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Phosphoinositide metabolism, lithium and manic depressive illness

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Abstract. Physiology underlying manic depressive illness and treating effects of its most commonly used remedy – "lithium" is yet to be elucidated. Recent years of psychopharmacology research witnessed sparkling developments in our understanding of the mechanisms underlying lithium's mood stabilizing effects. Recent data on molecular biology and *in vivo* magnetic resonance spectroscopy suggest that some of the initial actions of lithium may occur through the inhibition of the enzyme inositol monophosphatase (IMPase) and reduction of *myo*-inositol, which in turn initiate a cascade of events at different levels of signal transduction process and gene expression in brain; such as the effects on protein kinase C, myristoylated alenine rich C kinase substrate protein, glycogen synthase kinase 3β, B cell lymphoma-2 protein, and activator protein-I. It is likely that the enzyme IMPase other that being the key point in initiating lithium's therapeutic effects, may also play a critical role in the physiology underlying manic depressive illness.

Keywords: Lithium, phosphoinositide system, manic depressive illness

1. Introduction

Bipolar disorder is a chronic, disabling mental illness that affects at least 1–2% (in recent studies it has been reported as high as 5.5 to 8.3 %) of the adult population and is associated with a substantial risk of suicide among those affected [1,8,10,45]. Since its discovery in 1949, lithium has become the most commonly and successfully used treatment for bipolar disorder [5,26]. Despite the important impact that lithium has made on the lives of millions affected by this illness, the cellular and molecular basis for its mood stabilizing effects has yet to be elucidated.

The most widely accepted hypothetical mechanism of action for lithium proposes that when lithium inhibits the enzyme inositol monophosphatase (IMPase); phosphoinositides and second messengers derived from their hydrolysis are depleted due to inositol deficiency [3,4]. However, *in vivo* data on lithium's inhibitory effect on the enzyme IMPase is limited and inconsistent [19,30,32,40,41]. Moreover, in recent years the validity of "inositol depletion hypothesis" has been called into question with some remarkable criticisms [2,16]. In this article, first we summarized available data on the lithium's effect on the enzyme IMPase *in vivo*. Second, we discussed the validity of the "inositol depletion" hypothesis in the light of recent criticisms. Third, we provided a brief summary of the recent research data on the molecular mechanisms underlying therapeutic effects of lithium with their relevance to the lithium-induced reductions in *myo*-inositol levels. Finally, we made an evidence based discussion on the enzyme IMPase as a potential cornerstone in the pathophysiology of manic depressive illness.

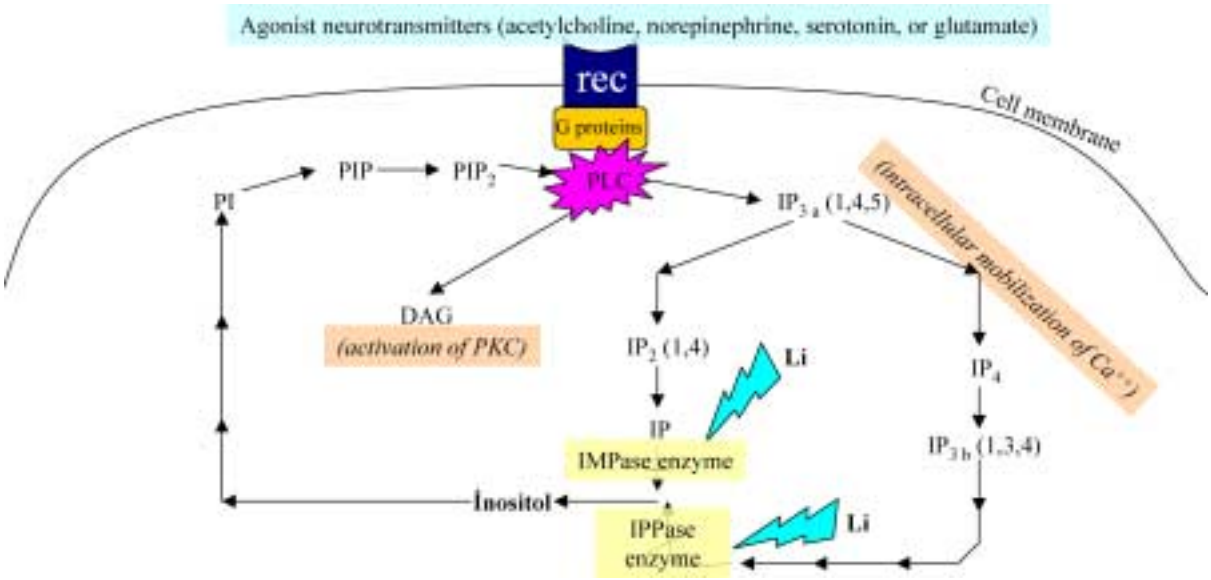

Fig. 1. Effect of lithium on phosphoinositide cycle. Abbreviations: PLC, phospholipase C; PIP_2, phosphatidylinositol-4,5-biphosphate; IP_{3a}, inositol-1,4,5-triphosphate; DAG, diacylglycerol; IP_4, inositol-1,3,4,5-tetraphosphate; IP_{3b}, inositol-1,3,4-triphosphate; IP_2, inositol-1,4-biphosphate; IP, inositol-1-monophosphate; PI, fosfatidylinositol; PIP, fosfatidyinositolphosphate, IMPase enzyme, inositol monophosphatase enzyme; IPPase enzyme, inositol poliphophate-1-phophatase enzyme. Adapted from [48].

2. *In vivo* data on the lithium's inhibitory effect on the enzyme IMPase

Neurotransmitters such as acetylcholine, norepinephrine, serotonin, and glutamate bind to specific cell surface receptors, which interact with G proteins [26]. These proteins stimulate the membrane enzyme, phospholipase C (PLC) [26]. Activated PLC catalyzes the conversion of phosphatidylinositol-4,5-biphosphate (PIP_2) to the second messengers, inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) [26]. IP_3 stimulates the mobilization of intracellular Ca^{++}, while DAG activates protein kinase-C (PKC) [26]. IP_3 can be phosphorylated or dephosphorylated, leading to other inositol phosphate compounds or to unphosphorylated inositol. Inositol may be converted to phoshatidylinositol (PI), which in turn is phosphorylated to phosphatidylinositolphosphate (PIP) and PIP_2, and recycled (Fig. 1) [26].

Since, brain *myo*-inositol is derived primarily from recycling of inositol phosphates, sufficient supplies of *myo*-inositol is crucial to a cell for the maintenance and efficacy of signaling [26]. Lithium, at therapeutically relevant concentrations, is an uncompetitive inhibitor of IMPase ($K_i = 0.8$ mM), and lithium administration is assumed to result in an accumulation of inositol-1-monophosphate (I1P), as well as a reduction in free inositol [26,37]. Lithium also inhibits inositol polyphosphate-1-phosphatase (IPP), which is involved in recycling inositol phosphates to inositol (Fig. 1) [26,37]. Thus, it has been hypothesized that a physiologic consequence of lithium's action is derived through a depletion of free *myo*-inositol in the brain [26].

However, data relating effects of lithium on receptor mediated phosphoinositide responses are inconsistent and are limited by considerable methodological variability. Therefore, many questions remain regarding the experimental basis of the "inositol depletion" hypothesis, and its applicability to *in vivo* response to lithium administration [16]. Considering the lack of animal models of bipolar illness, species selectivity of the pharmacological effects of psychotrophic drugs [16,38], and lithium's unique effects

on the brain, Magnetic Resonance Spectroscopy (MRS) offers a valuable tool to investigate the validity of the "inositol depletion" hypothesis *in vivo* human brain.

The effect of lithium on IMPase activity may be investigated using either proton (^{1}H) MRS, which has the potential to detect *myo*-inositol (the product of the enzyme), or phosphorous (^{31}P) MRS, which can estimate brain phosphomonoesters (PME), which include inositol-1-monophosphate (the substrate of the enzyme) [11,18]. When the enzyme IMPase is inhibited by lithium, *myo*-inositol levels would be expected to decrease while the substrate inositol-1-monophosphate would be expected to increase. However, direct *in vivo* measurement of *myo*-inositol level is not possible. With ^{1}H MRS, the *myo*-inositol resonance has contributions from three metabolites that can be affected by altered lithium concentrations *in vivo*, namely *myo*-inositol, inositol-1-monophosphate, and glycine. Therefore, the decrease in *myo*-inositol levels may be obscured by an increase in inositol-1-monophosphate and glycine levels [40].

A few human MRS studies have investigated the effect of lithium on the enzyme IMPase. Using ^{1}H MRS, Moore and associates (1999) documented a lithium-induced decrease in *myo*-inositol concentrations in the right frontal lobe of bipolar patients [30]. Although subject to a type II error by using ^{31}P MRS, Kato et al. (1993), found no change in PME concentrations in 6 manic patients, examined before and after initiation of lithium [19]. Another study utilizing ^{31}P MRS found that in 21 psychotic patients given lithium for two weeks, there were no changes in PME concentrations [32]. Silverstone and associates (1996, 1999) did not observe any significant effects of 7 days of lithium administration either on PME ratios or on *myo*-inositol levels in temporal lobe of healthy volunteers [40,41]. However, they reported a significant increase on the PME ratios after the stimulation of the phosphoinositide cycle by amphetamine [41].

In ^{31}P MR spectra of brain, typically seven peaks are resolved; PME, inorganic phosphate (P_i), phosphodiesters (PDE), phosphocreatine (PCr), as well as γ, α, and β nucleotide triphosphate (NTP), (Fig. 2). The PME peak contains signals from several metabolites, including metabolites related to membrane phospholipid synthesis, such as phosphocholine, phosphoethanolamine, and sugar phosphates such as α-glycerophosphate and inositol phosphates, including I1P [11,18]. It is estimated that inositol monophosphates constitute only about 10–15% of the total PME peak area [36]. Under normal circumstances, the concentration of inositol-1-phosphate is 0.05–0.1 mmol/l, whereas with ^{31}P MRS the minimum concentration for detection is 0.5–1 mmol/l [11,43]. Thus, a 5- to 10-fold increase in I1P concentration would be necessary for detection as an increase in the PME resonance [43].

There are a limited number of ways by which the sensitivity of ^{31}P MRS experiments may be increased. In particular, proton decoupling [18,31] and increasing the volume of tissue examined are the easiest methods to implement on an existing scanner (Fig. 2). With proton decoupling, protons that interact with phosphorous are irradiated to improve the spectral resolution deteriorated by spin–spin coupling [18]. Relying on that we collected proton decoupled ^{31}P MR spectra from healthy human subjects before and after the administration of lithium on a 1.5 T GE SIGNA MR system equipped for broadband spectroscopy [46]. Our a priori hypothesis was that the decoupled ^{31}P MR spectra obtained from a large volume of brain would reveal increases in the PME concentration after the chronic administration of lithium. 8 subjects, 4 women, and 4 men completed the full 14 days of the study period. With pair-wise comparisons of the mole percent PME values, we detected a significant increase following chronic lithium administration both on day 7 ($p < 0.04$, $t_{\mathrm{df}=7} = 2.57$, standard deviation (SD) of change $= 1.67$), and also on day 14 compared to baseline ($p < 0.04$, $t_{\mathrm{df}=7} = 2.58$, $\mathrm{SD}_{\mathrm{change}} = 1.34$) [46].

In this longitudinal study we have shown that lithium treatment increases PME levels in human brain [46]. This increase was observed at the 1_{st} week and maintained through the 2nd week of lithium administration [46]. Prior studies showing an increase in the PME peak following lithium administration

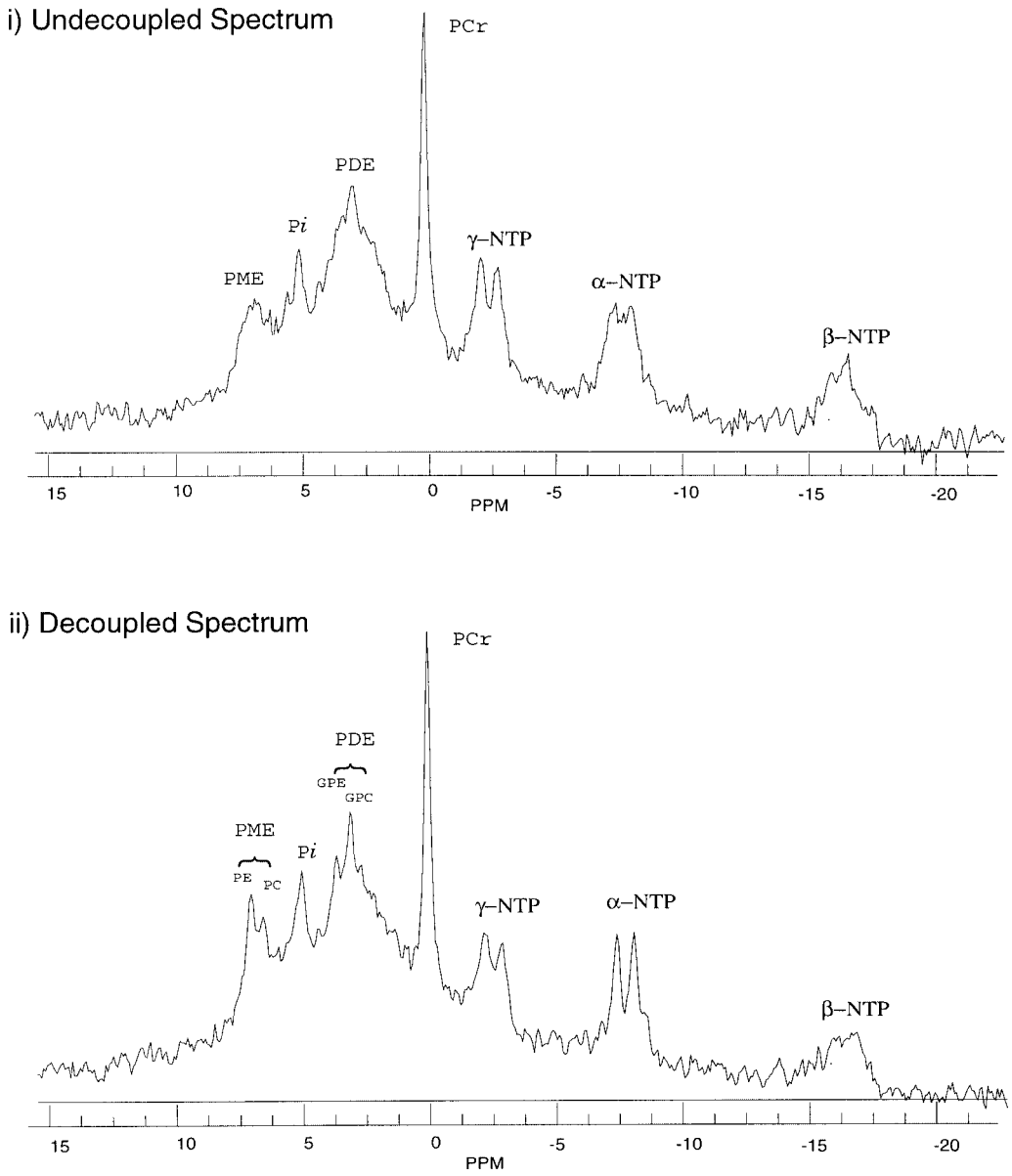

Fig. 2. Typical ^{31}P-MR spectra: (i) undecoupled, (ii) decoupled. Frequency units are expressed in parts per million (PPM). PME, phosphomonoesters; PE, phosphoethanolamine; PC, phosphocholine; Pi, inorganic phosphate; PDE, phosphodiesters; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; PCr, phosphocreatine; γ, α, and β-NTP, gamma, alpha, and beta nucleotidetriphosphates. Adapted from [46].

to cats and rats, using *in vitro* MRS, determined that this change was due to increases of inositol-1-monophosphate concentrations [34–36]. Another *in vivo* study showed that chronic lithium administration caused a significant decrease in platelet membrane phosphatidyl inositol-4,5-biphosphate levels in bipolar subjects [42]. Although, these findings provide evidence for the inositol-depleting effects of lithium, it should be considered that inositol depletion per se may not be responsible for lithium's therapeutic effects.

3. Validity of the "inositol depletion" hypothesis has been called into question

While data from preclinical studies have tended to demonstrate lithium-induced alterations in receptor-mediated phosphoinositide turnover, numerous methodologic criticisms have been raised recently, and the validity of the inositol depletion hypothesis, as originally articulated, has been called into question [2,16]. One methodologic criticism is that the inositol depleting effect of lithium observed in some cases in brain slices is dependent on the inositol-depleted state of the slices (inositol is already depleted by about 80% in the absence of lithium in washed rat brain slices). In addition, lithium's effects on IMPase are species-selective and can not be extrapolated to *in vivo* human brain [2,16]. Another major criticism is that with therapeutic concentrations of lithium, only partial inhibition of IMPase will be obtained and, even with toxic doses, lithium causes modest reductions in inositol concentrations (about 35% at most). It is unknown if such a modest decrease of brain inositol is sufficient to limit phosphoinositide synthesis and impair signal transduction [2,16]. Thus, inositol depletion by itself may not be responsible for lithium's therapeutic effects [28,29]. Instead, as some preclinical [9,21,44] and clinical data [30,46] suggest, some of the initial actions of lithium may occur through a reduction of *myo*-inositol, which in turn may initiate a cascade of secondary changes at different levels of signal transduction process and gene expression in brain, effects that are ultimately responsible for the anti-manic/mood stabilizing and neuroprotective effects of lithium [15,16,28,29]. Consistent with this model, recent findings in molecular biology established some remarkable effects of lithium at various levels of cellular function in brain [26].

4. The cascade of secondary changes presumably initiated by the lithium-induced reduction of myo-inositol

It has recently been demonstrated that lithium provoke some significant changes in signal transduction systems and gene expression in brain.

4.1. G proteins & lithium

Lithium is known to affect two major second messenger generating systems in the brain, namely the phosphoinositide turnover and adenylyl cyclase activity, which generates cyclic adenosine monophosphate (cAMP) [26]. Since, both of these systems are connected to cell surface receptors, and lithium affects these systems without influencing receptor levels per se, it has been considered that lithiums' effects are mediated through the G proteins that are directly connected to receptors [26].

Recent findings suggest that lithium modifies G protein function without effecting G protein levels [26]. It has been shown that lithium produce a significant increase in pertussis toxin-catalyzed adenine nucleotide diphosphate (ADP)-ribosylation in platelets of subjects receiving lithium treatment [13, 26]. Pertussis toxin selectively catalyzes the ADP-ribosylation of the undissociated, inactive $\alpha\beta\gamma$ heterotrimeric form of inhibitory G protein (G_i). Thus, this finding indicates that lithium inactivates G_i by stabilizing the inactive conformation [23,26]. It has also been shown that chronic lithium causes an enhancement of both basal and post-receptor stimulated adenylyl cyclase activity in human platelet preparations and rat brain. It is likely that this effect of lithium on adenylyl cyclase activity occurs as a consequence of the suppression of the tonic inhibitory control of G protein (Fig. 3) [23].

4.2. Gene expression & lithium

It is widely accepted that drugs, which require a lag period for onset of action and are not immediately reversed upon discontinuation such as the majority of the psychotropic medications, must exert their effects at the genomic level [15,26].

Activator protein-1 (AP-1), is a complex composed of products from two transcription factor families; "fos" and "jun". These products bind to a common DNA site in the regulatory domain of the gene, namely "12-*o*-tetradecanoyl-phorbol 13-acetate (TPA) response element-TRE" and activate gene transcription in response to protein kinase C (PK C) activators, growth factors, cytokines, and neurotransmitters [14,17]. It has been demonstrated that lithium at therapeutic concentrations produce a time-dependent increase in the DNA binding of AP-1 to TRE [6,33]; and this effect translates into alterations at the gene expression level [6,33,49].

It is yet to be delineated how lithium regulates AP-1 DNA binding, but may involve effects on mitogen-activated protein kinases. Supportively, lithium at therapeutic doses has been shown to inhibit the activity of glycogen synthase kinase-3β(GSK-3β), which is known to phosphorylate c-jun at 3 sites adjacent to the DNA binding domain, by this means reducing TRE binding [12,20]. In view of that, lithium by means of lessening GSK-3β activity may be enhancing AP-1 binding to the TRE, (Fig. 3) [48].

4.3. Protein kinase C & lithium

Protein kinase C family consists of at least 12 phosphorylating isozymes that are distributed heterogeneously in the brain. Each isozyme has different substrate affinities and second messenger activators suggesting distinct cellular functions [23].

It has been demonstrated that chronic lithium administration results in significant decrease in membrane-associated PK C-α and -ε isoforms [27]. PK C is a calcium activated diacylglycerol-dependent protein kinase and is known to be involved in regulation of signal transduction processes, ion channels' function and gene transcription in cell [24]. Besides, it has been shown that lithium induced down-regulation of PK C-α and -ε can be reversed by coadministration of *myo*-inositol *in vivo*, a finding that is confirming the hypothesis that lithium induced changes at different levels of signal transduction processes and gene expression may be secondary to lithium induced reductions in *myo*-inositol levels in brain.

Lenox and colleagues (1992) have revealed a decrease in myrstoylated alenine-rich C kinase substrate (MARCKS) by chronic administration of lithium [25]. MARCKS is protein kinase C substrate in brain and regulates cellular localization and function of PK C in cell through phosphorylation. MARCKS binds calmodulin in a calcium dependent manner and cross links actin at plasma membrane. Thus, MARCKS has been implicated in cellular processes associated with cytoskeletal restructuring and long-term neuroplastic changes. It has been shown that coadministration of *myo*-inositol *in vivo*, also reverses lithium-induced decrease in MARCKS, again supporting the hypothesis that *myo*-inositol reduction constitutes the initial step in lithium-induced alterations in brain (Fig. 3) [24].

4.4. B cell lymphoma protein-2 & lithium

B cell lymphoma protein-2 (Bcl-2), is a neuroprotective protein which enhances the survival of cells when exposed to adverse stimuli [22]. Chen and associates (1999) has found that chronic lithium at therapeutically relevant doses increases messenger RNA levels of the polyoma-virus enhancer-binding

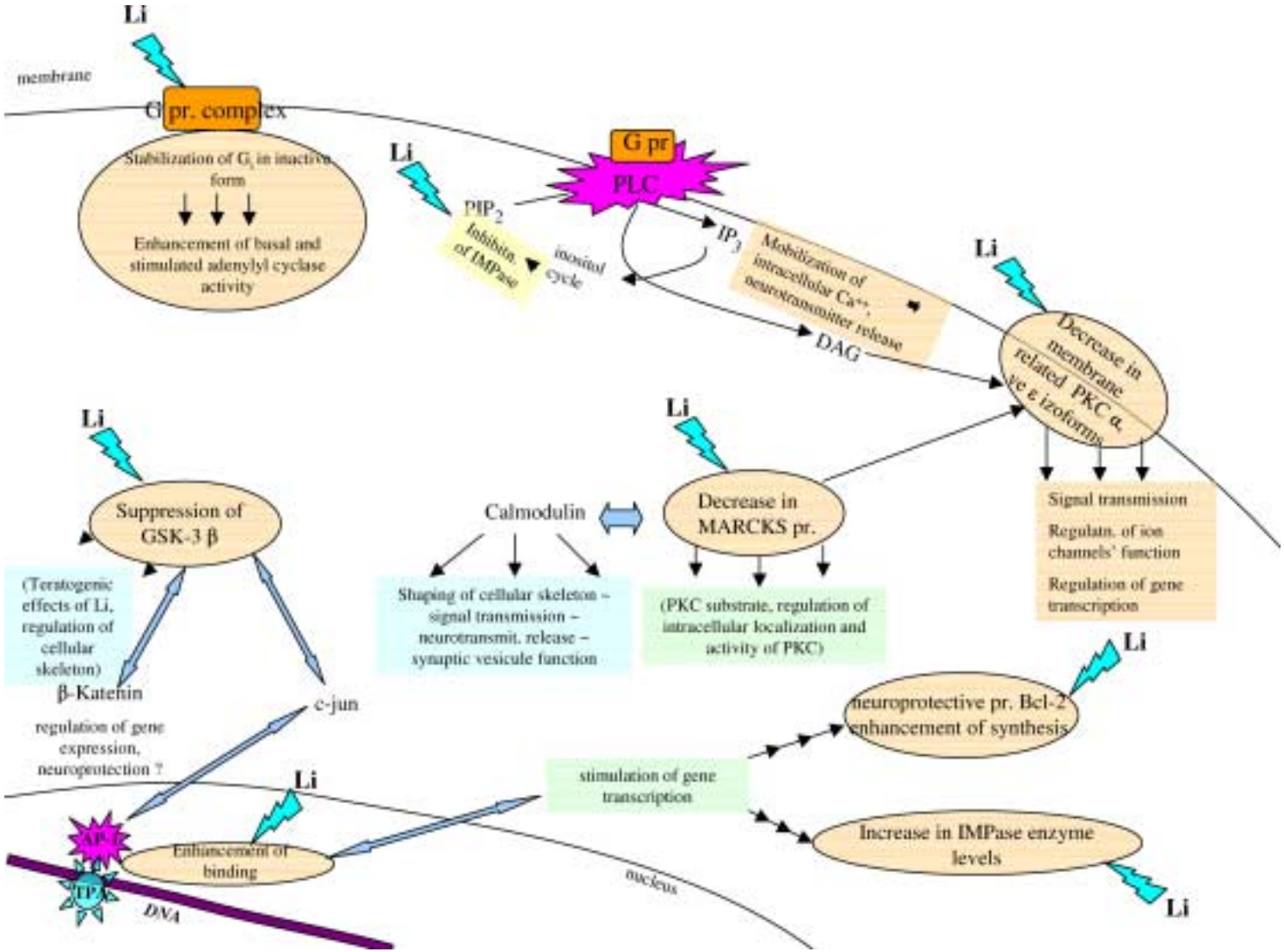

Fig. 3. Effects of lithium at different levels of cellular functions. Abbreviations: G_i, inhibituar G protein; PLC, phospholipase C; PIP_2 phosphatidylinositol-4,5-biphosphate; IMPase, inositol monophosphatase; IP_3, inositol-1,4,5-triphosphate; DAG, diacylglycerol; PKC, protein kinase C; MARCKS, myristoylated alenin-rich C kinase substrate; GSK-3 β, glycogen synthase kinase 3 β; AP-1, activator protein-1; Bcl-2 pr., B cell lymphoma protein-2. Adapted from [48].

protein-2β (PEBP-2β) transcription factors [7]. As, PEBP-2β regulates synthesis of Bcl-2 in brain; lithium, by increasing PEBP-2β levels produces a robust increase in Bcl-2 in brain [7].

Obviously, last decade has witnessed enormous progress in our understanding of mechanisms underlying lithium's therapeutic effects in manic-depressive illness. There is no question that it is not as simple as originally suggested by the "inositol depletion hypothesis". Nonetheless, there still exist some gaps in connecting all these data together and puzzling out the mechanism underlying lithium's mood stabilizing effects in bipolar illness. Yet, today's knowledge on the matter points toward the hypothesis that a lithium-induced reduction in *myo*-inositol levels (through inhibition of the IMPase) may initiate a cascade of events at various levels of cellular function, which results in treating effects of lithium in manic-depressive illness (Fig. 3).

5. If the enzyme IMPase is the key point in initiating lithium's therapeutic effects, could it be the case that it might also be involved in the pathophysiology of bipolar illness?

Today's data suggest that the enzyme IMPase plays an important role for the function of lithium in bipolar patient's brain. If this is the case for the treatment effects of lithium in bipolar illness, we asked if this enzyme could be affected in bipolar patients. To investigate this we run a meta-analysis including all the available *in vivo* data of human brain, and compared the PME values of bipolar patients in different mood states to healthy controls [47]. In this work, we detected lower PME levels in bipolar euthymics in comparison to healthy controls ($p = 0.014$) [47]. In regard to the state dependent alterations, bipolar patients with current depressive episodes had higher PME levels than bipolar euthymics ($p = 0.0005$); in manic vs. euthymic subjects however, we couldn't detect significance ($p = 0.247$) [47].

Since most of the subjects in the studies included in this meta-analysis were on lithium or off lithium for only one week; low PME levels in the euthymic and high PME levels in the depressive state of bipolar patients might be the reflections of lithium's long-term pharmacologic effects.

The PME resonance has contributions from the inositol-1-monophosphate [11,18]. Shamir et al. (1998) have reported that cell lines from bipolar patients have significantly lower inositol monophosphatase (IMPase) activity than cell lines from control subjects [39]. Besides, among bipolar patients, lithium responders exhibited significantly lower IMPase activity compared to nonresponders [39]. Lithium has been demonstrated to inhibit the enzyme IMPase, and increase PME *in vivo* human brain [37,46]. It is a heuristic hypothesis that bipolar subjects exhibit genetically determined low IMPase activity and lithium, by causing more inhibition of the enzyme activity, may cause an increase in inositol 1-monophosphate and/or a decrease in free inositol beyond a critical point which stimulates a compensatory transcriptional up-regulation of the enzyme's synthesis [47]. Supportively, it has been shown that chronic lithium administration causes an increase in IMPase and its messenger RNA levels [35,39]. When IMPase activity is increased, a bipolar patient's clinical status would be stabilized in euthymic state, and inositol 1-monophosphate levels as well as the PME levels would be decreased. Whenever the compensatory up regulating effect of lithium on the enzyme IMPase is lost, the patient's clinical status would be destabilized and the patient would have an episode of altered mood state [47]. In that case, since the enzyme is less active again, inositol-1-monophosphate levels and the PME levels would go up [47]. The results of our meta-analysis are compatible with this hypothesis for the eythymic and depressed states, but not for the manic state [47]. This may result from the inconsistency of the available data, and/or the small number of studies included in the meta-analysis of the PME in manic state.

In conclusion, lithium, by inhibiting the enzyme IMPase produce a reduction in *myo*-inositol and an increase in inositol-1-monophosphate levels. This initial decrease in *myo*-inositol or increase in I1P levels

in turn initiates a cascade of secondary effects at various levels of signal transduction processes and gene expression in brain including effects of lithium on PK C, MARKS, GSK-3β, and Bcl-2 proteins. Recent research data in molecular biology as well as *in vivo* MRS suggest that IMPase may have a critical role in the physiology underlying lithium's treating effects in bipolar illness as well as the disease process itself.

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