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Review

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Lithium and Autophagy

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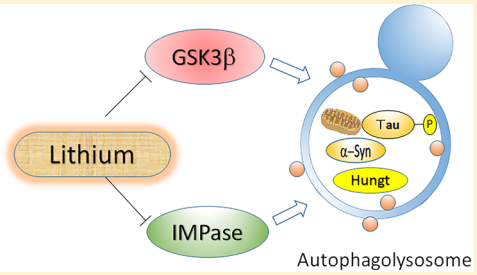
ABSTRACT: Lithium, a drug used to treat bipolar disorders, has a variety of neuroprotective mechanisms, including autophagy regulation, in various neuropsychiatric conditions. In neurodegenerative diseases, lithium enhances degradation of aggregate-prone proteins, including mutated huntingtin, phosphorylated tau, and α-synuclein, and causes damaged mitochondria to degrade, while in a mouse model of cerebral ischemia and Alzheimer's disease autophagy downregulation by lithium is observed. The signaling pathway of lithium as an autophagy enhancer might be associated with the mammalian target of rapamycin (mTOR)-independent pathway, which is involved in myo-inositol-1,4,5-trisphosphate (IP_3) in Huntington's disease and Parkinson's disease. However, the mTOR-dependent pathway might be involved in inhibiting glycogen synthase kinase-3β (GSK3β) in other diseases. Lithium's autophagy-enhancing property may contribute to the therapeutic benefit of patients with neuropsychiatric disorders.



KEYWORDS: *Lithium, autophagy, GSK3β, IMPase, Huntingtin, α-synuclein, tau, prion protein*

Lithium has been used clinically to treat bipolar disorders for over half a century, and various neuroprotective and neurotrophic properties have been described.[1] To the best of our knowledge, Sarkar et al. reported, for the first time, that lithium induced autophagy to enhance the degradation of mutant Huntingtin via the mTOR independent pathway in nonneuronal and neural precursor cells.[2] Since then, several papers have described lithium's autophagy regulation in various neuropsychiatric diseases such as Huntington's disease (HD), Alzheimer's disease (AD), Parkinson's disease (PD), prion disease, and amyotrophic lateral sclerosis (ALS). However, the signaling pathway to explain lithium's autophagy regulation has not been consistently described. Moreover, lithium did not always positively regulate autophagy in all pathological conditions. In a condition such as cerebral ischemia or AD, lithium has been shown to negatively regulate autophagy. In this review, we focus on lithium's autophagy-enhancing mechanism in various diseases.

I. THE BASICS OF AUTOPHAGY

I-1. Introducing Autophagy. Autophagy is the process of "self-eating." Under starvation conditions, bulk autophagy can be induced to catabolize cellular substrates to generate energy. There are three forms of autophagy: microautophagy, macroautophagy, and chaperone-mediated autophagy. The most common and well understood is macroautophagy, hereafter referred to simply as autophagy. For a more complete review of autophagy, see ref 3. Autophagy consists of four stages: initiation, elongation, maturation, and fusion (Figure 1). This process is initiated by formation of a cup-shaped membrane structure (the phagophore) in the cytoplasm. The phagophore accumulates additional proteins, which enables the membrane to elongate and form a double-membrane-bound structure called an autophagosome. A portion of the cytoplasm is enclosed in the autophagosome along with the cellular components to be degraded. Autophagosomes are then trafficked along microtubules to the perinuclear region of the cell (where lysosomes are clustered) to enhance the probability of autophagosome-lysosome fusion to form autophagolysosomes. After fusion with lysosomes, the protein and organelle contents of the autophagosome are degraded by acidic lysosomal hydrolases and recycled. Vacuolar H^+ATPase (V-ATPases) are proton pumps that reside within the lysosomal membrane and enable acidification of the autolysosome contents. This acidification is essential for the activation of lysosomal enzymes, such as cathepsins or other acid hydrolases, which are responsible for proteolysis of the components in the autophagolysosome.[4]

Macroautophagy has physiological roles in both health and disease. Upon nutrient deprivation, autophagy catabolizes cytoplasmic components nonselectively into building blocks, such as amino acids. Autophagy also occurs constitutively at low levels even under nutrient-rich conditions and mediates global turnover of cytoplasmic materials. Constitutive autophagy acts as the quality-control machinery for cytoplasmic components, and it is crucial for homeostasis of various postmitotic cells, such as neurons. Although this quality control could be partially achieved by nonselective autophagy,

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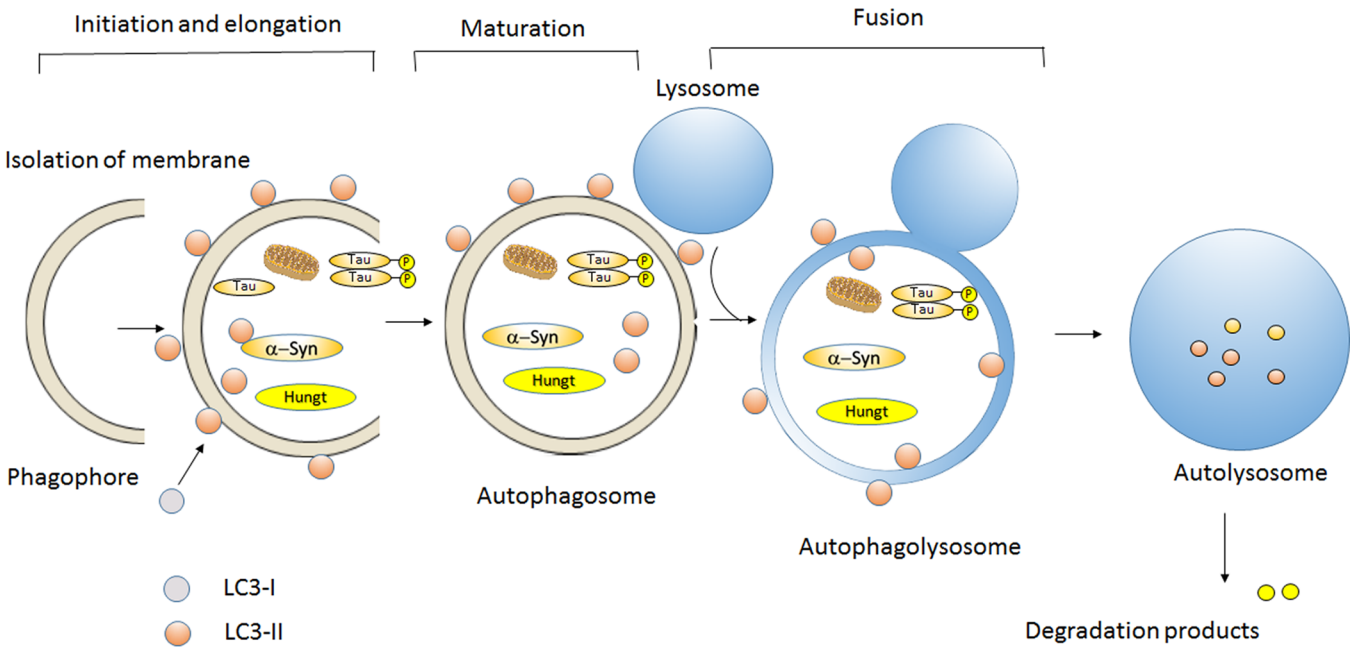

Figure 1. Stages of autophagy. A portion of cytoplasm, including organelles, is enclosed by an isolation membrane (also called a phagophore). On elongation of this membrane, LC3 protein is cleaved at its C-terminus to form cytosolic LC3-I. This protein is then conjugated with phosphatidylethanolamine to form LC3-II, which aids in the closure of the membrane to form an autophagosome. Autophagosomes ultimately fuse with lysosomes. Lysomal hydrolase then degrades their substrates in autophagolysosomes.

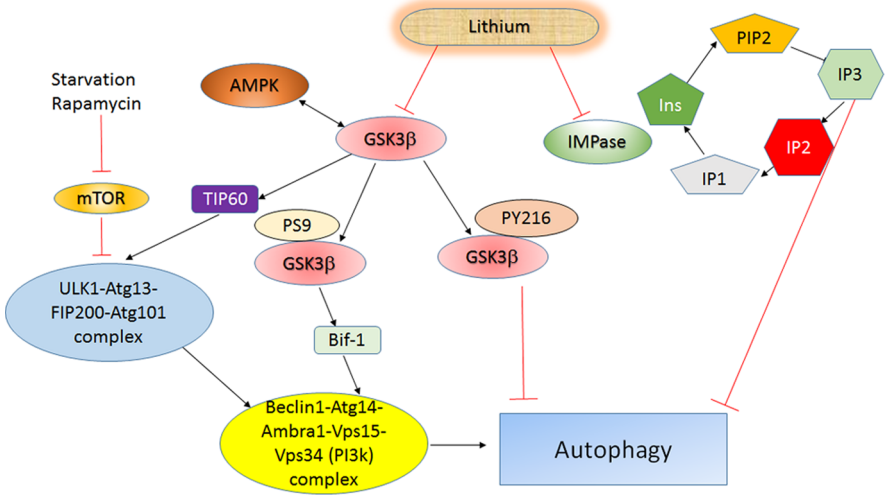

Figure 2. Lithium's autophagy regulation mechanism in various neuropsychiatric diseases. Signaling pathways involved in the regulation of autophagy of lithium In the mTOR independent pathway, lithium inhibits GSK3β as well as IMPase. The activity of GSK3β is downregulated by phosphorylation on Ser^{9} residue, and conversely, phosphorylation on Tyr216 upregulates GSK3β activity. GSK3β inhibition resulted in an elevation of Bif-1 which interacted with beclin-1-VPS complex and induced autophagy. AMPK can affect GSK3β activity and is also upregulated by GSK3β.[79] Under serum deprivation conditions, GSK3 activates TIP60 and stimulates ULK1. In nutrient-rich condition, mTOR suppresses the ULK1 (Atg1 homologue) complex under. Upon autophagy induction, the ULK1 complex (including ULK1, Atg13, FIP200, and Atg101) is activated and translocated to a certain domain of the endoplasmic reticulum (ER). In the ER, the ULK1 complex regulates the class III phosphatidyl inositol (PtdIns) 3-kinase complex (including Beclin 1, Atg14(L)/bakor, Vps15, Vps34, and Ambra 1). Abbreviations: IMPase, inositol monophosphatase; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-biphosphate; mTOR, mammalian target of rapamycin; GSK3β, glycogen synthase kinase-3β; TIP60, HIV-Tat interactive protein, 60 kDa; Bif-1, bax interacting factor 1; PS9, phosphorylation at Ser^{9}; PY^{216}, phosphorylation at Tyr^{216}.

increasing evidence indicates that "selective" autophagy degrades specific proteins, organelles, and invading bacteria.[5]

I-2. Autophagy in Neuropsychiatric Disease. Autophagy has physiological roles in both health and disease. Basal or constitutive autophagy is responsible for the quality control of essential cellular components by purging the cell of old or damaged organelles, such as peroxisomes and mitochondria, and by degrading long-lived or aggregate-prone proteins that are too large to be degraded by the proteasome[6,7] (Figure 1). Autophagic dysfunction might contribute to the pathogenesis of numerous neurodegenerative diseases, including forms of PD, AD, tauopathies, ALS, HD, and Lafora disease.[7−9] Patients with conditions that are associated with the accumulation of intracytoplasmic aggregate-prone proteins may benefit from

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pharmacological upregulation of autophagy. Studies showed that the CNS, in contrast to other organ systems, displayed only low levels of autophagosomes under normal conditions and even after starvation, but it was also demonstrated that constitutive turnover of cytosolic contents by autophagy is indispensable, even in the absence of the expression of any disease-associated mutant proteins.[10,11]

I-3. Regulation of Autophagy. Autophagosome formation is regulated by many signals that fall into two broad categories: mammalian target of rapamycin (mTOR)-dependent and mTOR-independent. The mTOR is a "classical" autophagy suppressor that acts by blocking the activity of the ULK1 complex. The activity of mTOR depends on various inputs from upstream signals that include the energy status and nutrient status of the cell, as well as the presence of amino acids and growth factors. Downstream of mTOR, numerous proteins encoded by *ATg* genes are essential for the execution of autophagy.[12] Pathways that act independently of mTOR include 5′-AMP-activated protein kinase (AMPK), the stress-activated enzyme Jun N-terminal kinase 1 (JNK1), BH3-only proteins, the inositol 1,4,5-trisphosphate receptor (IP3R), Erk1/2, and calcium.[13−15] Autophagy can also be pharmacologically induced by inhibiting negative regulators such as mTOR via the compound rapamycin[16] or by mTOR-independent inducers of autophagy such as trehalose.[17] Pharmacological inhibitors of autophagy include 3-methyladenine (3-MA), wortmannin, and LY294002.[18,19]

A number of enzymes have been proposed as potential targets of lithium action, including inositol monophosphatase (IMPase), a family of structurally related phosphomonoesterases, and the protein kinase glycogen synthase kinase-3.[20] Carmichael et al. found that the mood stabilizer lithium at 2.5−5 mM for 3 days (2−5 times human therapeutic plasma levels) reduced mutant huntingtin nuclear inclusions and apoptotic nuclear fragmentation in COS-7 African green monkey kidney cells and SKNSH human neuroblastoma cell lines transfected with mutant HTT exon 1 fragment possessing 74 CAG repeats.[21] Lithium's autophagy-inducing property was first described by Sarkar et al. to enhance the clearance of aggregate-prone proteins, such as mutant forms of huntingtion and α-synuclein.[2] Lithium 10 mM was added to CoS-7, PC12, and mouse embryonic fibroblast cells transfected mutant HTT exon 1 with 74 CAG repeats.[2,22,23] GSK3β inhibition by lithium reduced autophagy by activating the mTOR.[23] On the other hand, lithium induced autophagy independently of mTOR through the inhibition of inositol monophosphatase (IMPase).[20,22] IMPase catalyzes the hydrolysis of inositol monophosphate (IP_1) into free inositol required for the phosphoinositol signaling pathway.[24] Lithium affects this pathway by inhibiting IMPase, leading to free inositol depletion, which in turn decreases myo-inositol-1,4,5-trisphosphate (IP_3) levels (Figure 2). Increased inositol or IP3 levels inhibit autophagy, which reverse lithium's effect.[2] IP3 and the stimulation of its receptor have been seen to suppress autophagy.[14] Inositol depletion is a common mechanism of mood-stabilizing drugs such as lithium, carbamazepine (CBZ) and valproic acid (VPA).[25] Consistent with the role of inositol depletion in autophagy regulation, CBZ and VPA also enhanced the clearance of aggregate-prone proteins.[2] The mTOR inhibitor rapamycin in combination with lithium is more protective than treatment with either compound alone in a Huntington's disease model fly.[23] This combination enhances autophagy by mTOR-independent (IMPase inhibition by lithium) and mTOR-dependent (mTOR inhibition by rapamycin) pathways. This treatment showed greater protection against neurodegeneration in an HD fly model with mTOR inhibition and lithium than either pathway alone.

Lithium is a direct[26] and indirect[27] inhibitor of glycogen synthase kinase-3β (GSK3β), which has a wide range of cellular functions, including cell death, cell cycle, and carcinogenesis, and is an important regulator of various signal-transduction pathways. It is likely that GSK3β negatively regulates autophagy[28,29] (Figure 2). GSK3 activity is regulated by site-specific phosphorylation. The activity of GSK3β is upregulated by phosphorylation on the Tyr^{216} residue, and conversely, phosphorylation on Ser^{9} inhibits GSK3β activity. In epidermal cells, ultraviolet B activated autophagy as a protective response and inhibited GSK3β activation by simultaneously enhancing phosphorylation at Ser^{9}.[28] In acquired cadmium resistance, lithium augmented phosphorylation at Ser^{9} and increased autophagy while Thr^{216} inhibited autophagy.[29] Interestingly, under serum starvation condition, GSK3β suppression with chemical inhibitors or siRNAs induced cell death, enhanced autophagy and resulted in bax interacting factor 1 (Bif-1) protein levels,[30] although Ser^{9} phosphorylation has not been described. Bif-1 was found to modulate autophagy by interacting with beclin-1-VPS34 complex.[31] Under serum deprivation-induced autophagy, GSK3 activates acetyltransferase TIP60 (HIV-Tat interactive protein, 60 kDa) through phosphorylation on TIP60-Ser^{86}, which directly acetylates and stimulates the protein kinase ULK1, which is required for autophagy in metazoans.[32]

II. LITHIUM'S EFFECTS ON AUTOPHAGY IN VARIOUS NEUROPSYCHIATRIC DISEASES

II-1. Huntington's Disease. Huntington's disease (HD) is caused by mutant huntingtin protein resulting from an expanded polyglutamine cytosine-adenine-guanine (CAG) repeat sequence in the autosomal dominant gene *huntingtin*.[33] Mutant huntingtin accumulates in intraneuronal aggregates. Two studies in mice with deficiencies in either Atg7 or Atg5 demonstrated that constitutive autophagy is required for the clearance of cytosolic aggregate-prone proteins from neurons.[34,35] Induction of autophagy by lithium led to enhanced clearance of autophagy substrates, like mutant huntingtin fragments.[22] As described in subsection I-3, it was shown that lithium induced autophagy via IMPase inhibition, leading to decrease inositol levels.[2]

In mutant huntingtin-transfected HEK 293T and HeLa cells, lithium treatment down-regulated the histone deacetylase 1 protein level and facilitated autophagic degradation of mutant huntingtin.[36] Since GSK3β inhibitors did not alter the HDAC1 level, the authors conclude that lithium-facilitated down-regulation of HDAC1 is independent of GSK3β. Thus, it is likely that lithium's autophagy enhancing action related to mutated huntingtin is independent of GSK3β.

II-2. Alzheimer's Disease. Alzheimer's disease (AD) is the most common neurodegenerative disorder of dementia. The two pathological hallmarks of the disease are neurofibrillary tangles (NFTs), which are mainly composed of tau protein, and senile plaques, which consist of amyloid-β (Aβ). There is significant support for the possibility of defective autophagy in AD. Electron microscopic analysis of brain tissue from confirmed AD cases revealed that autophagic vacuoles accumulated in dystrophic neuritis and correlated with the presence of filamentous tau.[37] Similar findings were described

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in a mouse model of AD. In the hippocampus of 4- to 6-month-old presenilin 1 $(PS1)^{M146L}/APP^{751SL}$ mice, the protein levels of the autophagosome marker LC3 were increased.[38] By light microscopy, LC3-positive autophagosomes were localized in the axonal dystrophy, and election microscopy identified these vesicles as autophagic vesicles that fill and cause axonal swelling.

II-2-1. Tauopathies. Tau is an abundant microtubule (MT)-associated protein in the CNS that is implicated in the pathogenesis of neurodegenerative diseases known as tauopathies including AD. Abnormal aggregation of tau protein into filamentous structures and extensive neuronal loss are found in tauopathies.[39] It has been hypothesized that hyperphosphorylated tau misfolds, disassembles from microtubules, and forms aberrant filamentous aggregates that give rise to neurofibrillary tangles[40,41] (Figure 4). However, there is now significant evidence that pathologically modified monomeric and/or soluble oligomeric forms of tau are considered to be harmful species rather than insoluble aggregates.[42,43] The bulk of clearance of both physiological and pathological forms of tau is mediated by the proteasomal and autophagic degradation system.[44,45]

In our study, transgenic mice overexpressing human mutated tau (P301L) were treated with oral lithium chloride (LiC) for 4 months starting at the age of 5 months.[46] LiCl-treated mice showed a better score in sensory motor tasks as well as decreases in soluble and insoluble phosphorylated tau. Lithium treatment also showed LC3-positive autophagosome-like puncta in the spinal cord and a decreased level of P62, a substrate of autophagy. In the cervical spinal cord of LiCl-treated P301L mice, some LC3-positive puncta were immunostained with phosphorylated tau (AT8) (Figure 3).

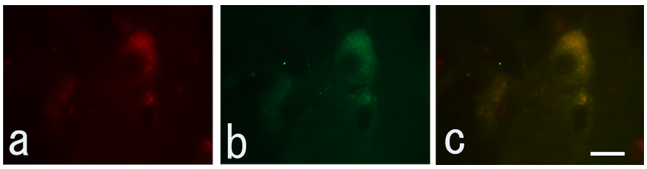

Figure 3. Lithium treatment increases autophagosome formation in tauopathy model mice. (a–c) Double labeling immunohistochemistry with phosphorylated tau, AT8 (red), and autophagosome marker, LC3 (green). LC3-positive puncta are immunostained by phosphorylated tau-specific antibodies such as AT8. It is said that granular phosphorylated tau represents a prestage of NFTs.[47] These results appear to show that only phosphorylated soluble tau could be degraded by the autophagosome pathway, whereas insoluble fibrillary tau remained. Bar = 10 μm.

In the AD brain, pre-NFT was defined as a cell containing diffuse phosphor-tau-positive staining within the cytoplasm, sometimes including phosphor-tau-positive punctate regions.[47] The appearance of these punctate regions is similar to LC3-positive autophagosome-like puncta. Neurons bearing LC3-positive puncta were relatively normal in size rather than atrophic. In order to examine the relationship between LC3-positive neurons and NFTs, we performed thioflavin-S staining after LC3 immunostaining. Interestingly thioflavin-S-positive neurons were completely LC3 negative. Since NFTs consist of insoluble tau, we speculate that soluble phosphorylated tau can be degraded by autophagy. Assuming that all studies were carried out and analyzed carefully, it remains to be determined whether tissue/brain region specificity or yet to be revealed factors led to the opposite outcome.

Lithium is a direct or indirect inhibitor of GSK3β. We administered another GSK3β inhibitor, AR-A014418, to tauopathy model mice for 2 weeks. AR-A014418 treatment decreased the P62 protein level accompanied by the inactive form of GSK3β (data not shown). Recently, it was shown that 4-month treatment with rapamycin ameliorated tau pathology with an increased level of the inactivated form of GSK3β (Phospho-Ser^9).[48] Taken together, in tauopathies, lithium may play a dual protective role by inhibiting GSK3β (Figure 4). At first, lithium inhibited GSK3β activity, reduced the phosphorylation of tau, enhanced the binding of tau to microtubules, and promoted microtubule assembly. As another mechanism, lithium may increase degradation of soluble phosphorylated tau, not insoluble filamentous tau, into autophagosomes via mTOR signaling. Both mechanisms finally contributed to enhancing the binding of tau to microtubules.

II-2-2. Amyloid β in Alzheimer's Disease. Aβ is produced from sequential endoproteolytic cleavage of the type 1 transmembrane glycoprotein amyloid-β protein precursor (AβPP) by β- and γ-secretases. Cleavage of AβPP by the β-site, AβPP cleaving enzyme 1 (BACE1), produces a soluble AβPP N-terminal fragment and a 99-residue C-terminal fragment (C99). C99 is then cleaved by γ-secretase to release Aβ. LiCl (0.18 mmol LiCl/mouse/day) was administered intraperitoneally to 10-month-old female double transgenic mice expressing $A\beta PP^{swe}/PS1^{A246E}$ for 3 months.[49] Lithium treatment decreased γ-cleavage of AβPP, Aβ production and senile plaque formation accompanied by the improvement of spatial learning. Lithium also elevated the inactive form of GSK3β and reduced autophagy, which was represented by decreased protein levels of LC3-II and Beclin 1. These findings are not consistent with our result in P301L mice in which lithium treatment enhanced autophagy as described under section II-2-1. Under serum starvation, GSK3β suppression promotes autophagic response via elevation of Bif-1 protein levels while this phenomenon does not occur under fetal-bovine-serum-supplied conditions.[30] Metabolic stress triggers the autophagic response in cells seeking survival. In P301L mice, there are mutated tau protein aggregates in neurons of the spinal cord, whereas in $A\beta PP^{swe}/PS1^{A246E}$ mice senile plaques consisting of insoluble Aβ accumulation are mainly localized in the extracellular spaces. Thus, the difference in the intracellular stress condition may explain the discrepancy between lithium-induced autophagy regulation in P301L mice and $A\beta PP^{swe}/PS1^{A246E}$ mice.

II-3. Prion Disease. Prion diseases are infections neurodegenerative disorders that can affect humans and animals. These diseases are designated as progressive spongiform encephalopathy, which manifests with a highly progressive loss of intellectual abilities. Prion propagation involves the endocytic pathway, and endosomal and lysosomal compartments are implicated in trafficking and recycling as well as in the final degradation of prions. It is conceivable that the basic process of autophagy has a physiological role in prion infection and might be used by cells for controlling or counteracting cellular prion infection.[50] The formation of an abnormally folded, protease-resistant isoform of the host-encoded cellular prion protein (PrP^c) is thought to be the mechanism responsible for prion diseases.[51] Ten mM LiCl reduced the amount of pathological prion protein (PrP^{sc}) in murine neuroblastoma cells persistently infected prions by inducing autophagy.[52] Trehalose also reduced PrP^{sc} in the same cell line while at the same time it induced autophagy.[53] Treatment of

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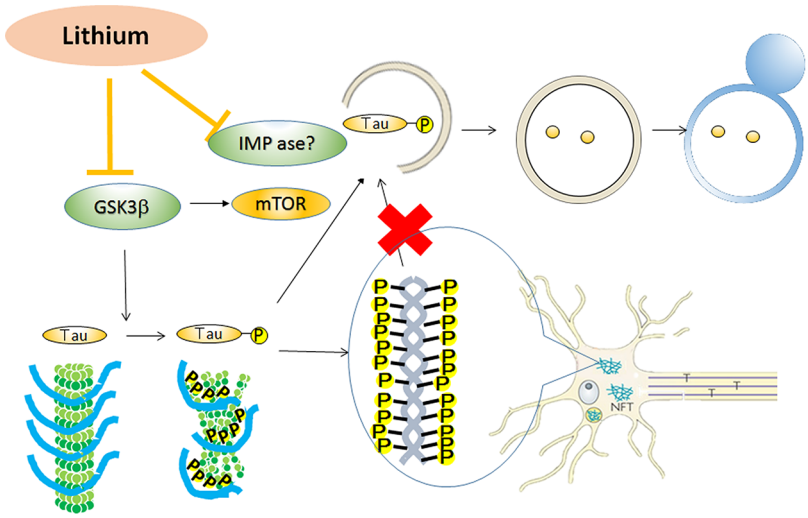

Figure 4. Hypothesis of the dual protective role of lithium's action against tauopathies.Normally, tau binds microtubules to promote microtubule assembly, while hyperphosphorylated tau misfolds, disassembles from microtubules, and forms aberrant filamentous aggregates that give rise to neurofibrillary tangles. Lithium inhibited GSK3β activity, reduced the phosphorylation of tau, and enhanced the binding of tau to microtubules. Lithium inhibited GSK3β activity, decreased mTOR signaling, and finally enhanced soluble phosphorylated tau to be degraded into autophagosomes. Only soluble phosphorylated tau, not insoluble fibrillar tau, was degraded by the autophagic pathway. Both mechanisms finally led to enhanced binding of tau to microtubules.

prion-infected cells with 2-methyladenine, a potent inhibitor of autophagy, counteracted the antiprion effect of lithium, demonstrating that induction of autophagy mediates the degradation of PrP^{sc}.

II-4. Amyotrophic Lateral Sclerosis. Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder with no effective treatment that usually leads to death within 3−5 years from diagnosis. Daily doses of lithium, leading to plasma levels ranging from 0.4 to 0.8 mM, delayed disease progression in human patients affected by ALS.[54] In a parallel study of ALS mutant copper−zinc superoxide dismutase 1 (SOD1) G93A male model mice, lithium treatment delayed disease onset and augmented the life span. The effect was accompanied by an increase in the number of the mitochondria and LC3-positive autophagosomes in the motor neurons in the spinal cord and activation of autophagy. Lithium had an effect on the removal of altered mitochondria and protein aggregates and also the biogenesis of well-structured mitochondria.[55] However, in female SOD1G93A mice, lithium neither exerted neuroprotective effects nor increased the expression of a marker of autophagy.[56]

II-5. Parkinson's Disease. Parkinson's disease (PD) is a relatively common disorder of the nervous system that affects patients with tremors, slowness of movement, gait instability, and rigidity. Currently, no neuroprotective candidate has been determined that has a disease-modifying effect on PD. Lewy bodies, which mainly consist of aggregated α-synuclein, are hallmark pathological features of PD. Lithium 10 mM treatment increased the clearance of A53T and A30P α-synuclein mutants using PC12 cell lines by inhibiting IMPase as well as mutated huntingtin.[2,22,23] The mitochondrial complex I inhibitors, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and rotenone were extensively used as neurotoxins to induce parkinsonian symptoms in vitro and in vivo.[57] Lithium treatment ameliorated Rotenon-induced toxicity in human neuroblastoma SH-SY5Y cells, which showed nuclear fragmentation and apoptosis.[58] A decrease in mitochondrial membrane potential, reduced reactive oxygen species generation and an increased number of lysosomes and autophagic vacuolar organelles was observed with 0.2 μm to 10 mM lithium treatment. A combination of valproate and lithium carbonate was injected intraperitoneally into C57BL/6 mice for 7 days following MPTP administration.[59] Mice showed the recovery of motor disturbance, dopaminergic neuron number, and dopamine metabolite dihydroxyphenyl acetic acid (DOPAC) with elevation of immunoreactivity for LC3.

II-6. Neuronal Ceroid Lipofuscinosis. Neuronal ceroid lipofuscinoses (NCL) are a group of severe neurodegenerative lysosomal storage disorders, and are considered the most common progressive encephalopathies of childhood.[60] Variant late-infantile NCL is caused by mutations in the *CLN6* gene. CLN6 is a nonglycosylated endoplasmic reticulum (ER)-resident membrane protein with unknown function. In the *nclf* mice, a naturally occurring model of the human CLN6 disease, prominent lysosomal strage was found accompanied by ubiquitinated proteins, and neuronal p62-positive aggregates in neurons.[61] The pathological marker of juvenile NCL (JNCL) is the accumulation of autofluorescence rich in subunit c of the mitochondrial ATP synthase complex within lysosomes and autophagosomes in central nerve system neurons of a $Cln3^{\Delta ex7/8\Delta ex7/862}$ knock-in mouse model. In JNCL patients, developmental abnormality of the cerebellum and neuronal loss of the fastigial pathway were seen.[63] In $Cln3^{\Delta ex7/8\Delta ex7/8}$ knock-in cerebellar cells, 10 mM lithium treatment restored LC3-positive autophagosomes and an IMPase inhibitor showed the same effect.[64] Lithium and IMP downregulation also protected against cell death induced by amino acid deprivation.

II-7. Cockayne Syndrome. Cockayne syndrome (CS) is a devastating autosomal recessive disease characterized by neurodegeneration, cachexia, and accelerated aging.[65] Overall, 80% of CS cases are caused by mutations in the CS complementation group B gene (CSB), known to be involved in transcription-coupled nucleotide excision DNA repair transcription. Recent evidence indicates that CSB is present in mitochondria, where it associates with mitochondrial DNA (mtDNA). Scheibye-Knusden et al. reported increased

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metabolism in $CSB^{m/m}$ mice and CSB-deficient cells.[66] In CSB-deficient cells, damaged mitochondria and free radical production are increased and autophagy is downregulated. Lithium chloride or rapamycin 10 mM reverses the bioenergetics phenotype of CBS-deficient cells.

II-8. Trimethyltin Toxicity. Organotin compounds are used as heat stabilizers in polyvinyl chloride polymers, industrial and agricultural biocides, and industrial catalysts in chemical reactions. Among these compounds, trimethyltin chloride (TMT), which was previously used as a fungicide and chemostabilizer, is characterized by the selective destruction of neurons in specific brain regions, particularly damaging the limbic system.[67] Ultrastructural studies performed on samples obtained from rodents intoxicated with TMT describe an increased number of lysosomes and vacuoles, suggestive of altered autophagy.[68] In TMT-induced primary mouse neurons, autophagy inhibitors (3-methyladenine and L-asparagine) greatly enhanced TMT toxicity, while 0.5−3 mM lithium and rapamycin displayed neuroprotection.[69] The neuroprotective effect of lithium against TMT in hippocampal neurons can be completely reversed by an excess of inositol and is possibly related to the inactivation of IMPase.

II-9. Cerebral Ischemia. Injection of lithium reduced infarct volume size and facilitated neurological recovery in a rat model of middle cerebral artery occlusion.[70] Pretreatment with lithium decreased neurological deficits and decreased ischemia-induced caspase-3 immunoreactivity and TUNEL staining in rats exposed to focal ischemia.[71] Recently, in a model of neonatal hypoxic-ischemic brain injury, lithium reduced the neuroprotective effect.[72] Lithium treatment reduced the ischemic-induced dephosphorylation of phosphor-GSK3β-Ser9 and extracellular signal-regulated kinase, the activation of calpain and caspase-3, the mitochondrial release of cytochrome *c*, and apoptosis-inducing factor as well as autophagy. In this study, under conditions such as late recovery after hypoxia-ischemia, lithium prevented autophagy, although most studies described in our review showed that lithium could induce autophagy. It remains elusive whether this is secondary to lithium-mediated tissue protection, resulting in less cellular damage and less intracellular debris.

III. CONTROVERSIES

III-1. Lithium Attenuates or Enhances Autophagy? Studies of cell cultures and flies, which are described in this review, indicated that lithium treatment showed neuroprotective effects by enhancing autophagy.[2,22,23,36,52,58,64,69] However, in vivo studies of rodent models did not show a consistent result for autophagy regulation by lithium, although behavioral abnormalities of all studies were improved.[46,49,54,66,72] This discrepancy is at least partly because of the lithium concentration. In tauopathy model mice, 4-month oral lithium treatment led to a 0.2 mM lithium plasma level and enhanced autophagy.[46] In SOD1G93A model mice, daily intraperitoneal injection of lithium for a couple of months reached 0.4−0.8 mM plasma concentration and enhanced autophagy.[54] In $A\beta PP^{swe}/PS1^{A246E}$ mice, 3-month intraperitoneal injection of lithium led to 0.65 mM/l, but attenuated autophagy. In a neonatal model of ischemia, the plasma lithium concentration was not noted but autophagy was prevented. On the other hand, in most cell culture studies, lithium concentration is 10 mM, which is much higher than in mouse model studies. However, these findings do not necessary indicate that a higher dose of lithium enhances autophagy while a low dose attenuates autophagy. Since lithium is used therapeutically at plasma concentrations between 0.2 and 1.5 mM in humans, a similar concentration should be examined in the treatment of cultured cells in order to establish a treatment for human diseases. Another interpretation of lithium's downregulation of autophagy is that this is secondary to lithium-mediated tissue protection, resulting in less cellular damage and less intracellular debris. Thus far, to the best of our knowledge, there is no report focusing on lithium's autophagy action using an adult mouse model of cerebral stroke. Lithium's regulation of autophagy should be examined in acute cerebral damage using adult mice.

III-2. The Role of IMPase and GSK3β in Lithium Effect on Autophagy. Lithium is a direct[26] and indirect[27] inhibitor of GSK3β. However, several studies support the concept that lithium's autophagy-enhancing properties cannot be attributed to GSK3β inhibition. A specific GSK3β inhibitor, SB21672, retarded the clearance of aggregate-prone huntingtin and α-synuclein, and impaired autophagosome synthesis.[23] In an AD model, a substrate competitive GSK3 inhibitor, L803-mts, restored the activity of mTOR, inhibited autophagy and ameliorated the Alzheimer's-like pathology.[73] In $A\beta PP^{swe}/PS1^{A246E}$ mice, lithium treatment upregulated the inactivated form of phosphorylated GSK3β(S9) and prevented autophagy.[49] These studies indicate that GSK3β is rather an autophagy-positive regulator by upregulating the mTOR pathway. On the other hand, an mTOR inhibitor, rapamycin, induced autophagy accompanying the elevation of the inactive form of GSK3β (Phospho-Ser^9) in mutant P301S tau transgenic mice.[48] Rather than GSK3β, IMPase was shown to play a role in lithium's autophagy-enhancing action for the clearance of huntingtin,[2,22,23] α-synuclein,[59,62,74] and ceroid lipofuscinosis[64] in in vitro studies. Using a mouse model, IMPase involvement in lithium's autophagy-enhancing properties will be examined in the near future.

IV. CONCLUSION

Overall, most data suggest that lithium always yields neuroprotective effects against neuropathological conditions, even though lithium negatively regulated autophagy. Many in vitro studies which showed lithium's autophagy-enhancing action used lithium concentrations that are higher than those that are used therapeutically. These studies are not easily translated for application in human studies. A phase 3 multicenter, randomized, double-blind, placebo-controlled trial of oral lithium in patients with ALS was conducted.[75] The trial found no evidence of benefit of lithium on survival in patients with ALS. The trial for polyglutamine disorders including Huntington disease and spinocerebellar ataxia type 3 have been described.[76,77] Lithium was safe and well tolerated, but it resulted in no improvement or effect on progression. However, it is worth noting that a recent placebo-controlled clinical trial in patients with amnestic mild cognitive impairment (MCI) showed that long-term lithium treatment may actually slow the conversion to AD with a reduced phosphorylated tau level in cerebrospinal fluid.[78] Lithium's autophagy-enhancing property may contribute to this improvement.

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Notes

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