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Article Addendum

Autophagy and amyotrophic lateral sclerosis

The multiple roles of lithium

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In a pilot clinical study that we recently published we found that lithium administration slows the progression of Amyotrophic Lateral Sclerosis (ALS) in human patients. This clinical study was published in addition with basic (in vitro) and pre-clinical (in vivo) data demonstrating a defect of autophagy as a final common pathway in the genesis of ALS. In fact, lithium was used as an autophagy inducer. In detailing the protective effects of lithium we found for the first time that this drug stimulates the biogenesis of mitochondria in the central nervous system and, uniquely in the spinal cord, it induces neuronogenesis and neuronal differentiation. In particular, the effects induced by lithium can be summarized as follows: (i) the removal of altered mitochondria and protein aggregates; (ii) the biogenesis of well-structured mitochondria; (iii) the suppression of glial proliferation; (iv) the differentiation of newly formed neurons in the spinal cord towards a specific phenotype.

In this addendum we focus on defective autophagy as a "leit motif" in ALS and the old and novel features of lithium which bridge autophagy activation to concomitant effects that may be useful for the treatment of a variety of neurodegenerative disorders. In particular, the biogenesis of mitochondria and the increase of calbindin D 28K-positive neurons, which are likely to support powerful neuroprotection towards autophagy failure, mitochondriopathy and neuronal loss in the spinal cord.

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset devastating neurodegenerative disease. The pathological hallmark of ALS is the progressive atrophy and final death of motor neurons (MN), preceded by swelling of perikarya and proximal axons, and accumulation of Bunina bodies (small cystatin C-containing neuronal inclusions and Lewy body-like inclusions). In addition activation and proliferation of astrocytes and microglia and the depositions of inclusions and ubiquitinated material are also common.[1] In the study we recently published[2] we found that lithium administration slowed down the progression of ALS in a small group of patients. Lithium is a well known mood-stabilizing drug used for the treatment of bipolar affective disorders. At the same time, lithium is increasingly recognized as neuroprotectant.[2-5] In fact, lithium has been shown to protect neurons from β-amyloid-induced degeneration associated with Alzheimer's disease,[6-8] to protect hyppocampal neurons from brain ischemia[5] and kainate-induced seizure and brain damage[4]. At the same time, lithium is shown to be an autophagy inducer[9,10] leading to upregulation of the autophagy-lysosomal degradative pathway.[9]

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Lithium, Autophagy and ALS

ALS is characterized by the presence within MN of intracellular aggregates and mitochondrial dysfunction.[11] In our recent paper we found that accumulation of altered mitochondria and intracellular aggregates within ALS MN was associated with defective autophagy. Moreover, when MN were challenged with an autophagy inhibitor such as 3-methyladenine this produced slight consequence in a normal MN but created devastating effects within ALS-affected MN. In line with this, when we stimulated autophagy by administering low doses of lithium, a significant decrease of intracellular aggregates containing alpha-synuclein, ubiquitin and mutant SOD1 within MN of the spinal cord occurred. The molecular mechanism through which lithium activates autophagy is not fully understood. The recent work by Rubinsztein and collaborators demonstrates that lithium-induced autophagy strictly depends on the inhibition of the inositol monophosphatase, which finally results in the reduction of inositol-1,4,5-triphosphate (IP3) cellular level.[9,10] IP3 and the stimulation of its receptor (IP3R) have been shown to suppress autophagy.[12] Thus, depletion of IP3 removes this inhibition and allows the autophagy response toward cellular stresses such as that provoked by protein aggregates (Fig. 1).

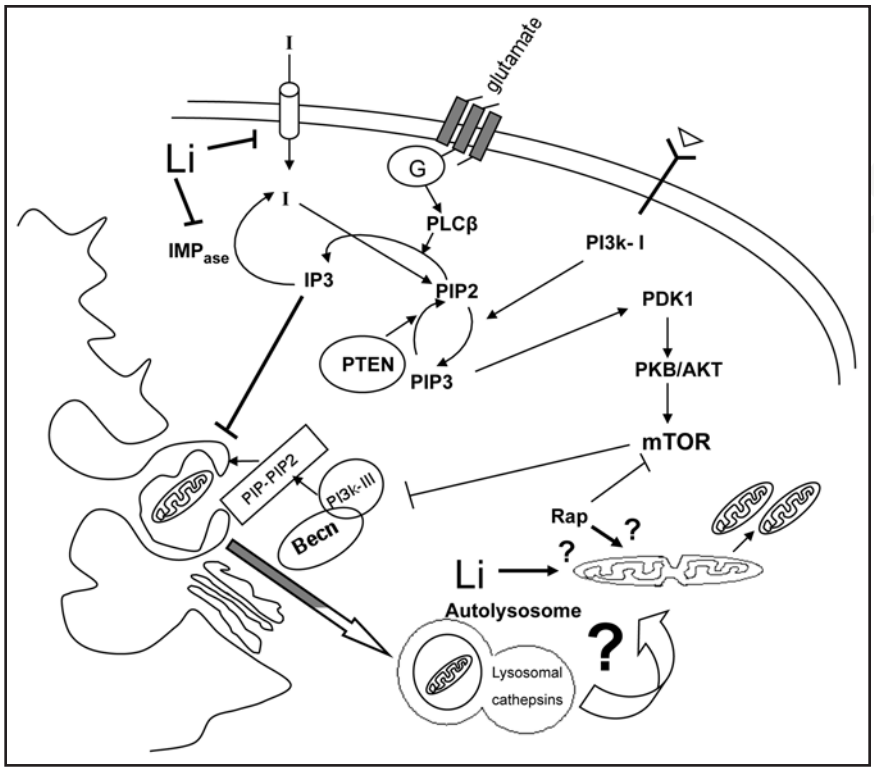

Figure 1. Scheme of Lithium targets and pathways activating autophagy and mitogenesis. The autophagy-inducing ability of lithium (Li) is attributed to its inhibitory effect on inositolmonophosphatase (IMPase) which limits the availability of free inositol (I) necessary for building phosphatidylinositol-4,5-phosphate (PIP2). The latter is the substrate of phospholipase C-β (PLCβ) which leads to the generation of (myo)-inositol-1,4,5-triphosphate (IP3) (and of diacylglycerol). IP3 has been shown to inhibit autophagy, therefore decreased levels of I and IP3 by lithium results in autophagy upregulation. Stimulation of membrane hormone/growth factor receptors activates class I phosphatidylinositol 3' kinase (PI3k I) which produces posphatidylinositol-3,4,phosphate (PIP3) from PIP2 (the phosphatase PTEN reverses this reaction). PIP3 is the donor for PKB/Akt phosphorylation and consequent activation of the mTOR pathway, which inhibits the Beclin 1 (BECN)-PI3k III-dependent activation of autophagy. Inactivation of mTOR by rapamycin (Rap) results in the activation of autophagy. Strikingly, both lithium and rapamycin, though activating autophagy through different and independent pathways, have mitochondriogenic effects, as the prolonged treatment with either drug results in the generation of small mitochondria. How lithium can exert such a mitochondriogenesis effect is at present unclear and deserves further investigation. It is tempting to suppose a link between the two stimulatory effects (on autophagy and on mitochondriogenesis), as if a 'sensor' triggered the mitochondriogenesis to compensate for mitophagy and so restore the normal equipment of cellular mitochondria.

Lithium, Mitochondria and ALS

As reported above, MN in ALS feature a number of altered mitochondria, and following lithium administration we found a normalization in the structure of mitochondria. This was initially interpreted as a further consequence of the induction of autophagy. However, when we counted the number of mitochondria we found a marked increase in their number. These appeared as normally structured, abundant and small organelles, which often appeared in the process of division (Fig. 2). This suggested a specific stimulatory effect of lithium on the biogenesis of mitochondria. Therefore, we focused on this effect and we found that in fact lithium promotes the biogenesis of neuronal mitochondria as shown by using a variety of approaches: counts by electron microscopy in vivo, cytofluorimetry following MitoTracker Green and Red in cell lines, immunofluorescence in ventral horn cultures, and by assaying the increase of mitochondrial mRNA and DNA in vivo. Altogether these findings provide strong evidence that lithium increases mitochondria independently of the specific biological matrix. In fact, the mitochondriogenesis of lithium that we found in the spinal cord in vivo or in neurons in vitro or in cells lines, was also recently demonstrated in endothelial cells of the aorta and seems to be a generalized effect of lithium, unrelated to a specific tissue.[13] Since in our experiments this was replicated by using another autophagy inducer, rapamycin, which owns a mechanism of action distinct from lithium, we suggest that induction of autophagy per se might be the trigger for the biogenesis of mitochondria (Fig. 1). This point is very intriguing since it may erase doubts concerning the potential risks of losing too many mitochondria when using a therapy based on autophagy inducers. In our opinion this point should be extensively investigated since it is a crucial aspect of autophagy and may lead to novel treatment for a variety of medical conditions beyond the central nervous system including oncology, immunology, and cardiovascular disease. A specific effort should be invested in elucidating the biochemical signaling bridging the activation of autophagy with the stimulation of mitochondriogenesis (Again, question points in Fig. 1).

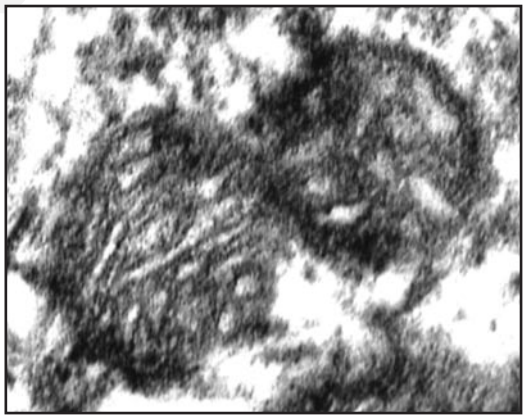

Figure 2. Lithium increases mitochondriogenesis. Representative picture showing a dividing mitochondria within the motor neurons, following lithium administration. Scale bar = 0.04 μm.

Lithium, Astrocytes and Neural Progenitor Cells

Astrocytic activation is a well-accepted feature of ALS.[14] Mutant astrocytes have an active role in MN degeneration by releasing toxic factor and amplifying the microglia-mediated inflammatory response.[15,16] We observed a significant decrease, following lithium administration, of GFAP immunopositivity in lamina IX of the SOD1^{G93A} mice spinal cord. Recently, this effect of lithium has been described in an "in vitro" model of rat cerebellar cultures, where lithium was able to enhance neuronal survival while inhibiting

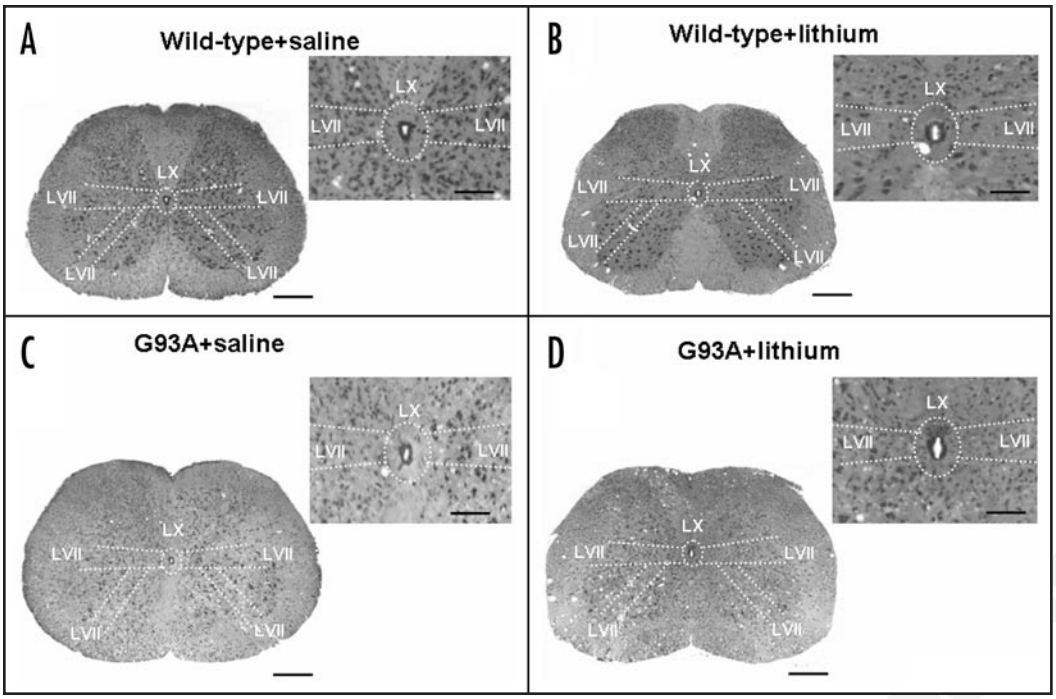

Figure 3. Motor neuron loss in the spinal cord of G93A mice. Low magnification of Nissl stained lumbar spinal cord sections of wild-type and G93A mice at the end of the disease. Note the loss of motor neurons in the anterior horn of the grey matter in the G93A mice (C and D), compared with wild-type mice (A and B). Despite the reduction of alpha motor neurons, chronic lithium treatment produced a marked increase in the cell density in the subependimal zone corresponding to the lamina X and lamina VII (insert at high magnification in D, compared with inserts in A–C). Scale Bars (A–D) = 440 μm; high magnification inserts = 176 μm.

astroglial growth.[17] So, we may hypothesize a dual effect of lithium. While inhibiting astroglial growth and proliferation, lithium treatment leads to neuronal differentiation. In turn by inhibiting glial cell proliferation, lithium hampers the release of some hypothetical glial-related toxic factors, further protecting the surrounding environment and the newly formed neurons.

Neurodegenerative and pathological processes promote neurogenesis as described for human Alzheimer's and Huntington's disease patients.[18,19] Previous papers found an increase in the number of neural progenitor cells (NPC) following chronic disease of the spinal cord (either for a long time interval following a spinal trauma or during the course of ALS).[20,21] Nonetheless, none of the studies provide evidence for a net increase in neuron number and the prevalent literature suggests that these NPC tend to differentiate into glial cells. In our case, the concomitancy of a chronic disease of the spinal cord joined with lithium administration leads to a net increase of NPC which specifically differentiate into Renshaw-like cells belonging to lamina VII. These neurons are critical in degenerative conditions of the spinal cord and seem to be affected earlier than MN in the course of ALS.[22,23] Strikingly, lithium alone is not able to alter the number of these neurons in the healthy spinal cord. The neurogenetic action of lithium is amplified in the diseased condition, at least in the spinal cord. In other brain areas, however, such as the hippocampus, where the differentiation of adult stem cells towards a neuronal phenotype is evident, lithium administration produces exactly the same effect we observed in lithium-treated ALS-affected spinal cords: suppression of glial differentiation and increase of calbindin 28K neurons.[24] The effect we describe in the spinal cord was mainly focused on the net increase of Renshaw-like cells of lamina VII, although in this addenda we wish to emphasize that also lamina X of the spinal cord undergo a massive neuronogenesis following lithium exposure (Fig. 3). In our opinion, the occurrence of such an effect should be regarded not merely as an advance in understanding the static pathology of ALS and the dynamic of stem cells proliferation but it rather represents an important advance to understand the clinical symptoms of ALS, the pathophysiology of the disease and the effects of lithium as a potential cure. In fact, the early loss of Renshaw cells is expected to leave MN more prone to the excitatory volley coming from descending and afferent pathways, thereby leading to uncontrolled contractions of single motor units (fasciculations). On the other hand, the absence of the tonic inhibitory influence played by Renshaw cells on the MN membrane might explain the vulnerability of these cells to the excitatory effects of glutamate. The rescue of these inhibitory neurons obtained by chronic lithium administration is expected to buffer the glutamate-surrounding neurons, thus reducing the potential concomitance of excitotoxicity.

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