Four weeks lithium treatment alters neuronal dendrites in the rat hippocampus

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Abstract

A large body of evidence from molecular, cellular and human studies suggests that lithium may enhance synaptic plasticity, which may be associated with its therapeutic efficacy. However, only a small number of studies have directly assessed this. To determine whether lithium treatment alters structural synaptic plasticity, this study examined the effect of 4 wk lithium treatment on the amount and distribution of dendrites in the dentate gyrus (DG) and hippocampal area CA1 of young adult rats. Following 4 wk lithium or control chow feeding, animals were decapitated, the hippocampi were prepared and stained using a rapid Golgi staining technique and the amount and distribution of the dendritic branching was evaluated using Sholl analyses (method of concentric circles). In the DG, lithium treatment increased the amount and distribution of dendritic branches in the proximal half of dendritic trees of the granule cells and reduced branching in the distal half. In area CA1, the same treatment also increased the number of dendritic branches in the proximal half of apical dendritic trees of CA1 pyramidal cells and reduced branching in the distal half of apical dendritic trees but had no effect on basilar dendritic trees. The lithium treatment altered the total density of dendritic trees in neither the DG nor area CA1. These findings suggest that, in the DG and apical CA1, chronic lithium treatment rearranges neuronal morphology to increase dendritic branching and distribution to where major afferent input is received.

Introduction

Lithium has been widely used in the treatment of bipolar disorder as well as other mood disorders (Geddes et al., 2004). However, the mechanism of action of lithium has not been fully investigated. Theories of neuroplasticity strongly suggest that lithium has diverse and robust effects on neuronal plasticity and these actions are associated with its therapeutic effects (Manji et al., 2001; Rowe and Chuang, 2004; Shim and Gould, 2010). For example, molecular biological studies have shown that lithium up-regulates molecules involved in neuronal plasticity (for review, see Shim and Gould, 2010). Among them, brain-derived neurotrophic factor (BDNF; Hashimoto et al., 2002; Einat et al., 2003; Son et al., 2003; Hammonds and Shim, 2009), B-cell CLL/lymphoma 2 (Bcl-2) (Manji et al., 1999; Manji and Chen, 2002; Hammonds and Shim, 2009) and nerve growth factor (Angelucci et al., 2003) have been most extensively studied. Lithium has also been shown to enhance the activation of cyclic adenosine monophosphate response element-binding protein (CREB), a major transcription factor in the gene expression of diverse proteins involved in neuroplasticity (Einat et al., 2003; Son et al., 2003; Omata et al., 2008; Hammonds and Shim, 2009; Heinrich et al., 2009). CREB and the above proteins play crucial roles in dendritic proliferation, synaptic formation and synaptic plasticity (Won and Silva, 2008; Finsterwald et al., 2010; Kwon et al., 2011). Cellular studies have shown that these molecules up-regulate synaptic plasticity by stimulating adult neurogenesis and protecting neurons from neurotoxic injuries and abnormal apoptotic processes (Chen et al., 2000; Tsujimoto and Shimizu, 2002; Boneva and Yamashima, 2011; Merz et al., 2011). One key issue in neuroplasticity research for bipolar disorder is the effect of lithium on synaptic plasticity. A substantial body of evidence suggests that impairment in synaptic plasticity is related to the pathophysiology of bipolar disorder (Sanacora, 2008; Schloesser et al., 2008). Post-mortem studies of bipolar patients indicate that synaptic proteins and/or their messenger RNA are down-regulated in
the case of synaptophysin, complexin II and growth-associated protein-43 (Eastwood and Harrison, 2001); synapsins Ia, Ila and IIla (Vawter et al., 2002); synaptosomal protein of 25 kDa (Scarr et al., 2006) and vesicle-associated membrane protein (Beasley et al., 2005). Diverse lines of evidence from molecular, cellular and clinical studies suggest neuropsychiatric theories of bipolar disorder where disease-induced changes in neuroplasticity lead to disruptive synaptic communications in neuronal circuits involved in the pathophysiology of this illness (Manji et al., 2001; Kapczinski et al., 2008; Fisˇar and Hroudová, 2010). Lithium may block these pathological processes and enhance synaptic plasticity, leading to a reversal of the neuroplastic impairments related to the disorder.

Although these theories have been widely accepted, few studies have directly assessed the effects of lithium on synaptic plasticity. We recently reported that lithium treatment increased hippocampal long-term potentiation (LTP) of field excitatory post-synaptic potentials, indicating that lithium treatment up-regulates functional synaptic plasticity in the hippocampus (Shim et al., 2007, 2012). This paper reports the effects of lithium treatment on structural synaptic plasticity. We examined whether 4 wk lithium treatment alters the amount and distribution of dendrites in dentate gyrus (DG) granule cells and hippocampal area CA1 pyramidal cells. Rats were fed with lithium chow for 4 wk. At the end of lithium feeding, the rats were euthanized and the amount and distribution of dendrites was quantitatively assessed using the Sholl analysis technique (Sholl, 1953).

Materials and method

Animals and drug treatment

Eighteen individually housed male Sprague–Dawley rats weighing 175–200 g (Harlan, USA; aged 45 d at the start of the experiment) were used. Animals were housed in a temperature regulated (22 ± 1 °C) and light-controlled (12-h light/dark cycle; lights on 07.00 hours) colony room with food and water available ad libitum for 1 wk to acclimatize. Lithium-treated animals were then fed rat chow containing 0.24% lithium carbonate (BioServ®, USA) for the next 4 wk. The cages of lithium-treated animals were equipped with two water bottles. One was a tap water bottle and the other contained 0.9% saline because free choice saline consumption has been shown to reduce renal toxicity associated with lithium administration (Smith and Amdisen, 1983). The cages of the control animals were equipped with tap water bottles only. During the period of drug treatment, the blood levels of sodium of both groups were within normal range and were not different between the two groups (Alda, 2006; Nocjar et al., 2007). All animal procedures were carried out in strict accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Cleveland Veterans Affairs Medical Center Institutional Animal Care and Use Committee. Animals were randomly divided into the lithium and control groups.

Rapid Golgi staining

Neuronal dendrites were stained using a modification of the rapid Golgi staining technique (Morest, 1981; Valverde, 1993). At the end of 4 wk of lithium chow feeding, animals were deeply anaesthetized with Nembutal® (75 mg/kg i.p.), the chest was opened, the left ventricle punctured and the right atrium incised to allow blood exit during saline injection. Blood was collected from the atrium as the infusion of saline pushed it out to measure levels of lithium. Samples were immediately centrifuged at 15 183 g at 4 °C for 10 min without anticoagulants and then stored at −80 °C for subsequent analysis. We used the method of Pybus and Bowers (1970) to analyse plasma lithium levels using an atomic absorption spectrophotometer (Model 1100A®; PerkinElmer, USA), a standard technique and one of the most sensitive methods available to measure lithium levels in animals. Please see our reference article (Nocjar et al., 2007) for feeding lithium to rodents and measuring blood levels of the drug. After saline was perfused thoroughly to flush out blood from the brain, a fixative solution (phosphate buffered 10% formalin) was perfused in to preserve the brain tissue. A coronal tissue block (2–3 mm thick) was dissected from each right hemisphere, which included the hippocampal formation. Each tissue block was stained using a modification of the Rapid Golgi method. Tissue blocks were placed in an ultraclean staining jar containing Rapid Golgi solution composed of 0.25% osmium tetroxide and 2.4% potassium dichromate. The jar was kept in the dark for 6 d while gently shaken. Tissue blocks were then thoroughly rinsed in a freshly prepared solution of 0.75% silver nitrate, blotted, then placed in another staining jar containing the silver nitrate solution and kept in the dark while gently shaken for 38–42 h. The blocks were then dehydrated in a series of graded alcohol and embedded in increasingly concentrated solutions of nitrocellulose. The nitrocellulose was then hardened by exposure to chloroform. These hardened nitrocellulose blocks of tissue were then affixed to a sectioning block and cut (at 120 μm in thickness) on an AO sliding microtome (Global Medical Instrumentation, Inc., USA). The sections were cleared in α-terpineol, thoroughly rinsed in xylenes, mounted on slides and overslipped using Permoun® (Fisher Scientific, USA). All slides were coded.

Morphological analysis of dendritic branches and dendritic spines

Sholl analysis was used to determine the amount and distribution of dendritic branches quantitatively (Sholl, 1953). The slides of Golgi-impregnated neurons were
mounted on a Zeiss digital microscope (Carl Zeiss MicroImaging Inc., USA) and were visualized using oil-immersion lenses (40×, 63× and 100×). Only neurons that met the following criteria were used in the Sholl analysis: (1) the cell body and dendrites were within the specified subregions of the hippocampus (CA1 and the upper blade of the DG); (2) dark and consistent impregnation was seen throughout the extent of the dendrites; (3) the chosen neuron was relatively isolated from neighbouring impregnated neurons; (4) the soma was located in the middle third of the tissue section. On Zeiss bright field research microscopes, camera lucida drawings were made of the selected neurons using a 40× objective and an intermediate magnification (optivar) of 1.25. The camera lucida drawings were then quantified using Sholl analysis. The Sholl analysis evaluates the amount and distribution of the dendritic material at increasing distances from the cell body. This is accomplished by placing a template comprising a series of concentric circles of larger diameters (i.e. shells) at equidistantly farther (10 μM) intervals from the soma and quantifying the numbers of intersections of the branches with each of the shells. Graphically, this generates a profile, which can be statistically compared with other profiles from other groups.

Statistical analysis

For the Sholl analysis of dendritic amounts and their distribution from the soma, the non-parametric Wilcoxon’s signed-rank test was used because of small sample sizes, non-normal distribution and to guard against outliers (Falke et al., 2003; Ron et al., 2011; Adamec et al., 2012). For total dendritic branch points per cell analyses, the unpaired Student’s t test was applied. The results were expressed as means ± S.E.M. Significance was set at p < 0.05. In Sholl analysis, the number of concentric ring intersections was used as an estimate of total branch length (Flores et al., 2005; Morales-Medina et al., 2007).

Results

Effects of 4 wk of lithium treatment on granule cell dendrite morphology in the DG

At the end of 4 wk lithium treatment, lithium-treated animals were killed and trunk blood was collected to determine the plasma levels of lithium. It was confirmed (data not shown, see Nocjar et al., 2007) that the blood levels of lithium were within the therapeutic range in humans (0.68–0.89 mEq/l). The dendritic trees of impregnated neurons were clearly visualized in photomicrographs of the upper blade of the DG in Fig. 1a. Granule cells with well-branched dendrites extending into the molecular layers in the upper blade of the DG are seen in Fig. 1b. An example of camera lucida drawings used for Sholl density analysis of dendritic tree arborization is seen in Fig. 1c.

Fig. 1. Low magnification photo of Golgi impregnated hippocampus with arrows indicating CA1 stratum radiatum and upper blade of the dentate gyrus (DG) (a). Photographs of representative dendritic trees of Golgi impregnated DG granule cells. Granule cells with well-branched dendrites extending into the inner and outer molecular layers of the DG (b). Camera lucida drawing of dendritic branches of a granule cell under a concentric shell template for the Sholl analysis are shown (c).

Table 1 provides a synopsis of the results that are described in detail below. Sholl analysis showed a remodelling of the dendritic trees of the granule cells (Fig. 2a). In the inner (proximal) half of the dendritic
trees, the lithium-treated group had significantly more dendritic material (Wilcoxon’s signed-rank test, $p = 0.0028$). Conversely, in the outer (distal) half of the trees, the control animals revealed more dendritic branching (Wilcoxon’s signed-rank test, $p = 0.0038$). This shift in the amount of dendritic material of the granule cells between the inner and outer parts of the dendritic trees is clearly demonstrated in Fig. 2a. As can be seen in Fig. 2a,
maximal dendritic intersections were observed for both groups between 60 and 170 μM away from the soma. The total dendritic branching per cell was 11.4% higher in the lithium-treated group (85.97 ± 5.05 μM) compared to the control group (79.13 ± 5.822 μM). However, the difference was not statistically significant between the two groups (unpaired Student’s t test, p = 0.4044; Fig. 2b).

Effects of 4 wk lithium treatment on apical dendrite morphology of hippocampal CA1 pyramidal neurons

The photomicrograph shows a representative Golgi-stained CA1 pyramidal cell (Fig. 3a). The dendritic branching is divided into two trees: (1) the relatively short basilar dendritic branches, which are in the stratum oriens; (2) the apical dendritic tree—emanating predominately from a thick main apical branch, which spreads the dendritic arbor through the stratum radiatum and stratum lacunosum/moleculare. An example of camera lucida drawings used for Sholl density analysis of dendritic tree arborization is seen in Fig. 3b. Sholl analysis indicated that in the proximal or inner half of the apical dendritic arbor there was significantly more dendritic branch material in the lithium-treated group (Wilcoxon’s signed-rank test, p = 0.0005), whereas in the distal or outer half of the apical tree there was more dendritic branching in the control group (Wilcoxon’s signed-rank test, p = 0.0009; Fig. 4a). Interestingly, the pattern in the above graph corresponded with the branching pattern seen in the granule cells of the DG (Fig. 2a), where maximum branching occurred at 60–170 μM away from the soma and lithium increased branching proximally compared to controls. In CA1 (Fig. 4a), maximum branching occurred at 60–130 μM away from the soma and the total apical dendritic branch points per cell was 4.5% more in the lithium-treated group (168.4 ± 15.74 μM) compared to the control group (161.1 ± 18.50 μM). The difference was not statistically significant (unpaired Student’s t test, p = 0.7663; Fig. 4b).

Effects of 4 wk lithium treatment on basal dendrite morphology of hippocampal CA1 pyramidal neurons

Main basal dendrites were shorter than apical dendrites in CA1 pyramidal cells and a tuft of well-branched short basilar dendrites were observed (Fig. 3a). Sholl analysis indicated that there was no significant difference in the amount and distribution of dendritic branching in either proximal or distal half-segments between lithium-treated (n = 25, N = 5) and control animals (n = 30, N = 6; Wilcoxon’s signed-rank test, p = 0.3496; Fig. 5a). The total dendritic branch points per cell were also not significantly different (unpaired Student’s t test, p > 0.05) between the lithium-treated group (24.05 ± 1.378 μM) and the control group (20.89 ± 1.68 μM; Fig. 5b).

Discussion

This study reports that 4 wk lithium treatment in naive young adult male rats increased the number and distribution of dendritic branches in specific segments of dendritic trees of principle cells in the DG and area CA1 of the hippocampus (see Table 1). These effects were not observed along the entire length of the dendritic trees in these regions, but were specific to the proximal segments of the DG and apical CA1 dendritic trees. More precisely,
dendritic branch amounts were increased by lithium only in the proximal half of dendritic trees in the DG granule cells (Fig. 2a) and in the proximal two-thirds of apical trees in hippocampal CA1 pyramidal cells (Fig. 4a). In DG and area CA1, 4 wk lithium treatment reduced dendrite branching in the distal segments of both the DG granule cells (Fig. 2a) and CA1 pyramidal cell apical trees (Fig. 4a).

Animal models of bipolar disorder

A pair of studies have provided evidence that lithium treatment did not change the morphology of dendritic branches in hippocampal CA3 pyramidal neurons (Wood et al., 2004) and in the amygdala (Johnson et al., 2009) in normal unstressed rats but, instead, attenuated stress-induced dendritic hypertrophy of those specific principal neurons (amygdala and area CA3). In those studies, the authors determined the effects of lithium on total dendritic branching and suggested a mechanism of action for lithium in a stress model. Our results specify that lithium increases dendrite distribution only in the proximal segments of dendritic arbors, where dendrites are highly branched and active synaptic transmission occurs, without altering the total amount of dendritic branches in the hippocampus (Table 1). Furthermore, our study shows that lithium decreases dendritic branching in the distal segments of dendritic trees (Table 1). Thus, our results strongly suggest that the effects of lithium or other psychotropic drugs on dendritic branching should be determined in the different segments of dendritic trees rather than in total dendritic branches alone. Contrasting the findings of Wood et al. (2004) and Johnson et al. (2009) with those of our present study in non-stressed (naive) rat hippocampal regions, where lithium increased particular segment branching, we contend that animal modelling of bipolar disorder remains a ripe area for future studies. We suggest that a more complete and
widely accepted animal model of bipolar disorder will need additional clarifying investigations in order to refine and explain the details, especially the impact of lithium on bipolar disorder in different cortical and subcortical systems and under different conditions, such as stress or traumatic injury.

Neurotransmission- and neurotrophic factor-induced dendritic remodelling

It is of interest that the lithium-induced increase in branching was seen in dendritic segments where branches are highly populated with synapses and, thus, where active synaptic transmission occurs. In the DG, dendrites in the stratum moleculare form most synapses with terminals of the glutamatergic excitatory perforant pathway (Blackstad, 1958; Amaral and Witter, 1995; Patton and McNaughton, 1995). The apical dendritic trees of CA1 pyramidal cells in the stratum radiatum form massive synapses with terminals of the glutamatergic excitatory Schaffer collaterals (Storm-Mathisen and Fonnum, 1972; Bannister and Larkman, 1995). In the stratum lacunosum/moleculare, distal to the stratum radiatum, the apical dendrites make additional synapses with terminals of Schaffer collaterals. The morphology of dendrites and dendritic spines is known to be highly plastic and dependent on synaptic transmissions (Hering and Sheng, 2001). Among synaptic transmissions, LTP is closely linked with plastic changes in dendrites and dendritic spines (Muller et al., 2002; Matsuzaki, 2007). Two electrophysiological studies produced findings wherein 4 wk lithium treatment increased LTP in both the DG (Son et al., 2003) and CA1 area (Shim et al., 2012). Our current finding that dendritic branches treated with lithium are increased and distributed to the same highly active synaptic segments of

Fig. 5. Effects of 4 wk lithium treatment on basal dendritic intersections in hippocampal area CA1 pyramidal cells. Sholl analysis shows that dendritic intersections of the basal dendrites of pyramidal cells were not significantly different between groups at any point from the soma, lithium-treated animals (n = 25, N = 5) and control animals (n = 30, N = 6; Wilcoxon’s signed-rank test, p = 3496) (a). Error bars were not included to highlight that the null hypothesis was confirmed and to see how similar the means were between the groups. Maximum branching was found 30–90 μm away from the soma. The total dendritic intersections per cell were also not significantly different between the lithium-treated group (24.05 ± 1.378) and the control group (20.89 ± 1.68; unpaired Student’s t test, p > 0.05) (b). Camera lucida drawing of CA1 pyramidal cell basilar dendritic tree from a representative control neuron (c). Camera lucida drawing of CA1 pyramidal cell basilar dendritic tree from a representative lithium treatment neuron (d).
dendritic trees, where lithium-increased LTP has been observed (Son et al., 2003; Shim et al., 2012), supports a mechanism that connects functional and structural plasticity in the effects produced by lithium.

In addition to the impact of neurotransmission alone on structural dendritic remodelling, multiple neurotrophic factors are involved in the regulation of functional (LTP) and structural plasticity (dendrites and dendritic spines). Among these factors, BDNF is the molecule that has been most extensively studied. Evidence shows that BDNF plays an crucial role in enhancing LTP (Bramham and Messaoudi, 2005; Shen and Cowan, 2010) as well as in mediating changes in functional synaptic plasticity (LTP) that yield positive plastic changes in the morphology of dendrites and dendritic spines (Bramham, 2007).

Taken together, previous studies provide evidence for multiple mechanisms underlying the effects of lithium on plasticity in general. The possibility of multiple mechanisms for lithium-mediated plastic changes correlates well with this present study. Herein, 4 wk of lithium increased branching in highly populated proximal segments of DG and apical CA1 dendritic trees and, conversely, reduced branching in less populated distal segments where less active synaptic transmission occurs.

**Why was basilar CA1 dendritic morphology unchanged?**

Curiously, in this study, lithium treatment did not increase CA1 dendritic branching amounts and distributions in the basal dendritic field, where dendrites are also highly populated and form synapses with terminals of recurrent collaterals as well as Schaffer collaterals (Amaral and Lavenex, 2006). This may be because of differing mechanisms for LTP induction in apical vs. basilar CA1 dendrites (Haley et al., 1996) or perhaps because basilar dendritic branching operates under a different structural mechanism for plasticity than apical branching. Even so, our morphological findings are consistent with electrophysiological studies, which reported that 4 wk lithium treatment increased LTP in the DG (Son et al., 2003; Yu et al., 2003). We have recently reported that 4 wk lithium treatment also increased LTP in the CA1 subregion of the hippocampus (Shim et al., 2012). In that investigation, LTP observations were recorded in the stratum molecular of the DG and in the stratum radiatum of CA1, where apical dendrites are highly populated. Those findings correlate well with this study, where 4 wk lithium treatment increased dendritic branch distribution in the same areas. Muller et al. (2000) and Bramham (2008) also indicated that LTP increases the formation of synapses and contributes to long-term changes in structural synaptic plasticity in the hippocampus.

**Impact of other antidepressants or antipsychotics**

Most studies reporting the effects of antidepressants on dendrites have examined the effect of the drugs on stress-induced atrophy of dendrites (for review, see Pittenger and Duman, 2008). To our knowledge, there have been no studies that examined the effects of antidepressants on dendritic amounts and distributions systemically (specifying different tree segments) in naive adult rodents. In normal rats, Chen et al. (2008) reported that imipramine treatment changed the density of different types of spine synapses in the hippocampus. Hajszan et al. (2005) also reported that fluoxetine treatment increased dendritic spines in hippocampi of normal rats. Those two studies suggest that, like lithium, antidepressants may also produce remodelling of structural synaptic plasticity in the hippocampus in naive stress-free rats. One recent study (Frost et al., 2010) focused on the effects of antipsychotic drugs on dendritic arbors and spines and reported that treatment with haloperidol or olanzapine in prenatal or early developmental stages reduced the density of pyramidal dendritic arbors and spines in the prefrontal cortex after the rats reached young adulthood.

Thus, we hypothesize that chronic lithium generally remodels the dendritic distribution of specific segments of principle hippocampal neurons to produce more branches in segments where synapses with major excitatory inputs are found and where the most active synaptic transmission occurs. Conversely, chronic lithium treatment generally diminishes branches in segments where less synapses form. This structural remodelling may be responsible for a more persistent enhancement in synaptic transmission or a more efficient manner of signalling mediated by lithium when it is used to treat mood disorders. These structural changes are likely preceded by changes in functional synaptic plasticity, such as an increase in LTP in these same hippocampal regions.

In conclusion, the main finding in this report is that 4 wk lithium treatment increased DG and apical CA1 dendritic amounts and distribution in specific segments of the dendritic trees, where highly active synaptic transmission occurs.

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**Statement of Interest**

None.

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