“Glycogen Synthase Kinase-3 (GSK-3) and Inositol depletion hypothesis - therapeutic intervention with Lithium in the Bipolar Disorder”

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Abstract

Lithium is often used to treat of bipolar disorder, but the procedure of its action is unknown in this condition. .Lithium has the ability to directly restrain the glycogen synthase kinase-3 (GSK3), a key multi-signal transduction regulator. GSK3 inhibition is a convincing explanation of several of the lithium's known actions including early development and production of insulin/glycogen. Their effectiveness is nevertheless limited to some extent and therapy could lead to some adverse outcomes. There is a little knowledge about the therapeutic mechanisms of these medicines, which impede more effective therapy. Among the many biochemical consequences of medicines, therapeutic effectiveness may be more often associated with the two. Two frequent results are the depletion of inositol and the inhibition of GSK3, suggested to explain valproic acid and lithium effectiveness. Some of these targets does lithium have behavioural or therapeutic effects in vivo are unknown. The purpose of this research is to investigate basic requirements for modelling systems in order to verify a hypothesised direct lithium target. For that we have gone through a collection of fundamental mouse behaviours that are significantly altered by chronic lithium and also by a number of structural GSK3 inhibitors. Additionally, we discuss the inositol depletion and GSK3 inhibition hypotheses in this section and offer a unified model for the connection between inositol depletion and GSK3 inhibition.

Declaration of Contribution

Contents

Abstract 2
1. Introduction 4
   1.1 Bipolar disorder 4
   1.2 Lithium 5
   1.3 Inositol depletion hypothesis and GSK-3 6
2. Literature Review 8
   2.1 History of Treatment by using Lithium 8
   2.2 Direct Targets of Lithium 8
   2.3 GSK-3 Function 9
   2.4 GSK-3 and Bipolar Disease 9
3. Inositol depletion hypothesis 11
   3.1 Inositol metabolism 11
   3.2 Altered levels of inositol in BD 12
   3.3 Lithium & VPA limit inositol synthesis 12
   3.4 Depletion of Inositol and PKC 14
   3.5 Differences between the inositol depletion hypothesis and evidences 15
4. GSK3 inhibition hypothesis 16
   4.1 Lithium inhibits the activation of GSK3 16
   4.2 VPA inhibition of GSK3 18
   4.3 GSK3 activity and HDAC inhibition 19
   4.4 GSK3 inhibition and its neurotropic effects 19
5. Inositol depletion and GSK3 inhibition model 21
   5.1 Lithium-induced behavioural depletion of inositol 22
6. Conclusion: 24
7. Future implications 25
References 26

1. Introduction

1.1 Bipolar disorder

Bipolar disorder (BD) is a characterized as severe mental illness characterised by severe and biphasic and extreme changes in mood, likewise neurocognitive dysfunction. Recurrent episodes of depression that alternate with mania, are experienced by BD patients which are usually separated by periods of normal functioning - euthymia. The general symptoms of a depressive episode are - reduced interest and pleasure, low moods, apathy, loss of energy, recurrent thoughts of death and suicide. Symptoms of a manic episode are elevated or irritable moods, increased self-esteem, flight of ideas, risk-taking behaviour and psychosis (Mahan et al., 2011).

Epidemiological studies suggest that up to 1-4.4% of the world's population are affected by BD, the disorder is highly prevalent. The illness is also debilitating and dominating, the prognosis is poor and it often comes with social, economic and psychiatric comorbidities. The mortality gap between individuals with BD and the general population is vast and growing. The risk of suicide is alarmingly high in those affected, with approximately 20%-65% of persons diagnosed having a history of suicide attempts.

BD is complex and heterogeneous; it may be considered a group of disorders. Only 30% of people with BD display classical symptoms, while the majority have a more severe and refractory diathesis. The disorder is difficult to treat and diagnose, which puts a significant burden on those affected. The evidence to research BD is therefore pressing (Smith, Whitham, & Ghaemi, 2012).

As is the case with many psychiatric disorders the etiology of BD is unknown. It is generally considered to be a multisystem disorder, that involves the interaction between genetic vulnerability (60-80% heritability) and environmental risk factors. History of neglect, abuse, adverse life events and stress are associated with elevated risk of the disease. Pathophysiology of BD seems to be underlined by epigenetic mechanisms, neurotrophic factors, neurotransmitter

release, changes in neurogenesis and neuroplasticity, elevated apoptosis, mitochondrial dysfunction, oxidative stress and inflammation. It is a disorder of multiple levels; causation of changes is highly complex to exactly determine the pathophysiology of the disorder (Sigitova, Fišar, Hroudová, Cikánková, & Raboch, 2017).

1.2 Lithium

Lithium is one of the oldest psychiatric drugs. It has been used as a treatment for BD for the last sixty years, since Cade described it as a drug that produces fast and dramatic improvements in patients with mania. Li+ remains a first line therapeutic and evidence for its effectiveness in BD only surges (Alda, 2015). It is the only medication that prevents both fresh and new manic episodes in the long run. Besides, lithium has well-established anti-suicidal efficacy, which is crucial. A recent study demonstrated that compared with other drugs used for treatment of BD (valproic acid, antidepressants antipsychotics and benzodiazepines), lithium was the only one that is related with a decreased risk of suicidal attempts, and a significant decline in suicide mortality (Toffol et al., 2015). Although some patients show remarkable improvement, others are refractory to treatment.

Lithium action mechanisms remain unclear, which is one of the biggest difficulties. In contrast to many other psychopharmacologic drugs, lithium does not bind to cell receptors. Instead, it performed its therapeutic effect by modifying intracellular second messengers downstream of metabolic neurotransmitter Systems via inhibition of the enzyme. The two major downstream targets of the inositol monophosphate (IMP) and glycogen-synthase-kinase-3 (GSK-3) may be components of a common mechanism across the whole range of biochemical actions of the medication. There are still many contrasting opinions, and explanations of these theories. Is it a singular downstream target that mediates the miraculous effects of the drug or is it the combined efforts of many kinases and other molecules? How can one drug ameliorate markedly distinct mania and depression?

Accumulated data indicates that Lithium is able to fight complicated BD molecular disease via the promotion of neuroproecive, antioxidant, anti-inflammatory, homeostatic protein, neurogenic and synaptic maintenance actions. Lithium's intricacy and method of action are linked with the

broad impacts of its known targets, particularly IMPase and GSK-3, which play critical roles in a variety of signalling pathways.

A greater knowledge of the processes of lithium may enable the creation of more bearable lithium-mimetic medicines that do not interact with objectives that encourage side effects - renal and thyroid dysfunction, arrhythmia, leucocytosis, neuromuscular effects. This would revolutionise the treatment of mental disease and clarify the biology of mood disorders (Cikankova et al., 2017).

1.3 Inositol depletion hypothesis and GSK-3

One of the earliest hypotheses which explained lithium action mechanisms was inositol depletion, which is the inactive and can be converted into inositol phosphates and inositol lipids. There are high levels of inositol in the brain, suggesting their critical role for normal functioning. Researchers suggested that lithium uncompetitive inhibits inositol monophosphates (IMPase) which limits recycling and metabolism of inositol, therefore, depleting free inositol which is required for phosphatidylinositol signalling pathways (Berridge, Downes, & Hanley, 1989). More precisely, due to blockage of IMPase, conversion of inositol-1-phosphate (IP1) to inositol is inhibited; PI synthase (PIS) becomes malfunctioned and cannot transform myoinositol into phosphatidylinositol, which triggers a reduction in synthesis of PIP2. This therefore limits PIP2 cleavage products - DAG and IP3, also the modulatory activity of PKC, which has significant effects on cell signalling (Machado-Vieira, Manji, & Zarate Jr, 2009). For instance, inositol signalling controls cell growth and proliferation, neuronal excitability, Ca2+ signalling, actin cytoskeleton remodelling, membrane dynamics, apoptosis, In individuals with BD, magnetic resonance spectroscopy (MRS) investigations reveal aberrant PI-cycle activity and increased myo-inositol concentrations. Higher levels of myo-inositol are also observed during manic episodes, contrasted by lower levels in depressive states (Yu & Greenberg, 2016).

The distinct kinase is widespread in neurons and glia where it is localised to cytoplasm, nuclei and mitochondria. It is one of the most active and influential kinases, with over 100 substrates in its possession. GSK-3 is considered constitutively active and exists in two isoforms - alpha and beta, which are paralogous proteins and share 84% sequence homology, the latter (GSK-3b) seems to be more prevalent and pivotal. Dysregulated activity of GSK-3 may be fundamental in

mood disorders, but there is little evidence. GSK-3 has a crucial role in cell survival, neurogenesis, and inflammation. Li+ can inhibit GSK-3 directly or indirectly. Directly by binding of Mg2+ which disrupts enzymes catalytic functions and indirectly, via PI3K mediated Akt activation which increases serine phosphorylation (Sato, 2021). The enzyme is best established in three pathways: first, the Wnt/b-catenin where activation determines cell connectivity, neurogenesis and neuroplasticity; second, the glycogen synthase pathway, where it has metabolic effects; and lastly, the neurotrophic signalling cascades - PI3K/Akt, that is mostly responsible for survival of neural and glial cells. GSK-3 is also phosporylated by mitogen activatetd protein kinases (MAPKs), PKC and PKA. GSK-3 substrates are b-catenin, CREB, other transcription factors, protein kinases and cytoskeletal protein. There are many downstream targets and upstream mediators of GSK-3, but not all of them are involved in BD pathophysiology or lithium mechanisms of action. This leads to a substantial interest in comprehending the role of this multimodal kinase as therapeutic targets in disease.

O'Brien and Klein have suggested four criteria that may be used to evaluate a putative direct lithium target in model systems

1. Therapeutic concentration of lithium inhibits the target in vivo and in vitro
2. Inhibitors of putative target mimic lithium action
3. Genetic evidence
4. Conversion of lithium effects by restoring lithium function or product

In this paper I will be comparing, evaluating and validating the two main downstream targets of LIthium - IMP and GSK-3, by applying the above criteria and discussing the mechanisms of action of the drug though the targets. I will attempt to link molecular mechanisms and behavioural effects of lithium action. Last, I will discuss future implications and comment on the significance of my work.

2. Literature Review

2.1 History of Treatment by using Lithium

In the late nineteenth and early twentieth century, water and lithium were used as medicated tablets by all medicines and health suppliers that were said to prevent or treat diseases induced due to uric acid. In 1948 lithium chloride was sold for low-sodium replacement table salt and resulted in numerous lithium toxicity reports, including fatal instances. After studying the calming effects of lithium in uric acid experiments conducted on guinea pigs and doing a self-administration safety evaluation of lithium, Australian psychiatrist John Cade reported that lithium citrate was beneficial in treating mania in hospitalised patients in 1949. In numerous individuals who were hospitalised for years Cade showed significant improvements and also that patients returned to mania when lithium was stopped. Cade's study has catalysed the current age of bipolar lithium treatment and is one of the first publications on rational therapy for severe mental illnesses (Cade, 1949). Longitudinal studies performed by Schou and colleagues in order to prevent the depressive episodes and recurrent manic have also shown the preventative benefits of lithium as mood stabilisers for treating bipolar disorder and depression (Schou, Juel-Nielsen, Strömgren, & Voldby, 1954). A basic test for plasma lithium levels was developed that has allowed for the establishment of a medicinal range for the element lithium. This has showed the limited therapeutic window of lithium. In 1970, in 1970, the US FDA authorised lithium to treat mania, and in 1974 approved it for the therapeutic maintenance (Shorter, 2009).

2.2 Direct Targets of Lithium

Direct lithium targets comprise of inositol monophosphatase and related phosphomonoesterases reliant on magnesium. These include fructose 1,6-bisphosphatase, inositol polyphosphate 1-phosphataseas well as bisphosphate nucleotidased in a consensus sequence of metal ions. While these three lithium target groups are structurally different, they all rely on magnesium and, in each instance; lithium with a magnesium-like ionic radius is in the competition with a magnesium binding site. Most magnesium-based enzymes, including almost all studied protein kinases, are not sensitive to lithium, however. Furthermore, it is interesting that the control of glucose or phosphoglucose levels is strongly related to all known targets of lithium. PGM isomerizes G6P to G1P; the gluconeogenesis of the FBPase; the G6P isomerizing IMP; and the

incorporation of glucose into glycogen is suppressed by GSK-3. These enzymes are sensitive to lithium in species from protozoans to humans (Phiel & Klein, 2001).

2.3 GSK-3 Function

GSK-3 has been initially classified as phosphorylate synthase among several protein kinases but many other direct objectives for GSK-3 have since been identified, including the protein β-catenin, Wnt effector and other transcription factors, translation regulators, multiple RNA splicing factors, cytoskeletal proteins and protein kinases. Targeted phosphorylation GSK-3 often affects the activity or stability of its objectives, including β-catenin and glycogen synthase, although GSK-3 may also stabilise substrate, including the reverbα nuclear hormone receptor, enhancing the function of goals including the 2-protein tuber sclerosis complex.

GSK-3 multi-site phosphorylation established a paradigm for a number of GSK-3 substrates, and GSK-3 relies on those substrates as well as phosphorylation. This phosphorylation "priming" increases the GSK-3 concentration on the substrates and phosphorylation of a serine residues at the N end of the priming region. GSK-3 substrates like GS and β-catenin often include numerous series or threonine that are divided into 4 residues, allowing for process phosphorylation in a C to N direction of the terminal. In GSK-3 itself, Phosphorylation of N terminal series produces a pseudo-substrate that inhibits the activity of primed substrate. However, several substrates of GSK-3, including Tau protein and inhibitor-2 (I-2), do not possess +4 priming sites and, in these instances, the methods used for substrate identification are less characteristic (Alon et al., 2011).

2.4 GSK-3 and Bipolar Disease

GSK-3 phosphorylates a wide range of substrates in a variety of essential cell activities, in addition to their conventional role in glycogen metabolism, as previously reported. The aetiology of human illness is clearly implicated in several of these processes, including autophagy, in the survival of cell and the regulatory cell cycles, to name a few examples. In order to better understand the function of GSK-3 as a medicinal target for these patterns, there has been a considerable increase in attention in this area (Doble & Woodgett, 2003).

The bipolar disorder is one of the main mental disorders with hyperactive episodes, high mood, and psychotic conditions (mania and hypomania), high depression periods, and a significant risk

of suicide. When it comes to bipolar disorder, Type I is characterised when one maniac episode is considered at, whereas Type II is characterised by many episodes of hypomania as well as severe depression episodes. Bipolar disorder is believed to affect 1-2 percent of the world's population worldwide. Despite the fact that lithium is considered as the first and foremost treatment for bipolar disorder, there is a small margin between the beneficial and hazardous lithium doses. Lithium is also used as a mood stabiliser, and it has been shown to reduce the risk of suicide in those suffering from affective disorders, either via immunotherapy or through supplementary treatment. Lithium is more efficient than other mood stabilisers and antidepressants in preventing suicide, but the reason is unclear. Lithium also offers excellent adjunctive therapy in conjunction with tricyclic antidepressants for treatment resistant depression (Philip, Carpenter, Tyrka, & Price, 2010).

Haploid Gsk3b Haploid replicates lithium therapy in lithium-responsive mouse behavioural tests, whereas CNS Gsk3b Haploid over Expression blunts lithium responsiveness without compromising the health or activity of the animal, indicating a particular function for lithium reactivity for GSK-3. In addition, several small molecular GSK-3 inhibitors similarly imitate the behavioural effects of lithium in mouse, which provide strong evidence for GSK-3 as a key lithium in targeting the behaviour of rat (Shaldubina et al., 2006).

GSK-3 remains a potential target of mood stabilisers, for this extra cells transduced by Akt lead to phosphorylation and GSK-3 inactivation. However, GSK-3 is opposed by at least two pathways to its own phosphorylation. (1) GSK-3 suppresses Akt by joining a β-arrestin complex and stabilising it, which leads to Akt's deactivation phosphatase PP2A. This avoids phosphorylation and GSK-3 inhibition by inactivating Act (Beaulieu et al., 2005).

3. Inositol depletion hypothesis

3.1 Inositol metabolism

Three methods are used to get inositol from eukaryotic cells. Inositol is taken by inositol carriers from the environment. When exogenous inositol is absent, glucose-6-phosphate (G6P) de novo is produced in a two-stage process. G6P is initially transformed to ISYNA1 and INO1 by myo-inositol-3-phosphate synthase (MIPS. The next step involves the conversion of inositol-3-phosphate by the enzyme IMPase into inositol. It is also possible to get inositol via recycling inositol phosphates. It is believed that high inositol levels in the brain are necessary for optimal brain function since brain inositol levels are more than blood and other tissues. Although inositol may be consumed by brain cells from the blood, the blood-brain barrier slows the absorption, indicating that the recycling process to get inositol phosphates is the considered as a way of obtaining inositol in the brain.

Inositol is a key phosphatidylinositol (PI) synthesis substratum from which phosphatidylinositol phosphates are generated. Alternatively, inositol phosphate kinase may be phosphorylated to produce IP4, IP5 and IP6 consecutively. These molecules transmit signals for many physiological activities, although inositol phosphate roles are not completely known. Inositol phosphates may be further phosphorylated to produce pyrophosphate in existing groups of phosphates, the activities of which include vesicular tracking, gene expression control, and DNA repair. There are many molecules that include inositol that act as metabolic sensors, which can control the neurotransmission and neuronal activities (Parthasarathy et al., 2006). For example, IP3 is a second transducer that stimulates the amount of calcium released from the cell's store. Calcium signalling controls the development of neurons, apoptosis and exocytosis. Many central nervous system receptors trigger PLC-determined PIP2 cleavage and enhance the release of IP3/calcium. Intracellular metabolism of inositol was disturbed by BD, Alzheimer's disease, diabetes, and cancer. The maintenance of steady inositol homeostasis is thus essential for proper cell function (Shi, Azab, Thompson, & Greenberg, 2006).

3.2 Altered levels of inositol in BD

There was a connection between BD and changed inositol levels in the brain. In live BD patients' brains, modified myo-inositol and phosphoinositide levels were detected using magnetic resonance spectroscopy. BD patients' brains showed increased myo-inositol signals during the manic phase, according to the findings of the study. Myo-inositol levels were significantly lower in the frontal cortex of the affected person with bipolar disorders in between their depressive period than in healthy controls. In post-mortem BD patients, frontal brain samples revealed lower amounts of myo-inositol than in healthy controls. Moreover, the levels of myo-inositol in the cerebrospinal fluid of those suffering from affective depression have been shown to be lower. It's worth noting that dietary inositol supplementation has been shown to be helpful during treating depression. In addition, in animal models, inositol alleviated depression. According to these researches, abnormal levels of inositol in the brain could play a huge role in the when we talk about how mood disorders are developed (Chengappa et al., 2000).

3.3 Lithium & VPA limit inositol synthesis

Although lithium has been utilised for BD therapy for more than 60 years, the therapeutic mechanism of the medicinal product is still unclear. Similarly, the VPA effectiveness mechanism is poorly known. Lithium has been shown to be an IMPase non-competitive inhibitor that catalyses the conversion to myo-inositol of inositol-3-phosphate. It was via these pivotal studies that the idea of inositol depletion as a medicinal procedure for lithium action was initially introduced. According to the findings of animal models, the mood-stabilizing effect of lithium is related to the reduction of inositol synthesis, providing more support for this hypothesis. The presence of lithium in the brain of rats resulted in lower levels of myo-inositol (Eickolt et al., 2005).

Inositol levels in the cerebral cortex of rat are dropped by 30% after 6 hours of injecting lithium and inositol decline continued for 24 hours. Furthermore, VPA and lithium therapy resulted to an intracellular IP3 concentration reduction. The decrease of lithium-induced inositol reduced PIP3. Inositol-deficient diet increased lithium effects in behavioural tests. These findings support the notion that medicines that stabilise the mood decrease PI via inositol metabolism. Depletion of inositol also impairs other cellular processes linked to mental disease. In yeast, VPA treatment

and inositol depletion have altered homeostasis and disrupted vascular ATPase activity; although relevant research have not yet been performed in human cells, such activities could be critical for the transmission of neural drugs and must be addressed. Exogenous inositol pre-treatment may have an inhibitory effect on the formation of synaptic connections between neurons in the hippocampus when combined with VPA and lithium. Inositol shortage have led to impaired craniofacial processing and functioning of brain in the model designed for rats, according to the researchers (Teo et al., 2009).

To clarify drug-related processes of inositol depletion it is obviously important to understand how inositol production is controlled. Surprisingly, inositol production regulation has not been extensively explored in mammalian cells. In contrast, the yeast Saccharomyces cerevisiae has been thoroughly described by inositol production. Lithium and VPA have been found to suppress the production of inositol in this yeast. Lithium lowers the quantity of intracellular inositol in the cells of yeast, much as it does in the cells of human, via blocking the enzyme IMPase. It is interesting to note that VPA interfered with the metabolism of inositol in the yeast. VPA depletes inositol via a process that is distinct from the one used for lithium. When it was initially discovered by Vaden et al. that VPA could lower the concentrations of inositol and intracellular inositol-3-phosphate in the cells of yeast; they concluded that this was due to MIPS inhibition, which is the catalyst for the inositol-3-phosphate production of G6P. However, they later discovered that this was due to MIPS inhibition, not MIPS inhibition. In fact, it has been shown that VPA may results to decrease 35% activity rate of the MIPS enzyme when studied in vivo that is administered clinically through calculated doses of the drug. Following studies, it was discovered that MIPS in yeast cells as well as in human cells is inhibited by VPA. To the contrary, in contrast to lithium's direct inhibition of the enzyme IMPase, the activity of MIPS is suppressed indirectly and not in vitro. It has also been discovered that VPA MIPS may be inhibited indirectly in the human brain. Chronic therapy with VPA, consistent with inositol deprivation, substantially have reduced PI production and have raised yeast CDP-DAG concentrations (Shaltiel et al., 2004), (Ju & Greenberg, 2003).

Phosphorylation of MIPs has affected the activity of both the human and yeast enzymes. Yeast MIPS has three phosphorylation sites that match to human MIPS sites Ser-177, Ser-357, and Ser-279. This phosphorylation in MIPS sites was classified and linked to the human MIPS residues

Ser-184, Ser-374, and Ser-296. It has been discovered that VPA increases the rate of phosphorylation within MIPS sites of yeast cells. The combination mutation of Ser-374 and Ser-184 into Ala has enhanced the activity of MIPS enzymes by fourfold while simultaneously decreasing the susceptibility of cells to VPA. The PKA, GSK3, AKT, and PKC signalling pathways are all impacted by VPA, despite the fact that it suppresses MIPS in an indirect manner. As a consequence, it is possible that MIPS inhibition by VPA will have an indirect effect on these kinases (Deranieh, He, Caruso, & Greenberg, 2013).

Animal studies have also shown VPA-mediated disturbances of inositol metabolism. Similar levels of inositol depletion were caused by VPA and lithium in rat brain. Acute VPA therapy decreased mouse brain inositol levels. Inositol inhibited VPA and lithium's inhibitory effects on the fall of sensory neuronal cones and the development of cones in cells of the rat ganglia. These findings show that depletion of inositol is a frequent result of structurally diverse antibiotic medicines.

3.4 Depletion of Inositol and PKC

Inositol has an impact on the BD-related PKC, lithium and VPA targets. PKC is classified as a serine/threonine kinases family element that is present in tissues and organs of mammals. It is significantly concentrated in the areas of brain, which impacts on a range of physiological processes, like the proliferation of cells, neurotransmission secretion, and extracellular receptor location throughout the body. DAG, the signalling molecule produced by the PIP2 breakdown is activated by a various procedure of PKC isoforms. A wide range of research has combined PKC with aetiology and BD therapy. The translocation of serotonin-induced PKC to platelets from persons with bipolar disorder was changed during the period of mania. The ratio between total PKC activity and membrane bound PKC was higher in BD patients when compared with respect to healthy person. When PKC levels and activity were examined after a brain post-Mortem BD technique, changes were found. In an association-wide genome analysis, triacylglycerol kinase H (DGKH) was also discovered as a risk gene for BD aetiology and is now being investigated further. DGKH is responsible for transforming DAG into metabolism of phosphatide acid. DAG is a cofactor for several PKC isoforms and DGKH is intended to inhibit PKC, since DAG is required to work. A post mortem brain tissue research showed that DGKH expression in pre-frontal BD patients' body cortex has risen; suggesting that altered DGKH expression may be

associated with illness genesis. The results indicates that a disruption of the PKC way of transmission is strongly linked with the development of BD (Moya, Murphy, McMahon, & Wendland, 2010)..

VPA and Lithium are used for the purpose of decreasing PKC levels, which is noteworthy to note. PKC-associated PKC was less in the rat hippocampus membrane after continuous lithium therapy. In hippocampal samples from lithium-treated rats, PKC″ and PKC″ levels were decreased up to myo-inositol administration in combination with lithium therapy. Chronic VPA therapy also reduced the PKC and PKC levels in rat cells. Tamoxifen, a powerful PKC inhibitor used extensively for breast cancer, has been proven to be helpful in decreasing manic symptoms but has been unsuccessful in treating BD patients during the depression stage of the illness. In short, the interruption of PKC activity contributes significantly to the development of BD. The down-regulation of lithium and VPA is supposed to lead to an inositol depletion, with therapeutic consequences that may change the downstream signalling pathways (DiazGranados & Zarate, 2008).

3.5 Differences between the inositol depletion hypothesis and evidences

Some studies have found no evidence to support the notion of inositol depletion. First and foremost, lowering inositol levels in the mouse did not result in improved mood stability. The quantity of inositol in brain tissue decreased by 33–37 percent in mice heterozygote for the SMIT1 null allel, a greater decrease than the 22–25 percent drop seen in the brain of mouse that was treated by lithium, according to the findings. After being subjected to a forced swim test, only the lithium-treated mouse, not the SMIT+/- animal, demonstrated a significant decrease in immobility. PI is not decreased as a result of inositol depletion, which is a second argument against the notion of inositol depletion, according to this argument (Shaldubina et al., 2006)..

The levels of phosphatidylinositol in the embryonic brain have been compared to those of SMIT and WT. Despite the fact that the SMIT/- mouse had a decrease of 92 percent in intracellular levels of inositol, and no change was detected in phosphatidylinositol levels between the two strains. But the remaining 8 percent of the WT inositol levels in SMIT/- mice are sufficient to maintain normal PI signalling. It is still not verified how reduction in inositol levels may be used to effectively treat mania and depressive symptoms. In their research, Cheng et al. proposed a process through which VPA and other mood stabilisers might act in two ways while yet maintaining constant PI signalling. In manic patients, inositol depletion may cause PI signals to

be reduced, while prolyl oligopeptidase inhibition induced by VPA may cause PI signals to be increased in depressed patients. In summary, although many of the typical side effects of anti-bipolar medications may be explained by inositol depletion, other research do not support this theory (Cheng, Lumb, Polgár, & Mudge, 2005), (Liang, Wendland, & Chuang, 2008).

4. GSK3 inhibition hypothesis

4.1 Lithium inhibits the activation of GSK3

Bipolar disorder (BD) is a specific disease, with mania and depression along with polar opposite mood symptoms of the same patient. Lithium is an alkaline metal used extensively for BD therapy. However, why lithium can regulate mood is mainly unclear. Lithium inhibit kinase 3β (GSK3 β) glycogen synthase. The active GSK3 β reduces neuronal activity both for the glutamatergic system and in the GABAergic system, while slowing the injection of GSK3 β enhances excitability of neuronal, indicating that activating GSK3 β that could cause depressed mood and the similarly decreasing the injection of GSK3 β could cause manic mood. Many kinases and phosphatases govern the activities of GSK3β. Thus, these complex regulatory mechanisms may swing GSK3β, the swing of neuronal excitability, the swing of GSK3β activity and ultimately the intrinsic mood swing, typically seen in healthy human beings. The magnitude of the mood swing is believed to be increased by several variables. Lithium may reduce the magnitude of the GSK3β swing depending on the dosage. Lithium also inhibits the activation of K+ channels, leading to an extension of the refractory period, which decreases neuronal excitability. In depressed humour, lithium can therefore increase neuronal activity by inhibiting neural activity GSK3beta, and lithium in manic mood could be controlled by the inhibition of neuronal excitability through inhibiting the activation of K+ channels, thereby reducing mood swing amplitude, e.g. reducing depression and manic mood and thus normalisation of the mood swing.

Klein and Melton revealed in 1996 that lithium is a GSK3β inhibitor in Xenopus. Further investigations revealed that in Drosophila lithium blocked the GSK3β, cultivated mammalian

cells, and rat brains. When lithium competes with the magnesium cofactor for enzyme binding, it may promote inhibitory serine phosphorylation. Lithium may inhibit GSK3 indirectly via a variety of pathways, including PI3K/AKT, PKA, PKC, and GSK3 self-regulation.. This has led to the idea that the therapeutic mechanism of mood stabilising medications may involve GSK3 inhibition (Costemale-Lacoste, Guilloux, & Gaillard, 2016).

GSK3 was originally discovered as protein kinase which inactivates glycogen synthase and phosphorylates. GSK3, a serine/threonine kinase, regulates numerous cell processes that influence the determination of cell destiny, cell survival and signal transmission. GSK3 has two isoforms in mammalian cells: GSK3a and GSK3b. GSK3a and GSK3b are the two isoforms of GSK3. The sequences of amino acid of the kinase domains of these two isoforms are almost identical (98 percent similar). It is the PI3K/AKT pathway that stabilizes the GSK3 activity, since it suppresses the phosphorylation of GSK3 on serine-21 and serine-9, which are found on the amino acid serine-21. A total of approximately 40 proteins are controlled by GSK3, the most prevalent version of the protein in the brain, in different cell signals. Some of these proteins are essential in the development of bipolar disorder as well as cancer and Alzheimer's disease.

Researchers investigated the relationship between intracellular GSK3 reduction and behavioural effectiveness in order to determine if GSK3 inhibition could be responsible for the medicinal effects of stabilizing the mode. Mice are cured with chronic lithium exhibited much reduced immobility during the forced bathing test, indicating a beneficial antidepressant impact on the mice. Interestingly, animals with heterozygous deletion of GSK3, which resulted in a significant reduction in GSK3 concentrations that were equivalent to those seen in mice treated with lithium. The observed behavioural changes in lithium-processed and GSK3+/- mice have been restored by overexpression of GSK3, demonstrating that GSK3 is a potential lithium target. Several GSK3 inhibitors were shown to have mood-stabilizing effects, which is consistent with the idea that GSK3 inhibition is a therapeutic strategy. The forced swimming test revealed that rats treated with AR-A014418 and L803-mts GSK3 inhibitors were less immobile than those who were not. GSK3 inhibitors, in addition to their antidepressant effects, were shown to substantially reduce manic behaviour in animal studies as well. A range of GSK3 inhibitors decreased manic mice hyperactivity. Mice with heterozygous GSK3β deletion also showed

reduced amphetamine-induced hyperactivity. These results indicate that decreased GSK3 activity helps manic behaviour in animal models to be alleviated (Takahashi-Yanaga, 2013).

4.2 VPA inhibition of GSK3

Some, but not all, investigations have demonstrated VPA to inhibit GSK3β. Chen et al. showed that in vivo and in vitro tests, VPA inhibit GSK3. The activity of GSK3 and GSK3a, as measured in vitro using 32P in CREB phosphopeptides, was shown to be decreased in a manner limited by the concentration- in the presence of VPA. These findings support the use of VPA as a GSK3ß inhibitor. GSK3 human phosphorylation of tau protein was decreased in a concentration-dependent manner in the presence of VPA, in a manner similar to that seen with VPA. Furthermore, in brain cells, VPA treatment decreased MAP1B phosphorylation by in vivo GSK3, which was previously observed. In contrast to Chen et alfindings, .'s VPA in Hall et alstudy .'s did not inhibit GSK3 in vitro, suggesting that VPA's reduction of GSK3 may be an indirect effect.. Further evidence for VPA's inhibitory impact on GSKβ was the determination of an increased amount of GSK3β serine-9 phosphorylation in human neuroblastoma cells treated with VPA. Furthermore, like lithium, VPA has behavioural effects due to disruption of the signalling pathway AKT/GSK3.

Although the aforementioned data indicate that VPA suppresses GSK3β, these results are not generally accepted. In addition, higher β-catenin levels were found in lithium-treated, non-VPA, dorsal root ganglia cells. In additional investigations, similar findings were found. The range of models and methods employed in these studies probably contributes to the differences in findings and more research is required to understand the impact of VPA on GSK3 (Feng et al., 2008).

4.3 GSK3 activity and HDAC inhibition

HDAC was found to suppress therapeutic amounts of VPA. Interestingly, HDAC inhibitors have antidepressant effects in animal depression models. HDAC inhibition modifies the transcription profile significantly and changes cellular signalling, which may possibly take account of VPA therapeutic benefits. For instance, long-term HDAC Cpd-60 inhibitor therapy has substantially enhanced the expression of SGK1 in the mouse brain. In rats treated with lithium and other antidepressants, Upregulation of SGK1 has also been observed. SGK1 encodes and inhibits GSK3β, a protein kinase that phosphorylates. As previously stated, the GSK3 activity is adversely controlled by the AKT signalling route. VPA enhanced AKT phosphorylation related to activation and GSK3β phosphorylation associated with inhibition. The impact of VPA in activating AKT and GSK3β has also been mimicked by two additional HDAC-inhibitors, TSA and sodium butyrate, indicating that the inhibition of HDAC increases GSK3β inhibitions through means of the AKT route. Although the disagreement remains whether VPA is affecting GSK3 directly, such results indicate that VPA is indirectly reducing GSK3β via inhibition of HDAC, which may be a basis for the drug's antidepressant effects (Van den Hove et al., 2013).

4.4 GSK3 inhibition and its neurotropic effects

Numerous studies have shown neuronal and glial cell death in BD brains as a result of increased apoptosis. The GSK3 enzyme is a proapoptotic enzyme, which means that when it is activated, apoptosis is made simpler. GSK3 inhibition may, as a result, exert its neurotrophic and antibacterial effects in the brain via decreasing the apoptosis of the brain's neurons. G-CSF is a factor in the growth of neuroprotective that counteracts apoptosis by controlling the enzyme GSK3 in the body. Further evidence was discovered to support the connection between apoptosis and modified GSK3 activity, and that a variety of selective GSK3 inhibitors, antibiotics, including lithium and VPA, were also important protective agents against apoptotic death of cells, according to the findings. VPA has an anti-apoptotic effect on B-cell lymphoma 2 in a mouse model, which is shown by up-regulating the expression of this gene (Bcl-2). In human brain cells, VPA and lithium increased the amount of Bcl-2 that was present intracellularly. In addition, VPA inhibited Bcl-2 ubiquitination in human endothelial cells, resulting in a reduction in apoptosis. Furthermore, lithium stimulated the proliferation of brain precursor cells by activating the GSK-3-NF-AT signalling pathway. The inhibition of GSK3 has been found to

have neuroprotective effects against acute toxicity. Because of GSK3 inhibition, our result indicates that lithium and VPA may be able to show their medicinal effects via promoting proliferation and neuronal cell survival.

Moreover by applying anti-apoptotic signalling, the control of GSK3 results in the stimulation of the Wnt transmission path and the overexpression of -catenin. In its role as a transcription factor, beta-catenin is an important component in the control of neural connections, and it is involved in a variety of neuronal functions. A potential BD treatment strategy, according to some evidence, is to increase intracellular -catenin levels in the cells. In the hippocampus of the mouse, L803-mts, a selective antidepressant GSK3 inhibitor, increased the expression of -catenin by a significant amount. Overexpression of -catenin in the mouse brain, as well as lithium treatment, resulted in behavioural alterations that were similar, including decreased immobility during the forced swim test. In addition, overexpression of -catenin decreased hyperlocomotion induced by amphetamine, which mimicked the anti-manic effect of lithium in the laboratory. In a recent study, using BD patient-derived pluripotent stem cell lines, researchers discovered abnormal neurogenesis and gene expression in the absence of Wnt signalling. Increased GSK3 expression in BD-induced pluripotent stem cells has been shown to improve the proliferation of these cells. The upregulation of -catenin may demonstrate the therapeutic efficacy of GSK3 inhibition in the treatment of BD when used together. The use of Wnt/-catenin as a BD treatment target may be a feasible option.

In short, GSK3 could play an important role in the development of bipolar disorder treatment therapies. A decrease in GSK3 activity and a rise in protein levels have been seen in BD patients. Animal behaviour may be mimicked by reducing GSK3 levels via genetic ablation or inhibitor treatment, which is similar to the mood-stabilizing effect of antibiotics. Furthermore, many studies have shown that VPA leads to the inhibition of the activity of the GSK3 enzyme. In conclusion, our results suggest that GSK3 inhibition is a frequent side effect of a wide range of structurally different mood stabilising medications, on a par with inositol depletion (Harwood & Agam, 2003).

5. Inositol depletion and GSK3 inhibition model

Lithium inhibits GSK3 in vivo and alternate GSK3 inhibitors replicate many of the lithium's behavioural effects. Both the AR-A014418 and the L803mts peptide inhibitor, a GSK3 inhibitor for ATP which penetrates the blood brain barrier, injecting into the ventricles, decrease immobility in a forced swimming test. The TDZD-8 thiadiazolidinone decreases the tail suspension test, resistance to associated behaviour, and reduces latency from dark to light. Lithium diminishes amphetamin-induced hyperactivity and is thought to be related to enhanced dopamine signals in dopamine transporting knockout mice (DAT-KO) In addition, lithium and a number of inhibitors of GSK3, including SB216763, alsterpaullone, 6-bromo-5′-indirubin-3′-oxime (6BIO), and TDZD, lower hyperactivity in DAT KO mice. Open field operations are similarly reduced in wild-type mice by IP injection of lithium (like LiCl), TDZD, or AR-A014418. These findings may indicate that the open field is often an indicator of overall animal status. However, we prefer an alternate interpretation that IP injection produces greater lithium or other inhibitors peaks and that decreased activity is a particular result of the more powerful GSK3 inhibition. Our conclusion is that, although neither oral lithium nor Gsk3ß haploinsufficiency alone changes the open field activities, Gsk3ß+/− oral lithium therapy reduces the open field activity of the mouse. In addition, overexpression of a Gsk3-resistant phosphorylation mutant enhances the locomotive activity, providing a model for manic hyperactivity.

To provide genetic data supporting GSK3 as the goal of lithium, we investigated whether the deletion of the Gsk3ß gene in mice impacts lithium-like behaviour and, therefore, our behavioural experiments with Gsk3ß KO mice were conducted on animals crossed back into a specified strain. Mice die during pregnancy, but the heterozygotes are feasible and seem to be developing properly, despite a 50 per cent decrease in brain, hippocampus, hypothalamus and cerebellum levels of the GSK3ß protein. Similar to the lithium-treated FST, TST, amphetamine-induced hyperactivity, exploratory behaviour and increased null labyrinth, Gsk3ß+/− mice behave. In addition, production in the brain of a ß-catenin mutant lacking at regions of GSK3 phosphorylation simulates FST lithium effects, and hyperactivity with an amphetamine effect, although the conditional loss of ß-catenin has so far only been somewhat affected by lithium-

sensitive behaviour. Finally, early evidence, published elsewhere in detail, shows that the effects from lithium on the FST, exploratory behaviour and a high zero labyrinth are reversed by Gsk3ß overexpression in the brain, strongly indicating that GSK3 is the particular goal of lithium in these activities.

5.1 Lithium-induced behavioural depletion of inositol

Chronic lithium also decreases mouse brain inositol by 10-25%. If this partial decrease of inositol may explain for the behavioural alterations seen, an alternate route to inositol reduction in the intelligence would be very useful, but presently, no IMPase inhibitors are available that pass the blood brain barrier. However, the homozygous knock-out of the mouse sodium myo-inositol transporter (SMIT1) gene lowers foetal brain inositol levels by >90% and has no global PI impact, suggesting that the small drop seen with lithium is likely to limit PI production. Accordingly, lithium was never proven to decrease PI or PIP2 in vivo. However, the decrease of PI/PIP2 may be confined to particular regions in the brain which global monitoring of PI would not reveal. Therefore, behaviours in SMIT1 KO mice are still worth testing. Even if SMIT1−/− mice die during gestation, heterozygotes SMIT1+/− are viable. Inositol levels in adult SMIT1+/− mice are decreased by 33-37 percent.

There are no effects on forced-swimming tests, an amphetamine-induced hyperactivity, or the susceptibility to pilocarpine-induced seizures, which show that global inositol depletion is not enough to produce lithium sensitive behaviour, to a degree higher than that seen with lithium. However, it is not apparent, in these studies, that SMIT1 is expressed in neurons and that inositol is particularly decreased in SMIT1 mutant mouse neurons.

More recent studies have shown that homozygous SMIT1 KO may be brought up to adult years by the addition of inositol to the moms. When treatment with inositol is stopped, individuals exhibit a 60% decrease of brain inositol. Under those conditions of greater inositol depletion, animals have shown lower immobilisation in FSTs and increased sensitivity towards pilocarpine induced seizures, in parallel with the effects of lithium; it has not been reported how this mutation affects the animals' state in open-field for example. Moreover, lithium therapy does not reach this degree of inositol depletion, thus it will require to further investigate the significance of these findings for lithium action.

Mouse knockouts were reported for both IMPase genes, IMPA1 and IMPA2. Homozygous IMPA1 knockdown is deadly but may be restored by inositol supplement from pregnant mothers. While the inositol content in the adult brain does not change, IMPase activity is decreased by up to 65%. Thus, although this mouse strain is not a model for global inositol depletion, localised inositol reductions may occur which can be difficult to detect but nonetheless trigger IMPase lithium-mediated inhibition. These mices exhibited increased swimming activity and lithium-like sensitivity to pilocarpine in the FST. The deletion of IMPA1 also causes significant open-field hyperactivity and the baseline hyperactivity of IMPA1−/− mices is a major perplexing feature of this mutant line's FST interpretation as the lithium improves swimming activity in FST. The IMPA2 deletion does not emulate lithium-sensitive behaviour and does not reduce inositol and IMPase brain activity, which means IMP redundancy.

6. Conclusion:

The research on lithium action in various modelling set-ups has given rise to a variety of methods to testing putative lithium action targets. In this study, we focused on direct molecular objectives and evaluated the different methods to validating these enzymes in specific biological settings as potential lithium targets. Evidence supports GSK3 as an essential lithium objective, which will most probably affect the cell fates and patterns of the organisms in the development of dictyostelium, sea urchins, xenopus, and zebrafish, by activation of the Wnt signal pathway as well as inhibition of GSK3 in metazoans. On the other hand, inositol phosphatase suppression is seen in the lithium transmission of invertebrates seen in vivo. The impact of lithium as well as other BPD on the growth of cultivated neurons are not clear with both the inositol depletion and GSK 3 inhibition; the hypothesis established to make same observations is that the GSK3 inositol-synthesis is regulated through GSK-3, supported by the yeast data, but not yet mammalian tests Finally, despite the fact that IMPA1 and SMIT1 have shown some similarities in behaviour to lithium therapy, we could believe that the pharmaceutical and genetic information includes the effects of variousGSC3 inhibitors and the Gsk3ß+/knockout excludes the possibility of inositol reduction in the brain. Hence the depletion of inositol and inhibition of GSK3 are supported by many research as the results of antibiotic medicines.

7. Future implications

The findings provided in this study demonstrate that the depletion of inositol as well as the inhibition of GSK3 is common side effects of lithium therapy and VPA, respectively. The revealing that GSK3 is needed for optimum inositol synthesis as well as identifying possible GSK3 phosphorylation sites for the inositol enzyme of MIPS suggests that the suppression of GSK3 may be caused by the inositol shortage in the body caused by VPA. A biochemical connection is shown in a single unified theory between the depletion of medication induced inositol and GSK3 inhibition. It is still unknown how these biological findings affect one's mood. The depletion of inositol and GSK3 inhibition have an impact on a variety of downstream pathways, including the previously described HDAC and PKC inhibition, as well as anti-apoptotic factor expression, PI synthesis, and -catenin regulation, among others. In order to develop feasible BD therapy therapies for the future, it is essential to find medically relevant areas of target for the depletion of inositol and inhibition of GSK3.

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