthesis, increased degradation and/or enhanced release, since trophic factors are locally produced in the cortex and hippocampus and retrogradely transported to other brain areas, including the striatum (Mufson et al., 1999). An additional possibility is that lithium altered BDNF and GDNF distribution in specific substrutures of these brain regions. It would be of interest to investigate the gene expression for these neurotrophic factors after lithium treatment to elucidate, in part, these mechanisms.

In summary, our data support the notion that neurotrophic factors play a role in the antidepressive action of lithium. Since these molecules display distinct and/or overlapping action on brain cells, one hypothesis is that lithium, via regulation of neurotrophic factors, can improve symptoms of depression. This assumption is consistent with the recent findings that low expression of BDNF may be associated with depression (Chen et al., 2001; Karege et al., 2002) and that BDNF can lead to the recovery of behavioural deficits in an animal model of depression (learned helplessness) and in the forced swim test (Sucjak et al., 1997).

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