Lithium Induced Neural Plasticity

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Neural plasticity refers to the brain’s ability to make new cellular connections. Drugs that can induce neural plasticity are of basic as well as clinical interest. Lithium, a drug already in use, has been demonstrated to be neuroprotective and is likely to find wider use. The spectrum of diseases that can be potentially treated with lithium suggests that there could be a common cellular mechanism, such as neural plasticity, in operation. We review effects of lithium on major cellular processes that comprise neuroplasticity – alterations, \textit{in vitro} and \textit{in vivo}, in neurites, axons and synapse formation. Lithium is known to support extension of cytoplasmic outgrowths. Lithium alters patterns of axonal modifications including their extensions or retractions and sprouting of new branches. However, there are few studies directly demonstrating lithium action of synapse formation. The molecular basis of lithium action is complex with various pathways involved in cross talk. Of these multiple pathways, we have focused on lithium induced inhibition of glycogen synthase kinase-3β, block of inositol phosphate pathway and up regulation of neurotrophins as there are direct evidences of involvement of these in lithium induced neuroplasticity. This review provides a bird’s eye view of studies that could provide insight into special aspect of lithium action, induction of plasticity, which have implication for treating a wide variety of neurological conditions.

INTRODUCTION

Neural plasticity is the crux of research in psychology, depression, and aging in the present times. It refers to the brain’s ability of making new connections by repeated disciplined actions. The phrase ‘Use it or lose it’ or \textit{le-su-rung-wa}, meaning pliability in Tibetan, can be used to summarise neural plasticity as the way to change brain activity by repeated practises. So called “brain workouts” are other useful stimuli for reorganization of connections when the brain is not capable of reorganizing on its own. Challenging intellectual environment and physical activity too are suggested for boosting general growth of nerve connectivity. Any change in neural plasticity modulates cellular microarchitecture by growth of neurite and branching of axons, which influences changes in synaptic sites and neurogenesis (Gary and McEwen, 2013).
What is important to note is that these stimulations alone are not effective for stabilizing mood disorders or other pathological or degenerative brain conditions. Hence, drugs that can demonstrate induction of neural plasticity are important candidates for brain science today. Lithium has been a drug of choice for bipolar disorder (BD) for several decades (Quiroz et al., 2004) and meets these criteria. Its mechanism of action has been investigated extensively, yet the clarity about how it brings symptomatic relief to BD patients has been shown to be due to a handful of genes in a signalling pathway. Lithium protects neuronal cells, \textit{in vivo} and \textit{in vitro}, against a variety of stressors (Chiu et al., 2013), is anti-apoptotic and is known to enhance neuroplasticity (Quiroz et al., 2010) and synaptic plasticity (Contestabile et al., 2013; Nelson et al., 2013). In another report the mechanism of lithium action was related to stimulation of stem cells and increase in neurogenesis in adult brain (Chen et al., 2000). The neuroprotective properties of this small molecule have made it a possible candidate for treating neurodegenerative diseases such as Alzheimer's, Parkinson, Huntington's disease and Amyotrophic Lateral Sclerosis (Dell'Ossò et al., 2016; Forlenza et al., 2014). It has been reported to reduce brain damage associated with AIDS (Dou et al., 2005), ischemia and trauma induced brain injury (Leeds et al., 2014; Wada et al., 2005). The spectrum of diseases that can be treated with lithium suggests there could be a central mechanism of action. It was established beyond doubt that lithium at therapeutic concentrations competed with magnesium and affected cellular activity of a number of magnesium dependent enzymes (Amari et al., 1999; Quiroz et al., 2004). It also inhibited enzymes and molecular pathways by non-competitive inhibition (Nahorski et al., 1991). The three major enzymes viz., glycogen synthase kinase 3\(\beta\) (GSK-3\(\beta\)) (Ryves and Harwood, 2001), inositol monophosphatase (Atack, 2000) and Akt (De Sarno et al., 2002), which have a cascading change in molecular dynamics also accounted for major cellular effects of lithium. These three major pathways initiate neuroprotective effects of lithium and may also be responsible for lithium induced neuroplasticity.

In this mini review, we narrate some of these molecular pathways and their specific roles in lithium induced neuroplasticity (Fig. 1). We review the
major processes that comprise neuroplasticity alterations in neurites, axons and synapses that eventually reflect as a change in brain microarchitecture and results in clinical relief. We further review what is known this far about molecular basis of lithium induced cellular changes under these conditions.

**Lithium Alters Neurite Extensions**

A number of studies demonstrate that lithium supports extension of cytoplasmic out- growths, the neurites. It induces neuritogenesis in SH-SY-5Y cells (Nciri et al., 2015), hippocampal neural progenitor cells in *vitro* and *in vivo* (Kim et al., 2004), in mouse N2a neuroblastoma cells (Wang et al., 2011), in mouse N1E-115 neuroblastoma cells (Mizutani et al., 2009), in mouse model of severe combined immunodeficiency (Dou et al., 2005), in primary cultures of dissociated spiral ganglion neurons from adult mice (Shah et al., 2013), and in cultured chick dorsal root ganglia (DRG).
neurons (Hollander and Bennett, 1991). Our own studies using SK-N-MC cells show lithium induced neurite extensions (Italia et al., 2011). A study on primary cultures of rat hippocampal neurons used immune-histochemical staining to show dose-dependent inhibition of neurite growth in presence of lithium (Takahashi et al., 1999). Lithium was also shown to inhibit nerve growth factor induced neurite outgrowth (Burstein et al., 1985), although there was an increase spreading of growth cone filopodia. Likewise, chronic lithium treatment inhibiting pilocarpine induced mossy fibre sprouting was seen in rat hippocampus (Cadotte et al., 2003). Lithium blocked stress induced decrease in dendrite length and branching of hippocampal neurons though no changes in dendrites were observed in unstressed animals. Though there are no clear molecular explanations to these contradictory observations, several molecules have been demonstrated to be associated with lithium induced alterations in neurites. More recent results of stress responsive neuroplasticity related genes from PLP family and their expression related to depression (Fuchsova et al., 2015) could be of some cue.

**Lithium Alters Axonal Micro-architecture**

Plasticity in the brain is defined by axon microarchitecture dynamics and its constant modifications. Patterns of axonal modifications include their extensions or retractions and sprouting of new branches. Lithium has been found to alter neuroplasticity by causing changes in both these processes. The axons have a growing cytoplasmic extension called the growth cone which respond to environmental cues that in turn signal extension / retraction of the axon. Lithium has been demonstrated to alter morphology of growth cones and enhance axonal branching *in vivo* (Hall et al., 2000) and *in vitro* (Nciri et al., 2015). Interestingly, it is known to cause enhancement of growth cone surface area associated with decrease in axonal length (Lucas et al., 1998; Williams et al., 2002). Lithium has been observed to cause sprouting of pyramidal neurons in corticospinal tracts (Dill et al., 2008).

**Lithium Alters Synaptic Connections**

Synapses are dynamic communicating ends of neurite extensions connecting neurons. Alteration in their number and strength affects the plasticity of the brain. Though a number of clinical studies
suggest that efficacy of lithium is associated with synaptic plasticity (Hee and Stanley, 2009; Lopez et al., 2010; Tomasetti et al., 2017), there are a few studies directly demonstrating lithium's action on synapse formation or on strengthening pre-existing synapses (Shim et al., 2013). Increase in synaptic connections between hippocampal neurons *in vitro* has been observed using confocal imaging. Lithium treatment for four weeks has been reported to rearrange neuronal morphology with increase in dendritic branching and distribution in dentate gyrus and C1 hippocampal region (Shim et al., 2013).

In addition to increase in actual synapse formation, lithium is known to alter synaptic proteins that are involved in neurotransmission. A protein implicated in neurite outgrowth and maintenance of neural network has been found to be significantly high in lithium treated rats (Murdoch et al., 2003). Clustering of Gephyrin, a post synaptogenic molecular scaffold that regulates synaptic plasticity of GABAergic neurons is induced *in vitro* and *in vivo* (Tyagarajana et al., 2011). The post synaptic density proteome in rat hippocampus have 40 proteins expressed differentially in control versus lithium treated animals. Of these synaptic proteins Ank3 and GluR3 were seen to be significantly increased in the lithium treated group of animals (Nanavati et al., 2011). SNARE, the soluble N ethylmaleimide – sensitive factor attachment protein complex is part of the molecular docking machinery at the synapse and is obligatory for neurotransmission. In a recent study it has been demonstrated that post nerve injury reduction in levels of SNARE could be reversed by lithium. Thus, increased abundance of SNARE enhances neurotransmission and improves cognitive functions in rat model (Carlson et al., 2017). Alterations in synaptic currents have also been reported in hippocampal pyramidal neurons treated with therapeutic concentrations of lithium (Ankolekar and Sikdar, 2015). Only one report showed that the number of active synapses and basic presynaptic functions in the hippocampal neuronal network are not affected by lithium (Lueke et al., 2014).

On the contrary acute treatment with high concentration of lithium has been reported to act preferentially on presynaptic terminals of isolated rat hippocampal pyramidal cells, inhibiting transmission of excitatory signals than
inhibitory ones (Wakita et al., 2015).

**Molecular Basis of Lithium Induced Neuroplasticity**

The process of neurite and axonal sprouting, extension and characteristics of the synapse are altered by lithium and could be responsible for the lithium induced neuroplasticity. Its molecular basis is complex with various pathways involved in cross talks. An excellent review by Dell’Osso lists most of the pathways known so far and could be involved in lithium action (Dell’Osso et al., 2016). As an outcome of its effect on three major pathways mentioned earlier, lithium upregulates cell survival molecules such as B cell lymphoma 2, [Bcl2] (Chen and Chuang, 1999), cyclic adenosine monophosphate-responsive element binding protein [CREB] (Kopnisky et al., 2003) and heat shock proteins (Nciri et al., 2014). Lithium downregulates pro-apoptotic molecules such as p53 (Ngok-Ngam et al., 2013), Bax (Liechti et al., 2014) and caspase 3 (Mora et al., 2001). It also down regulates cytochrome c release (Li et al., 2010), β-amyloid peptide accumulation (Yu et al., 2012), and tau hyperphosphorylation (Muñoz-Montaño et al., 1997). It inactivates N-methyl-d-aspartate (NMDA) receptors (Hasimoto, 2002). Again, alterations in GPM6A, GPM6B, and PLP1 gene expressions in depression/suicides result in dysregulation of neuroplasticity involving neurite outgrowth and filopodium formation (Fuchsova et al., 2015). Of these multiple effects, we have reviewed the major molecular events related to lithium induced plasticity only.

**Lithium Inhibits GSK-3β**

Involvement of GSK-3β in normal neuronal development has been documented as it is known to be highly expressed in the cells. It is known to regulate neuronal polarity and maturation of neurites as they form axons (Jiang, 2005; Yoshimura et al., 2005). Inhibition of GSK-3β a central molecule in wnt/β-catenin signalling by lithium, leads to cytoplasmic stabilization of β-catenin. This accumulated β-catenin is translocated to the nucleus and regulates the TCF/Lef dependent gene transcription which include genes that support survival, neurogenesis and cytoskeletal regulation leading to neuronal plasticity (Quiroz et al., 2010). Changes in arborization of neuronal cells is expected to involve the cytoskeleton (Dent and Gertler, 2003). Both actin

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(Kerstein et al., 2015) and microtubules (Kahn and Baas, 2016) are responsible for micro guidance of the growth cones and branching of axons. Lithium influences actin polymerization (Colombo et al., 1991) and microtubule stability (Goold et al., 1999). Lithium treated, enlarged growth cones alter microtubule dynamics (Nicri, 2015). Lithium appears to redistribute F actin to the periphery with induction of F actin rich growth cone protrusion (Shimshoni et al., 2009). Changes in growth cone architecture has been associated with level of GSK-3β mediated phosphorylation of MAP 1B (Goold et al., 1999; Lucas et al., 1998) and inhibition of GSK-3β by lithium block collapse of growth cones that are induced upon molecular signals from the micro environment (Eickholt et al., 2002). Several observations however indicate that growth cone spreading associated with lithium treatment may not arise due to GSK-3β inhibition (Williams et al., 2004).

**Lithium Blocks Inositol Phosphate Pathway**

There is an alternative possibility that lithium induces depletion of inositol and may be responsible for complex chemotaxis observed in growth cones. Lithium inhibits the enzyme inositol monophosphatase which converts inositol mono phosphate to inositol. Thus, lithium treatment effectively blocks recycling of inositol and leads to depletion of this molecule. It is not clear how exactly blocking of this signalling pathway increase growth cone size. It is hypothesised that growth cone chemotaxis is regulated by intracellular calcium which is mediated by Inositol phosphate pathway. Lithium thus indirectly affects intracellular calcium causing increased growth cone surface area and spreading. Intracellular calcium also stimulates PKC that post translationally phosphorylates myristoylated alanine rich C kinase substrate (MARCKS), a molecule seen preferentially in dendritic branches and axonal terminals of developing brain (McNamara and Lenox, 1998). At the synaptic level, MARCKS co-localizes with synaptic vesicles and has been implicated in neurotransmission (Ouimet et al., 1990; Yang et al., 2002). Interestingly, chronic but not acute, lithium treatment in rats significantly downregulates MARCKS and this reduction appears to be mediated via the phosphor inositol pathway (Lenox et al.,

**Lithium Up-regulates Neurotrophins**

Another family of molecules found responsible for lithium induced neuroplasticity are the neurotrophins. These are well-established mediators in differentiation of neurons and modulators of synapse. At therapeutic concentrations, lithium has been shown to upregulate brain-derived neurotrophic factor [BDNF] *in vivo* (Hashimoto *et al.*, 2002a) and *in vitro* (Mai *et al.*, 2002), Vascular Endothelial Growth Factor [VEGF] (Guo *et al.*, 2009), Nerve Growth Factor [NGF] (Hellweg *et al.*, 2002) and glial cell line derived neurotrophic factor [GDNF] (Emamghoreishi *et al.*, 2015), all known for participation in neuronal survival and plasticity. BDNF and its receptor, tropomyosin related kinase B (Trk B) was increased in cultures of cortical neurons treated with lithium (Hashimoto *et al.*, 2002b). *In vivo* studies have demonstrated that lithium increases levels of BDNF in circulation (Leyhe *et al.*, 2009) and brain tissue (Fukumoto *et al.*, 2001), though human studies do not equivocally report this increase (Gray *et al.*, 2013). Lithium selectively increases levels of exon IV BDNF mRNA and activity of its promoter and receptor, TrkB (Yasuda *et al.*, 2009). This action of lithium is not only via inhibition of GSK-3β but also by direct modulation of the cAMP-PKA-CREB-BDNF signalling (Quiroz *et al.*, 2010). Lithium is known to activate ERK/MAP kinase cascade at therapeutic concentrations which is responsible for upregulation of transcription of BDNF *via* CREB (Mai *et al.*, 2002).

**CONCLUSION**

Today the literature available on various aspects of lithium as a drug is enormous. There have been investigations ranging from cell specific systems, animal models and clinical trials to use lithium as a supplement. As new possibilities appear for its use the underpinnings of its action that is likely to have maximum impact need to be revisited as has been done in this review. Neuroplasticity induced by lithium is well documented in animal models and in brain cells *in vitro*. Although exact mechanism is not completely understood, neuroplasticity has been stressed as one of the major
as well (Benedetti et al., 2011). Lithium alters connectivity and networking of neurons which manifests as changes in cortical maps (van Erp et al., 2012; Foland-Ross et al., 2011) and leads to subsequent change in behaviour. Cellular processes that cause lithium induced neuroplasticity involve all the major molecular pathways reported so far. This review provides a bird’s eye view of relevant studies that insight into the special aspect of lithium action that is, induction of neural plasticity. It may have implications for treating varieties of neurological conditions in future.

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