REVIEW

BIOLOGICAL EFFECTS OF LITHIUM – FUNDAMENTAL AND MEDICAL ASPECTS

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In this review, the authors present data on the biological effects of lithium in human and animal body and the subsequent manifestations of these effects. Since the lithium ion radius is close to that of magnesium, lithium ion can compete with magnesium as cofactor of certain magnesium-dependent enzymes. Numerous studies have shown lithium to be an inhibitor of glycogen synthase kinase 3. It also inhibits the phosphatases involved in the metabolism of phosphoinositides and phosphoadenylate 3'-nucleotidase. As these enzymes play an important and sometimes crucial role in signaling systems activity, lithium is able to affect the essential physiological, adaptive and pathogenetic processes.

K e y   w o r d s: lithium, glycogen synthase kinase 3, β-amyloid peptide, phosphoinositides, Wnt signaling cascade, diabetes.

The research of the biological activity of lithium began in the 19th century, and even the first studies revealed it impacts the process of development and metamorphosis. In 1942, Joseph Needham, a prominent British biochemist, noted that it is of no use to look for a biochemical effect of lithium of equal significance as its morphological effect (cited by Voinar A.I. [1]). However, several years later, it was found that lithium has therapeutic effect in treatment of bipolar mood disorder, and the study of mechanism of its action resulted in the discovery of impressive number of biochemical effects of lithium.

In contrast to many pharmaceuticals, lithium does not bind to cellular receptors, but interacts with intracellular messengers and alters signal transduction. Today, lithium morphogenic effects are mainly associated with Wnt-dependent signaling pathway. In embryogenesis this signaling system regulates cell proliferation, cell polarity and differentiation [2]; in adult it controls tissue regeneration, stem cell homeostasis, cell proliferation. Within the CNS Wnt-dependent signaling system defines the neu-

rons growth, axonal remodeling, and synapses formation [3]. A special role in Wnt signaling pathway belongs to a glycogen synthase kinase 3 (GSK-3, EC 2.7.11.26). This enzyme was first discovered in research of glycogen metabolism, but since it has been identified to play important role in the regulation of many other biochemical processes. Lithium was found to inhibit this enzyme.

Inhibition of GSK-3 has been demonstrated by some researchers to be important in implementation of Wnt signaling. Lithium, being introduced in the Hamburger-Hamilton stage 3 of chic embryonic development, caused abnormalities in heart development, and these defects were particularly associated with Wnt signaling system [4]. An important role in embryonic development of heart plays connexin 43, which is involved in synchronization of cardiomyocytes contractions throughout lifetime. It was investigated whether lithium can attenuate post-infarction arrhythmias by increasing of connexin 43 induction via GSK3β/β-catenin system and activating Wnt-dependent and phosphoinositide 3-kinase/Akt signaling pathway [5]. Rats after coronary artery ligation were

given lithium for 4 weeks. The level of connexin 43 in heart substantially decreased after infarction, and this change was significantly attenuated by lithium. Arrhythmia scores were reduced. Phosphorylation of Akt in the border zone increased 4.5-fold in animals receiving lithium. GSK3β phosphorylation and β-catenin translocation into nucleus increased 11.2- and 3.6-fold, respectively. These data indicate that chronic treatment with lithium after myocardial infarction leads to rise in level of connexin 43 through Wnt- and phosphoinositide 3-kinase/Akt - dependent GSK3β systems and reduces arrhythmia parameters.

In some research it was shown that patients receiving lithium were less likely to have bone fractures [6], although other studies did not confirm these findings [7]. Older people taking lithium were reported to have less risk of fractures [8]. It is known that alcoholism impairs healing of broken bones. In mice with the tibia fracture, alcohol slowed the formation of cartilage, reduced the formation of active β-catenin and increased the level of inactive β-catenin. Lithium decreased the GSK3β activity in cartilage, increased the activity of β-catenin and restored the formation of cartilage and its ossification [9]. These findings point out important role of Wnt-dependent system to be played in bone growth and bone formation.

Very interesting results were obtained in research on mice with a genetic defect of Wnt coreceptor - low density lipoprotein LRP-5. Due to the absence of Wnt signaling, bone mass reduced and bone structure impaired [10]. The administration of lithium led to activation of Wnt-catenin system, increased bone mass and improved bone structure. A similar effect was observed in SAMP6 mice (line shows accelerating aging and develops osteoporosis early in life). In cultured calvarial osteoblasts from LRP-5 knockout mice, lithium activated canonical Wnt-dependent signaling pathway [10].

Important results were obtained when studying effect of lithium on the differentiation of mesenchymal stem cells [11]. These cells, present in human bone marrow, are the precursors of osteoblasts, chondrocytes and adipocytes, and can be used in regenerative medicine. Lithium was found to enhance osteogenic differentiation of these cells in animal models; however, results obtained on human cells were not so clear. The authors conducted a high-throughput study of the transcriptome of lithium-treated human stem cells to assess the ability to undergo osteogenic differentiation. Upon lithium treatment, suppression of proliferation and increase in alkaline phosphatase activity were observed. Using microarray analysis, downregulation of adipogenic genes expression and expression of genes involved in lipid biosynthesis were shown. Expression of the osteoclast factors and immune response genes (*Il7*, *Il8*, *CXCL1*, *CXCL12*, *CCL20*) also decreased. Negative transcriptional regulators of the osteogenic program (TWIST1 and PBX1) were suppressed, whereas genes involved in mineralization were induced. Gene ontology analysis showed that expression of genes related to the mesenchymal cell differentiation and signaling were upregulated. The authors concluded that lithium enhances the differentiation of mesenchymal stem cells into osteoblasts. Earlier, others researchers had discovered that in lithium-exposed human mesenchymal stem cells expression of genes responsible for their differentiation and intracellular signaling was upregulated [12]. It should be noted that in this work authors studied the role of Wnt signaling and involvement of lithium in stem cell differentiation in the presence of dexamethasone - activator of differentiation [12].

To some extent the contradictions of these data were solved when studying differentiation of mouse pre-osteoblasts MC3T3-E1 and pluripotent mesenchymal cell line C2C12 [13]. It was shown that lithium inhibits bone morphogenic protein 2 (BMP-2) signaling in both cell lines. Lithium treatment decreased SMAD1,5,8 phosphorylation and did not affect dephosphorylation. Lithium was suggested to affect the early stages of osteogenic differentiation via both canonical Wnt -dependent signaling and a previously unknown GSK3/Wnt - independent pathways [13].

Function of the Wnt/β-catenin system as a messenger is obviously universal. It was shown that the activation of Wnt/β-catenin-dependent signaling pathway stimulates the proliferation of cultured human Sertoli cells [14]. A significant increase in the mRNA and protein c-myc levels was observed upon lithium treatment. Knockdown of c-myc expression attenuated the ability of Wnt/β-catenin system to stimulate proliferation. It was concluded that the activating effect of Wnt/β-catenin system on Sertoli cell proliferation is mediated by *c-myc* gene.

As we mentioned above, an impetus for the study of lithium effect on the fundamental biochemical processes has been given by successful treatment with lithium of bipolar disorders. However, the posi-

tive effect of lithium has not always been observed. An attempt was made to find a link between the response to treatment with lithium and gene expression [15]. Microarray analysis was used to study gene expressions in blood of both depressed patients successfully treated with lithium and patients resistant to the therapy. The differences were found in 127 genes. The most significant ones were observed among genes involved in the regulation of apoptosis. Several anti-apoptotic genes including *BCL2* and insulin receptor substrate 2 (*IRS2*) were up-regulated in patients successfully treated with lithium, at the same time pro-apoptotic genes, including BCL2 antagonist/killer 1 (*BAK1*) and agonist of cell death associated with BCL2 (*BAD*), were down-regulated. In patient resistant to lithium therapy *BCL2* and *IRS2* were down-regulated, whereas *BAK1* and *BAD* were up-regulated after one-month treatment with lithium. Attempts to evaluate the prospects of lithium therapy using GWAS (gene-wide association study) are made.

It is known that depression, stress and aging are accompanied by shortening of leukocyte telomeres. The highest activity of telomerase in brain was found in hippocampus. However, it is unclear, whether depression is associated with telomerase dysfunction in hippocampus [16]. To assess telomerase activity in hippocampus and effect of lithium, the authors used a genetic model of depression in rats. Telomere length and telomerase reverse transcriptase (Tert) expression were determined in this study. It was found that naïve Flinders Sensitive Line rats with genetic modeled depression had shorter telomeres, down-regulated Tert and brain-derived neurotrophic factor and reduced telomerase activity compared to the Flinders Resistant Line controls. Lithium treatment normalized Tert expression and telomerase activity, as well as increased level of β-catenin.

Interesting results were obtained when analyzing pathogenesis of oculopharyngeal muscular dystrophy, a genetic disorder that generally developing in middle aged individuals [17]. It is associated with the number of encoding alanine GCG triplets in PABPN1 gene (poly (A)-binding protein nuclear 1). The normal gene contains (GCG)6 sequence which encodes polyalanine tract, while upon the disease the (GCG)8-13 sequence was observed. Thus, this disease develops due to the expansion of polyalanine tract in PABPN1 protein. Since pathogenesis of polyalanine pathology is associated with GSK3, the authors used an inhibitor of this enzyme - lithium. Lithium had a protective effect on cultured mouse

myoblasts transfected with a gene encoding exp-PABPN-1 (nuclear protein with the polyalanine expansion). Lithium was shown to prevent death of these cells.

Promising results were obtained while studying fragile X syndrome (Martin-Bell syndrome), a sex-linked genetic mental retardation. This disease is associated with CGG triplet repeat expansion in *Fmr1* gene on X chromosome, resulting in a failure to express *Fmr1* gene required for normal development of the nervous system. It was previously shown that GSK3 activity increased in mice with *Fmr1* gene knockout. Lithium administration led to suppression of GSK3 activity in different brain regions of the knockout mice. GSK3 phosphorylation at serine was substantially increased. Physiological parameters in knockout mice were also ameliorated upon lithium administration [18]. Analysis of the physiological parameters in mice with *Fmr1* gene knockout (used as a model of fragile X syndrome in humans) showed that lithium administration significantly improved these parameters [19]. Assessment of correlation between lithium effect and single nucleotide polymorphisms was presented in [20].

Now it is known that lithium exhibits therapeutic effect in treatment of some neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) [21], amyotrophic lateral sclerosis [22]. Lithium may alter the biochemical processes that determine the pathogenesis of these diseases. Lithium was found to reduce tau protein phosphorylation [23]. Apparently, lithium prevented neuronal death caused by fibrillar β-amyloid [24]. It inhibited the formation of β-amyloid peptide (Aβ) in HEK293 cells stably transfected with the Swedish amyloid precursor protein APP 751, and in the brain of transgenic mice PDAPP (APPv717f) with modeled AD [25]. These studies showed that lithium affects APP processing, in which GSK-3 is involved. Inhibition of GSK-3 by antisense oligonucleotide mimics the effect of lithium. In the brain of transgenic mice PDAPP (APPv717f) lithium prevented increase in Aβ level and reduced plaque formation.

The importance of Aβ protein in the nervous system pathology was demonstrated in the experiments with fruit fly Drosophila [26]. In this work authors used several Drosophila lines with adult-onset expression of Aβ42 protein. The Aβ42 accumulation induced neuronal changes and increased flies mortality. Lithium was shown to reduce Aβ42 level in Drosophila and to prevent toxic effect of Aβ42.

Locomotor disorders reduced at various doses of lithium; however only low doses extended Drosophila lifespan. Increased flies life expectancy at lithium treatment was observed in [27]. The mechanism of this effect, according to the authors, includes inhibition of GSK-3 activity and activation of NRF-2 transcription factor.

An increase in the β-amyloid level is observed not only at Alzheimer's disease, but also at traumatic brain injuries that may predispose to the disease. Lithium administration to rats in 30 min after injury and during the next 5 days was accompanied by GSK3β phosphorylation, accumulation of β-catenin, and a decrease in hippocampus CA3 neurons loss [28]. Lithium treatment led to reduced deficiency of hippocampal-dependent cognitive processes assessed on 14-28 days post-injury. Post-injury lithium treatment decreased accumulation of β-amyloid, its precursor APP and β-APP-cleaving enzyme-1 overexpression in the mice brain on the 3d day [29]. Lithium also reduced tau phosphorylation in the thalamus, which was increased after injury. Physiological parameters of neural activity, determined on the 10-20 days post-injury, were significantly better in the animals treated with lithium. To date, most researchers associated the therapeutic effect of lithium with its inhibitory effect on GSK-3 [30].

Above we have presented data on the effect of lithium on differentiation of mesenchymal stem cells. Neurogenesis in the hippocampus is impaired in Down syndrome patients. The ability of lithium to stimulate transition from neuronal precursor cells to differentiated cells was studied on the Ts65Dn mouse model of Down syndrome [31]. The lithium administration restored hippocampal neuron population and memory parameters in adult mice with Down syndrom. GSK-3α/β knockout mice, in which the replacement of serine to alanine blocks serine phosphorylation in both enzyme isoforms resulting in the enzyme hyperactivity, displayed changes in behavioral performance, impairment of cognitive skills and precursor-cells differentiation in the hippocampus [32]. Comparison of each GSK-3 isoform knockouts revealed that hippocampal precursor cell proliferation did not change in GSK-3α knockouts, whereas was greatly suppressed in GSK-3β knockouts.

Since GSK-3 is involved in the regulation of carbohydrate metabolism and many other biochemical processes, changes in the activity of this enzyme may lead to various shifts of regulatory and pathologic nature. It is known that insulin receptor binding to hormone induces Akt activation that, in turn, increases GSK-3 phosphorylation and its inactivation. Tissue GSK-3 activities in various animal models of carbohydrate metabolism disorder were determined.

It was found that both basal and induced by high-fat diet GSK-3 activities in adipose tissue of inbred diabetes- and obesity-prone mice (C57BL/6J strain) were higher 2.3- and 3.2-fold, respectively than in mice resistant to obesity and diabetes (A/J). However, high-fat diet did not lead to an increase in the GSK-3 activity in liver and muscles of C57BL/6J mice [33]. Glucose uptake by muscle of insulin-resistant Zucker diabetic fatty rats *in vitro* increased at lithium treatment in both control conditions and in the presence of insulin in the incubation medium [34]. Lithium activated glycogen synthase (EC 2.4.1.11) in muscle of both lean and fat animals.

The study of GSK-3 activity and expression in biopsies of vastus lateralis from type 2 diabetic patients revealed that both parameters were increased regardless of patients' body weight [35]. The GSK-3 activity was inversely proportional to the activity of glycogen synthase. Lithium salts were found to activate glycogen synthase, increase glycogen level and stimulate glucose transport, thus showing antidiabetic effect [36-38].

Analysis of the mechanism of lithium salts action in the experiments with rat hepatocyte suspension reaffirmed glycogen synthase activation and GSK-3 inhibition [39]. Neither cycloheximide nor actinomycin D modulated the effects of lithium. Glycogen synthase mRNA level also did not change. Amount of glycogen synthase protein determined by Western blot analysis was increased in the presence of lithium, and, moreover, was further profoundly increased after addition of calpain and proteasome inhibitor ALLN to the medium.

The important role that GSK-3 plays in the development of insulin resistance and type 2 diabetes, as well as the results obtained on the effect of non-specific GSK-3 inhibitor lithium allowed to suggest that specific inhibitors of GSK-3 may be promising drugs for the treatment of this disease [40]. It was hypothesized that pathogenesis of type 2 diabetes is associated with hypersecretion of incretins GIP glucose-dependent insulinotropic peptide [41]. An increase in the serum GIP level induced desensitization of insulin receptor *in vivo*. Insulinotropic effect of GIP in patients with type 2 diabetes and in their

first-degree relatives was lost [42]. GIP secretion is regulated by the Wnt/β-catenin system. The authors investigated the effect of lithium on the GIP formation by entero-endocrine cells. The analysis of the functional elements of *GIP* gene promoter and their involvement in the regulation of expression was performed. Lithium induced expression of *GIP* gene affecting a conserved region of the promoter-proximal element. Lithium facilitated binding of lymphoid enhancer-1/β-catenin with *GIP* promoter and reduced immunoprecipitation of chromatin with T-cell factor-4 and histone deacetylase 1. It was concluded that Wnt and lithium are incretin inducers.

An important component of diabetes pathogenesis and, particularly, its complications is an increase in reactive oxygen species (ROS) production resulting from hyperglycemia. The possible protective effect of lithium upon hyperglycemia was studied on PC12 cells *in vitro* [43]. The cells were pretreated with lithium and incubated for 24 h with glucose. Lithium was shown to protect PC12 cells from apoptotic death owing to a decrease in the ROS formation and the Bax/Bcl-2 ratio, and inhibition of caspase-3 activity as well as Jnk and P38MAPK phosphorylation. The authors suggested that at hyperglycemia lithium can prevent mitochondrial apoptosis via both Jnk- and P38-signaling pathways.

Intensive apoptosis was observed when renal proximal tubular cells were cultured in the absence of growth factors [44]. The authors studied the possibility of enhancing cell survival under these conditions by activating Wnt-dependent signaling pathway via GSK-3 inhibition by, in particular, lithium. Lithium treatment led to decrease in GSK-3 activity and β-catenin phosphorylation, and to increase in cyclin D1 expression (promitogenic response factor). Furthermore, lithium significantly increased Akt phosphorylation (activation) and inhibited caspase 3. Lithium also increased the expression of insulin-like growth factor II (IGF-II). These results indicate that activation of GSK-3 is important to trigger apoptosis. The authors suggested that lithium promotes survival of kidney epithelial cells, inhibiting apoptosis via inhibition of GSK-3 and activation of Wnt-signaling pathway followed by IGF-II secretion. Extracellular IGF-II in the medium is an autocrine growth factor that activates, particularly, antiapoptotic phosphatidylinositol-3-kinase/Akt pathway.

Analyzing the apoptosis process in human adrenal cortex, we found that lithium effects largely depend on the nature of tissue [45]. Lithium *in vitro* exhibited antiapoptotic effect in adenocarcinoma tis-

sue. In contrast to tumor tissue, lithium enhanced DNA fragmentation in adrenal tissue upon Cushing disease - in hypertrophied tissue of adrenal glands.

Development of apoptosis, to a large extent, related to the changes in mitochondria. Swelling of brain mitochondria, their depolarization and a release of cytochrome c in response to Ca^{2+} were suppressed by replacement of KCl in the medium for LiCl [46]. The authors consider this effect of lithium to be a result of counteracting Ca^{2+}-induced opening of mitochondrial permeability transition pores.

It was reported that upon bipolar disorder, successfully treated with lithium, dysfunctions of neuronal mitochondria, in particular, respiratory complex 1 as well as alteration in DNA methylation were observed. Scola et al. [47] examined the effect of lithium on these dysfunctions. Neuronal cells of E18 line rats were treated with lithium, and rotenone was added to the incubation medium. Rotenone decreased the complex 1 activity and the ATP production, induced apoptosis and increased number of dead cells. The DNA methylation (methylcytosine) level and hydroxymethyl level increased. Lithium prevented changes in the complex 1 activity and methylation. Cell death decreased.

Sleep deprivation (one of the models of mania) in mice for 96 h caused changes in the activity of respiratory complexes in some parts of brain [48]. Lithium administration prior to sleep deprivation prevented these changes.

The initial stage of mitochondrial changes leading to apoptosis is a transition to high permeability: opening the pores and release of cytochrome c into the cytoplasm. Using various biochemical and molecular biological approaches, Juhaszova et al. found that hypoxia/reoxygenation led to a decrease in the threshold for the mitochondrial permeability transition in cardiomyocytes [49]. Cell survival inversely related to mitochondrial depolarization. A wide range of cardio/neuroprotectors affects cells reducing opening of permeability pores. It was reported that GSK-3β isoform (but not GSK-3α), which prevents pore opening, is the effector element in protection signaling. However, the mechanism of GSK-3β translocation to mitochondria remains unclear. This question was investigated in the study [50]. Cardiomyocytes from H9c2 mouse line were transfected with mutant constructions, including GSK-3β and fluorescent protein. Following H_2O_2 treatment, GSK-3β of wild strain cells was translocated from the cytosol to mitochondria. After this

phase a mitochondria permeability transtion and cell death were observed. Moreover, signal intensity of nine spots of antiGSK-3β precipitates on two-dimensional electrophoresis increased more than 3-fold. MALDI-TOF/MS test identified that one of the spots contains VDAC2 - voltage-dependent anion channel protein 2 involved in pore formation. Knockdown VDAC2, but not VDAC1 and VDAC3 by miRNA impaired GSK-3β translocation and pore opening under oxidative stress. GSK-3β translocation was also attenuated at Lys15 replacement to alanine in N-terminal site of GSK-3β molecule. Both GSK-3β translocation from cytosol to mitochondria and H_2O_2 induced cell death were suppressed in the presence of lithium, a GSK-3β inhibitor. The interaction of GSK-3β and VDAC2 depended on kinase activity, and was also suppressed by lithium [50].

Extensive experience in clinical use of lithium in treatment of bipolar disoreder revealed that lithium can cause disorder in thyroid function [51, 52]. The most common complication is a goiter, less often - hypothyroidism. In patients with tendency to autoimmune processes, thyroiditis, hyperthyroidism and less often Graves' disease can be developed [53]. However, in some cases, lithium was reported to have therapeutic effect on thyroid. Thus, lithium has been successfully applied in patient with Graves' disease exhibiting methimazole intolerance [54].

Goiter can be developed via two pathways. A decrease in thyroid function results in an increase in the secretion of pituitary thyrotropic hormone inducing the thyroid tissue proliferation. Lithium may also stimulate the proliferative processes by activating Wnt/β-catenin signaling [55].

In 1996, two research groups independently demonstrated that lithium affects GSK-3 *in vitro* [56, 57]. However, the inhibition constants were determined to be significantly higher than the actual lithium concentration in cells. Analysis of this fact allowed the authors to suggest that a low activity of lithium in these experiments was determined by the concentration of Mg^{2+} in the incubation medium [58]. It was hypothesized that lithium competes with magnesium in inhibiting of GSK-3 activity. It was shown that the enzyme requires magnesium ions. An increase in concentration of ATP which chelates magnesium *in vitro* led to reduced activity of GSK-3 [58]. It is known that the lithium and magnesium ionic radii are very close. With respect to ATP and substrate, lithium is not a competitive inhibitor.

Function of Wnt/β-catenin-dependent signaling system is, to great extent, associated with the activi-

ty of glycogen synthase kinase 3 (GSK-3) (tau-protein kinase, 2.7.11.26). This enzyme is an antagonist of Wnt signaling and should be inhibited for successful signal transduction. GSK-3 in cytoplasm binds to transcription factor β-catenin; to protein, which is encoded by gene adenomatous polyposis coli (APC); to casein kinase 1α and to adaptor scaffold protein Axin to form complex, in which β-catenin phosphorylation is activated (destructive complex). β-catenin phosphorylation by GSK-3 facilitates its degradation by proteasomes [59]. This scenario occurs in the absence of Wnt ligands. Since lithium inhibits GSK-3, the multiprotein complex does not phosphorylates β-catenin, thus, it accumulates and translocates to nucleus, where it acts as a co-activator and transcription factor of the TCF/LEF family [2].

In organism another pathway for lithium regulation of the GSK-3 activity involving phosphoinositides and Akt is possible. Akt can phosphorylate N-terminal serine of GSK-3 molecule that lead to inactivation of the latter. GSK-3 phosphorylation occurs via serine 21 in isozyme GSK-3α and serine 9 in isozyme GSK-3β. Akt in cells is a part of signaling complex composed of Akt, β-arrestin 2, and protein phosphatase A2, in which Akt is inactive. Lithium is able to disrupt the signaling complex, releasing and activating Akt [60, 61]. This mechanism is supported by the findings that lithium is not able to increase phosphorylation of GSK-3 in mice with reduced Akt activity [62]. The mechanism for lithium-induced Akt release from complex is not entirely clear. Lithium is likely to compete with magnesium which is essential for the complex formation [61].

The study of the molecular mechanisms of lithium action revealed long ago that lithium affects phosphoinositides metabolism [63]. This effect is associated with inhibition of inositol monophosphate phosphatase (IMPase, EC 3.1.3.25). Lithium was later found to inhibit the phosphatase activity of several genetically related enzymes. Along with IMPase this group includes inositol polyphosphate-1-phosphatase (IPPase), fructose-1,6-bisphosphatase (FBPase), 3'-phosphoadenosine 5'-phosphate phosphatase (PAPase) and the enzyme capable of hydrolyzing both inositol-1,4-bisphosphate and PAP. All these enzymes exhibit obligatory need for Mg^{2+} for catalysis. By crystallographic and genetic approaches it was shown that they have common sequence motif Asp-Pro- (Ile or Leu) -Asp- (Gly or Ser) - (Thr or Ser) that participates in metal binding and catalysis [63]. The authors compared the 6 α-carbon

coordinates of this motif in inositol polyphosphate-1-phosphatase, inositol monophosphate phosphatase and fructose-1,6-bisphosphatase, and found that they are comparable with a standard deviation of 0.27 to 0,60 Å. Authors found that in the indicated proteins with the aligned motif (mentioned above) a common core structure emerged comprising 5 α-helices, 11 β-sheets and 155 amino acid residues. IMPase-substrate complex contains three Mg ions. The metal binding sites differ. The first binding site of human IMPase includes Glu 70, Asp 90 and the carbonyl oxygen of Ile 92 [64]. The second binding site of Mg^{2+} includes Asp 90, Asp 93 and Asp 220 [65]. The third site of human IMPase is formed by Glu 70. It has been shown that lithium displaced Mg^{2+} from the second binding site [66].

E. coli suhB gene encodes a protein which exerts an IMPase-like activity and is strongly inhibited by lithium [67]. This enzyme is similar to the human IMPase and has the same amino acids in its active site. It was shown by 7Li-NMR spectroscopy that lithium binds to bacterial IMPase in the site 2 with aspartate residues 84, 87, 212. Using X-ray crystallography it was established that lithium binds to Staphylococcal IMPase at the second binding site of Mg^{2+} [66]. Lithium acts as a stabilizer of the post-catalyst complex and inhibits subsequent catalytic cycle. IMPase and IPPase are key enzymes in cyclic transformation of myo-inositol into PIP2 and, thereby in maintaining its pool. Lithium, inhibiting both IMPase and IPPase may cause a decrease in the level of inositol and modulate phosphoinositide signaling system involved in numerous regulatory processes.

While studying the regulatory effect of K^+ on the hormones formation in the human cortex adrenal glands cortex, we have shown that in this process inositide messengers play essential role. With increasing of the K^+ concentration in the incubation medium, accumulation of inozitol-, mono-, di- and triphosphates was observed [68]. The concentration of inositol triphosphate increased in the presence of lithium due to inhibition of inositol triphosphatases. Lithium at physiological concentrations of K^+ (without stimulation) exerted only slight effect. Increasing of the K^+ concentration in the medium during incubation of guinea pig adrenal slices stimulated [3H]-uridine incorporation into nuclear RNA fraction. The preincubation of slices with lithium prevented this effect of K^+ [69].

Adding of lithium to the medium during incubation of slices of conventionally normal human adrenal tissue prevented expression of mRNA of constitutive and inducible NO-synthases that had been activated by an increase in the medium K^+ concentration [70]. Since lithium inhibited activation of protein kinase C in the adrenal tissues under these conditions, this can indicate a possible mechanism for inhibition of NO-synthases expression.

Lithium was suggested to regulate autophagy by inhibiting of inositol phosphatase [71]. Recently, the notion about involvement of inositide signaling pathway in the pathogenesis of bipolar disorders has been confirmed [72, 73]. The authors found that knockout mice of *IMPA1* and *Slc5a3* genes exhibit lithium-like neurochemical and behavioral alterations. The authors, using DNA-microarray and PCR analysis, studied the alterations caused by both knockouts and lithium, and established that mitochondria associated genes were upregulated. Genes *Cox5a*, *Ndufab1* and *Ndufs7* encode proteins of the mitochondrial respiratory chain. Mitochondrial dysfunctions (observed upon bipolar disorders) were modeled in mice by administration of low doses of rotenone. It was revealed in the forced-swim test and the amphetamine-induced hyperlocomotion test that lithium and rotenone exert opposite effects. Given that *IMPA1* and *Slc5a3* genes encode proteins related to inositide metabolism, the authors speculate that lithium effects may be due to changes in inositol metabolism.

Inhibiting another phosphatase, namely phosphoadenylate 3'-nucleotidase (PAPase), lithium affects processes of sulfation, DNA repair and apoptosis. It was demonstrated that lithium inhibits Golgi-resident PAPase [74]. Inactivation of this phosphatase by molecular and biological methods led to neonatal lethality, abnormal lung development (similar to atelectasis), and dwarfism, associated with changes in cartilage [74]. PAPase inhibition was reported to cause accumulation of 3′-phosphoadenosine 5′-phosphate in cells [75]. It is formed from 3′-phosphoadenosine 5′-phosphosulfate - a universal donor of sulfate groups. The further transfer of sulfur to various acceptors is catalyzed by sulfotransferase and is very important. Generated 3′-phosphoadenosine 5′-phosphate is a potent inhibitor of PARP-1. *In vitro*, in micromolar range, it inhibited PARP-1 and reduced both PARP-1 automodification and heteromodification of histones.

The data presented in this review demonstrate an extremely wide range of biological effects of lithium. Some manifestations of these effects are
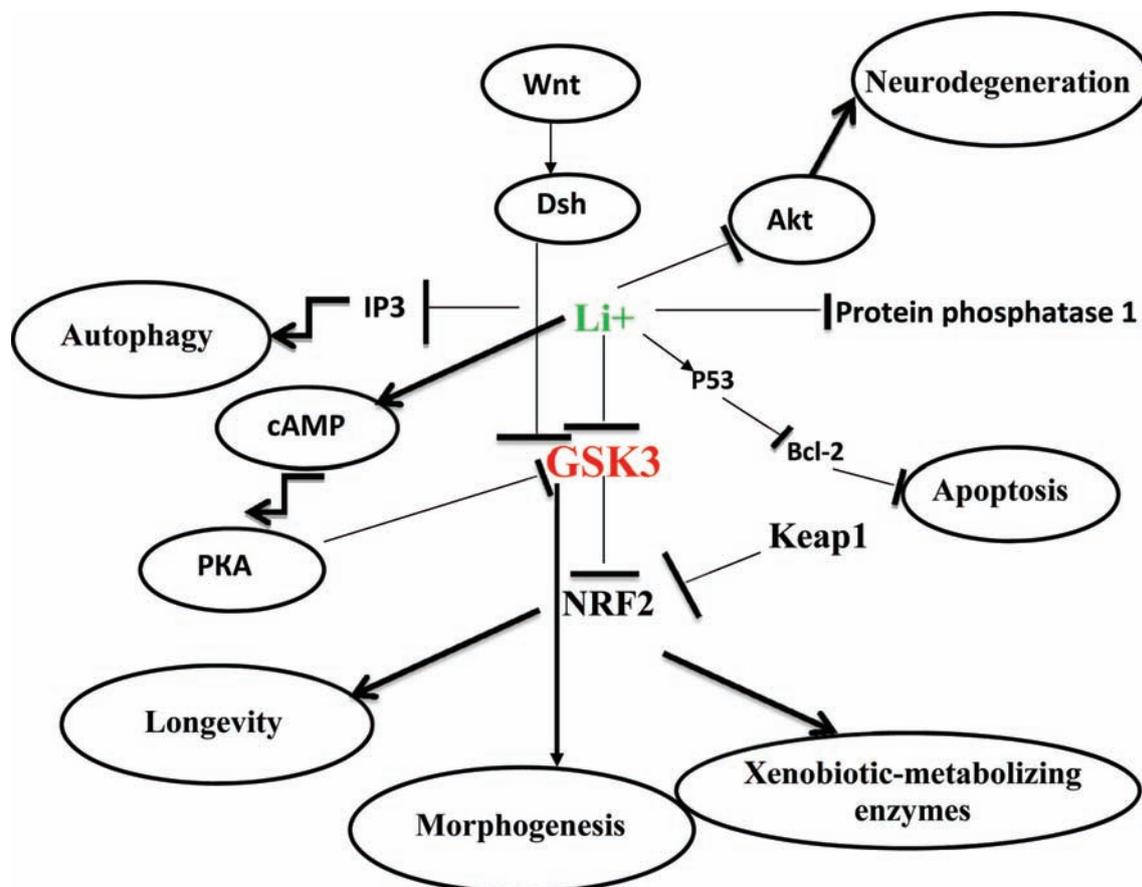
Pathways for lithium effects: Akt – protein kinase B, serine/threonine kinase; Dsh – Dishevelled proteins; IP3 – inositol 3-phosphate; GSK3 – glycogen synthase kinase 3; Keap-1 – Kelch-like ECH-associated protein 1; NRF2 – nuclear factor; PKA – protein kinase A; PP-1 – protein phosphatase 1; P53 – tumor suppressor protein; Wnt – peptide, from which activation of Wnt/β-catenin signaling pathway begins

summarized in Fig. These effects are regulated through involvement of various signaling systems. Since these messenger systems interact with each other, modulating the function of one of them leads to changes in a number of biological features. Due to such complexity, many effects of lithium still remain to be explained and interpreted.

БІОЛОГІЧНА РОЛЬ ЛІТІЮ – ФУНДАМЕНТАЛЬНІ І МЕДИЧНІ АСПЕКТИ

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В огляді представлені відомості про біологічні ефекти літію в організмі людини і тварин та наступних проявах цих ефектів. Так як іонний радіус літію близький до розміру іона магнію літій здатний конкурувати з магнієм як кофактор деяких магнійзалежних ензимів. Численні роботи свідчать, що літій є інгібітором кінази глікогенсинтази 3. Він також пригнічує фосфатази, які беруть участь в обміні фосфоінозитидів і фосфор аденілат 3′-нуклео-тидаз. Так як ці ензими відіграють важливу, а іноді ключову роль в діяльності сигнальних систем, літій здатний впливати на найважливіші фізіологічні, адаптаційні та патогенетичні процеси.

К л ю ч о в і   с л о в а: літій, кіназа глікогенсинтази 3, β-амілоїдний пептид, фосфоінозитиди, Wnt-сигнальний каскад, діабет.

БИОЛОГИЧЕСКАЯ РОЛЬ ЛИТИЯ – ФУНДАМЕНТАЛЬНЫЕ И МЕДИЦИНСКИЕ АСПЕКТЫ

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В обзоре представлены данные о биологических эффектах лития в организме человека и животных и о последующих проявлениях этих эффектов. Так как ионный радиус лития соизмерим с радиусом иона магния, литий способен конкурировать с магнием в качестве кофактора некоторых магнийзависимых энзимов. Многочисленные исследования свидетельствуют о том, что литий является ингибитором киназы гликогенсинтазы 3. Он также ингибирует фосфатазы, которые участвуют в обмене фосфоинозитидов и фосфор аденилат 3′-нуклеотидаз. Так как эти энзимы играют важную, а иногда и ключевую роль в сигнальных системах, литий способен влиять на важнейшие физиологические, адаптационные и патогенетические процессы.

К л ю ч е в ы е   с л о в а: литий, киназа гликогенсинтазы 3, β-амилоидный пептид, фосфоинозитиды, Wnt-сигнальный каскад, диабет.

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