# Apoptoza – mogući patofiziološki mehanizam u poremećajima raspoloženja kojeg mijenjaju litijeve soli

# Apoptosis – the potential pathophysiological mechanism in mood disorders modifiable by lithium salts

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**Sažetak**

Zadnjih godina naše znanje o patofiziološkim i patoanatomskim promjenama u središnjem živčanom sustavu bolesnika, koji boluju od širokog spektra kliničkih slika poremećenog raspoloženja, doživjelo je znatan napredak. Posebno, u tom smislu, naglasak treba staviti na istraživanja koja poremećaje raspoloženja opisuju kao neurodegenerativne bolesti u čijoj podlozi posebno značajno mjesto zauzima propadanje glija stanica.

Istraživanja koja opisuju apoptotski način propadanja glija stanica, ali i ostalih stanica CNS-a (engl. *central nervous system*, središnji živčani sustav) nude vrlo uvjerljive dokaze da je upravo apoptotski mehanizam, molekularno gledano, ključan u etiologiji poremećaja raspoloženja. Ove spoznaje nisu samo promijenile dosadašnji stav o tome kako su poremećaji raspoloženja uzrokovani poremećajem u monoaminergičkom sustavu neurotransmitera, nego su promijenila i razmišljanja o glija stanicama. Naime, do nedavno se mislilo kako su glija stanice isključivo potporno tkivo CNS-a, na neki način „vezivno tkivo". Međutim, danas sa sigurnošću možemo tvrditi da su glija stanice i funkcionalni dio CNS-a. Drugim riječima, glija stanice su glavno skladište ekscitatornog neurotransmitera glutamata, te o njihovom metabolizmu i doziranju otpuštanja glutamata ovisi podražljivost CNS-a. Na taj način glija stanice zajedno s neuronom predstavljaju funkcionalnu cjelinu. Nadalje, na glija stanicama su dokazani i ostali neurotransmiterski receptori, primjerice dopaminergički i serotonergički.

S druge strane, u psihofarmakoterapiji poremećaja raspoloženja, koja je danas temeljni dio terapije bolesnika s poremećajem raspoloženja, uključuje se primjena različitih psihofarmaka od antidepresiva do stabilizatora raspoloženja. Od ovih potonjih, soli litija zauzimaju posebno mjesto u terapiji poremećaja raspoloženja, bilo kao lijek u suzbijanju simptoma manično promijenjenog raspoloženja, ili kao stabilizator raspoloženja, pojačivač antidepresivnog djelovanja uz antidepresive ili lijek koji sprječava pojavu novih epizoda promijenjenog raspoloženja. Zadnja navedena indikacija litija čini se posebno zanimljivom jer time litij dobiva posebno mjesto u psihofarmakoterapiji, ali i farmakoterapiji općenito, jer je to lijek koji se koristi i za prevenciju, dok su ostali lijekovi pretežito kurativnog djelovanja. Uostalom, činjenica je da djelovanje litija, ali i antidepresiva ne nastupa odmah nego tek nakon nekoliko dana i tjedana uzimanja. Drugim riječima, da je terapijsko djelovanje litija samo u činjenici kako se to dosada shvaćalo, u promjeni neurotransmitera, promjena

**Abstract**

In recent years, our knowledge about the pathophysiological and pathoanatomical changes in the central nervous system (CNS) of patients suffering from a broad spectrum of clinical pictures of mood disorder has seen considerable progress. In this context, mention should be made of the studies that tend to characterize mood disorders as neurodegenerative diseases underlain to a significant extent by glial cell destruction. The studies that describe apoptotic destruction of glial cells and other CNS cells have provided strong evidence for the mechanism of apoptosis to be crucial in the etiology of mood disorder at the molecular level. These concepts have not only changed previous opinions on mood disorders to be caused by impairment in the monoaminergic system of neurotransmitters but have also modified current considerations about glial cells. Until recently, glial cells were believed to act exclusively as a CNS supportive tissue, a sort of "connective tissue". Now, however, glial cells can be with certainty described as a functional part of the CNS. In other words, glial cells are the main store of the excitatory neurotransmitter, glutamate, and CNS excitability depends on the glutamate metabolism and release by glial cells. In this way, glial cells with the neuron constitute a functional unity. Furthermore, other neurotransmitter receptors, e.g., dopaminergic and serotonergic receptors have also been demonstrated on glial cells. On the other hand, psychopharmacotherapy as the main treatment in the management of mood disorders includes the use of various psychopharmaceuticals, from antidepressants through mood stabilizers. Of the latter, lithium salts have a prominent place in the treatment of mood disorders, either as a drug to suppress the symptoms of manic mood disorders or as a mood stabilizer, for enhancement of antidepressant action along with antidepressants, or as a drug to prevent the occurrence of new episodes of mood disorder. The latter indication for lithium administration appears to be quite interesting, conferring a special place for lithium in psychopharmacotherapy as well as in pharmacotherapy in general, since it is also used for prevention, whereas other drugs mostly have curative action. The action of lithium as well as of antidepressants is known to only occur after a period, days or even weeks, of administration. Thus, had the therapeutic effect of lithium been strictly limited to the action on neurotransmitters, as believed to date, change in the clinical picture would occur immediately. Therefore, lithium should be postulated to act *via* a more subtle mechanism through CNS cell

kliničke slike nastupila bi trenutačno. Stoga treba pretpostaviti da litij djeluje puno finijim mehanizmom koji se zbiva kroz prilagodbu stanica CNS-a na nove uvjete, štoviše danas je sve više dokaza da litij može mijenjati živčanu plastičnost djelovanjem na apoptotske mehanizme.

**Ključne riječi:** litijeve soli; poremećaji raspoloženja; apoptoza; stabilizatori raspoloženja

adjustment to the new conditions. The more so, there is ever more evidence for lithium to be able to modify neuronal plasticity by its action on apoptotic mechanisms.

**Key words:** lithium salts; mood disorders; apoptosis; mood stabilizers

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## Pregled dosadašnjih spoznaja o biološkim promjenama u poremećajima raspoloženja

Danas poremećaji raspoloženja uz shizofreniju, anksiozne poremećaje te ovisnost o alkoholu i druge ovisnosti predstavljaju poseban istraživački izazov za kliničara-psihijatra, ali i za neuroznanstvenika. Sve je više dokaza o biologijskim uzročnim čimbenicima koji zajedno s okolišnim socijalnim čimbenicima dovode do duševnih bolesti (1). Interakcija je, svakako, vrlo složena i isprepletena nizom okolišnih čimbenika koji mogu mijenjati biologijsku ravnotežu središnjeg živčanog sustava, ali i obrnuto, čitav sustav od molekula i iona, preko staničnih struktura, neurona i glija stanica do neurotransmitera, u određenim makroanatomskim strukturama središnjeg živčanog sustava, može utjecati na izražavanje nečije osobnosti, a time i utjecati na socijalno okruženje pojedinca (2).

Poremećaji raspoloženja, u tom smislu, čine posebno zanimljive poremećaje jer po svojoj kvaliteti predstavljaju otklone u raspoloženju u konstantnoj noti svačije osobnosti. Raspoloženje je pak dugotrajno, unutarnje i predvidivo čuvstveno stanje pojedinca. Nekada su se isti poremećaji nazivali i afektivnim poremećajima, što bi svakako trebalo napustiti, jer je raspoloženje u odnosu na afekt trajna kvaliteta, pa bi odnos raspoloženja prema afektu mogli opisati kroz odnos klime prema trenutačnom vremenu (2).

Općenito govoreći, raspoloženje se može mijenjati prema nižem – depresivnijem i prema višem – euforičnom, maničnom. Svakako treba razgraničiti normalne promjene raspoloženja uzrokovane svakodnevnim životnim prilikama od onih koje su psihopatološke. Drugim riječima, oscilacije raspoloženja koje po svojoj jačini, trajanju, i nemogućnosti da ih osoba kontrolira odstupaju od svakodnevnih „životnih" promjena raspoloženja klasificiramo kao poremećaj raspoloženja. Najčešće se takve promjene raspoloženja javljaju bez ikakvog očiglednog vanjskog uzroka.

## A survey of previous concepts on biological changes in mood disorders

In addition to schizophrenia, anxious disorders, alcohol and other addictions, mood disorders pose a major research challenge for both clinician psychiatrists and neuroscientists. There is ever more evidence for the biological causative factors which, together with environmental social factors lead to mental disorders (1). This interaction is very complex and imbued with an array of environmental factors that may modify the biological balance of the CNS, and *vice versa*, the whole system from molecules and ions, through cellular structures, neurons and glial cells to neurotransmitters may in certain macroanatomical structures of the CNS influence the expression of a personality, thus exerting an impact on the individual's social environment (2).

In this sense, mood disorders are of special interest because by quality they represent mood deviation in the constant pattern of everybody's personality. Mood is a long-standing, inner and predictable emotional state of the individual. Mood disorders used to be referred to as affective disorders, which should definitely be abandoned because mood, unlike affect, is a permanent quality, so the mood to affect relationship could be described as the climate to current weather relationship (2).

Generally, mood can vary toward low, depressive mood or toward high, euphoric, manic mood. Normal mood variation caused by daily living should certainly be distinguished from psychopathological ones. In other words, mood oscillations that depart from the daily living mood variation by severity, duration and impossibility to control are classified as mood disorders. These mood changes usually occur without any overt external cause.

As mentioned above, the biological, social and psychological factors all play a major role in the onset of mood disorders. Therefore, biological, psychological and social therapeutic procedures are used in the management of

Kako je prethodno navedeno, i biologijski i socijalni i psihološki čimbenici igraju ulogu u nastanku poremećaja raspoloženja, sukladno tome i u liječenju poremećaja raspoloženja koristimo biologijske, psihološke i socijalne postupke liječenja (2). Biologijski postupci, ovdje posebno mislim na psihofarmakološko liječenje, danas ipak predstavljaju prvi izbor i najvažniji postupak u liječenju poremećaja raspoloženja. Odista velik je izbor psihofaramaka koji nam stoje na raspolaganju, od antidepresiva, antipsihotika, anksiolitika i stabilizatora raspoloženja, da spomenem samo najčešće, u liječenju poremećaja raspoloženja (1,2). Ovi potonji, stabilizatori raspoloženja, osobito najstariji predstavnik ove skupine litij je posebno zanimljiv. Naime, stabilizatori raspoloženja, a time i soli litija, su lijekovi koji se koriste za liječenje, ublažavanje tegoba, ali, za razliku od ostalih psihofarmaka, kao i lijekova općenito, i za prevenciju ponovnih epizoda bolesti (3).
Biološki etiološki čimbenici danas su ipak najviše istraženi i daju najviše odgovora o uzrocima poremećaja raspoloženja. Iz didaktičkih razloga najčešće se dijele na genetske, neuroendokrine, cirkadijalne i one povezane sa spavanjem, te neurokemijske i neuroanatomske (3).

**Genetski čimbenici**

Čini se da su genetski čimbenici vrlo važni u poremećajima raspoloženja (4). Posebice jaki dokazi o utjecaju nasljednih čimbenika važe za bipolarni afektivni poremećaj. Ipak treba napomenuti da se poremećaji raspoloženja ne nasljeđuju dominantno ili recesivno nego je najvjerojatnije riječ o poligenskim bolestima (4-6). Drugim riječima, vjerojatno je više različitih gena umiješano u nastanak poremećaja raspoloženja. Najčešće se opisuju geni na kromosomima 5, 11, i X (7). Danas se intenzivno istražuju polimorfizmi gena za serotoninske receptore, serotoninski transporter, kao i dopaminski transporter ili dopaminske receptore. Prvi rezultati nude kontroverzne nalaze.
Inače, bipolarni afektivni poremećaj javlja se kod 25% djece, ako je bolestan jedan od roditelja. Ukoliko oba roditelja boluju od bipolarnog afektivnog poremećaja, vjerojatnost da će dijete oboljeti je 50–70%. Rizik za jednojajčane blizance je čak 90%. Za depresivne poremećaje postoci su niži, tako primjerice ukoliko jednojajčani blizanac boluje od depresivnog poremećaja kod drugog blizanaca postoji 50%-tna mogućnost obolijevanja, a ako je samo jedan roditelj bolestan, vjerojatnosti da dijete oboli od depresije je 10% (8).

**Neuroendokrini pokazatelji**

Zbog činjenice da je endokrini sustav glavninom reguliran kroz os hipotalamus-hipofiza-ciljana žlijezda (štitnjača, nadbubrežna žlijezda, gonade), odnosno da je cijeli sustav preko hipotalamusa povezan i dio je središnjeg živčanog sustava – limbičkog režnja, logično je očekivati da hormoni mogu biti periferni pokazatelji neurokemij-

mood disorders (2). Currently, biological procedures, primarily psychopharmacological therapy, are the first-choice and most important approach in the management of mood disorders. There is a wide array of psychopharmaceuticals available, including antidepressants, antipsychotics, anxiolytics and mood stabilizers, to mention only the most common agents used in the treatment of mood disorders (1,2). The latter, mood stabilizers, in particular lithium as the oldest representative of the group, are of special interest. Mood stabilizers, including lithium salts, are used to treat and alleviate discomforts, and, unlike other psychopharmaceuticals and drugs in general, also for the prevention of recurrent disease episodes (3).
The biological etiological factors have been best studied to date and therefore provide most information on the causes of mood disorders. For didactic reasons, they are usually categorized as genetic, neuroendocrine, circadian and sleep-related, neurochemical and neuroanatomical factors (3).

**Genetic factors**

Genetic factors appear to play an important role in mood disorders (4). Evidence for the impact of hereditary factors is especially strong in case of bipolar affective disorder. Yet, it should be noted that mood disorders are not inherited as a dominant or recessive trait but most probably are polygenic diseases (4-6). In other words, a number of different genes are probably involved in the occurrence of mood disorders. Genes on the chromosomes 5, 11 and X are most frequently mentioned (7). Polymorphisms of the genes for serotonin receptors, serotonin transporter, dopamine transporter and dopamine receptors have currently been intensively studied. Initial results have produced controversial findings.
Bipolar affective disorder will occur in 25% of children with one of the parents affected with the disease. The probability for child affection rises to 50%–70% if both parents suffer from bipolar affective disorder, while the risk in monozygotic twins is as high as 90%. These rates are lower for depressive disorders. When a monozygotic twin suffers from depressive disorder, the likelihood for the disorder to develop in the other twin is 50%. If one of the parents suffers from depressive disorder, the probability of the child to develop depression is 10% (8).

**Neuroendocrine indicators**

As endocrine system is mostly regulated *via* hypothalamic-pituitary-target glands (thyroid gland, adrenal gland, gonads), i.e. the entire system is inter-related *via* hypothalamus and is an integral part of the CNS – limbic lobe, it is quite logical to expect that hormones may act as peripheral indicators of the neurochemical function of the brain (9,10). Indeed, pituitary hormones are primarily influenced by monoaminergic neurons. So, serotonin, no-

ske funkcije mozga (9,10). I zaista, hipofizarni hormoni su pod utjecajem prvenstveno monoaminergičkih neurona. Tako u izlučivanju adenokortikotropnog hormona (ACTH) odnosno kortizola ili lučenju tireostimulirajućeg hormona (TSH), odnosno triiodotironina (T3), tironina (T4) ili prolaktina (PRL), odlučujuću ulogu imaju serotonin, noradrenalin i dopamin (11). Posebno je zanimljiva os hipotalamus-hipofiza-nadbubrežna žlijezda u bolesnika s depresivnim poremećajem. Tako je deksametazonski supresijski test jedan od najpouzdanijih pokazatelja psihičke bolesti uopće. Naime, oko 50% bolesnika s depresijom su nesupresori na deksametazonskom testu (11). Također, gotovo svi bolesnici imaju veće koncentracije serumskog kortizola i niske vrijednosti ACTH. Isto tako, praktički svaki bolesnik s poremećajem funkcije štitne žlijezde ima i neki simptom poremećenog raspoloženja, ovisno je li riječ o hiper- ili hipotireozi (9,10). S druge strane, bolesnici s poremećajem raspoloženja imaju često povišene vrijednosti slobodnih hormona T3 i T4. Ipak, za razliku od deksametazonskog testa, TRH test (engl. *thyrotropin-releasing hormone test*, TRHt) nije toliko specifičan u bolesnika s depresijom (9,10).

**Poremećaji spavanja i cirkadijalni ritmovi**

Poremećeno spavanje jedan je od najznačajnijih tjelesnih simptoma poremećenog raspoloženja, bilo maničnog ili depresivnog. U spavanju su vrlo jasno izražene elektroencefalografijske (EEG) abnormalnosti u bolesnika s poremećajima raspoloženja. Primjerice, najznačajnija abnormalnost jest u dužini NREM (engl. *non rapid eye movement*) spavanja i REM (engl. *rapid eye movement*) spavanja (12). Naime, depresivni će bolesnici gotovo u pravilu imati skraćene i manje brojne epizode REM spavanja. Nadalje, cirkadijalni su ritmovi predmet intenzivnih istraživanja. Primjerice, u tom su smislu sezonski afektivni poremećaji posebno interesantni. Proučavan je i odnos raspoloženja i melatonina – hormona „biološkog sata" (12).

**Neurotransmiteri**

Poremećaji raspoloženja također se dovode u vezu sa smanjenjem monoamina (serotonin, dopamin, noradrenalin) kod depresije ili povišenjem navedenih neurotransmitera kod manije (13). Istraživanja su, u nekim slučajevima, potvrdila takva očekivanja, ali rezultati nekih studija su i kontradiktorni. Nadalje, lijekovi za liječenje depresije povećavaju koncentraciju monoamina u sinaptičkoj pukotini, doduše različitim mehanizmima (13). Kod poremećaja raspoloženja istraživali su se gore navedeni neurotransmiteri u moždanom tkivu, cerebrospinalnoj tekućini, plazmi ili mokraći bolesnika (13). Također su se istraživali i metaboliti monoamnina kao 5-hidroksiindoloctena kiselina (5-HIAA), 3-metoksi-4-hidroksifenol octena kiselina (HVA), 3-metoksi-4-hidroksifenilglikol (MHPG). Međutim, ne bi bilo primjereno bez sumnje i provjere prihvatiti hi-

repinephrine and dopamine play crucial role in the secretion of adrenocorticotropic hormone (ACTH) and cortisol, thyroid-stimulating hormone (TSH), triiodothyronine ($T_3$), thyroxine ($T_4$) and prolactin (PRL) (11). The hypothalamic-pituitary-adrenal (HPA) axis is of particular interest in patients with depressive disorder. The dexamethasone suppression test is one of the most reliable indicators of mental disease in general. About 50% of depression patients show the lack of suppression on dexamethasone testing (11). In addition, almost all these patients have elevated concentrations of serum cortisol and low ACTH levels. Virtually every patient with thyroid disease, either hyperthyroidism or hypothyroidism, has some symptoms of mood disorder (9,10). On the other hand, patients with mood disorder frequently show elevated levels of free $T_3$ and $T_4$ hormones. Yet, in contrast to dexamethasone test, the thyrotropin-releasing hormone test (TRHt) is not so highly specific in depression patients (9,10).

**Sleep disorders and circadian rhythms**

Sleep disturbances are one of the major physical symptoms of mood disorder, either manic or depressive. Electroencephalography (EEG) abnormalities in sleep are very pronounced in patients with mood disorder. For example, the most common abnormality refers to the length of non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep (12). As a rule, depressive patients have REM sleep episodes reduced in length and number. Circadian rhythms have also been intensively investigated, with seasonal affective disorders attracting high interest. The interrelationship of mood and melatonin "biological clock" has been studied (12).

**Neurotransmitters**

Mood disorders have also been associated to monoamine (serotonin, dopamine and norepinephrine) decrease in depression or their increase in mania (13). Some studies have confirmed these observations, whereas others report contradictory results. Furthermore, the agents used in the treatment of depression increase the concentration of monoamines in the synaptic cleft through various mechanisms (13). In mood disorders, the above mentioned neurotransmitters have been investigated in cerebral tissue, cerebrospinal fluid (CSF), plasma and urine. Monoamine metabolites such as 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) have also been investigated. It would be too naïve to take the hypothesis on a single neurotransmitter system seriously. Namely, it is quite clear that neurotransmitter systems in the CNS behave according to the principle of communicating vessels, i.e. a deviation in one neurotransmitter system immediately entails modification in another (14) resulting in

potezu o jednom neurotransmiterskom sustavu. Naime, sasvim je jasno da se neurotransmiterski sustavi u središnjem živčanom sustavu (engl. *central nervous system*, CNS) ponašaju po principu „spojenih posuda", tj. otklon u jednom neurotransmiterskom sustavu trenutno dovodi i do promjene u drugom neurotransmiterskom sustavu (14). Odatle potječu i neujednačeni nalazi istraživanja neurotransmitera u poremećajima raspoloženja.

Nadalje, koncentracije neurotransmitera ili njihovih metabolita mogu i biti u granicama normale, ali ukoliko postoji manjak ili višak receptora za neurotransmitere njihovo će se djelovanje očitovati kao smanjeno ili povećano (15). Novija istraživanja djelovanja antidepresiva pokazuju da se njihov terapijski učinak u konačnici ostvaruje utišavanjem monoaminskih receptora (15). Neurotransmitere, inače, pored brojnih podjela, za ovu svrhu možemo podijeliti na eksictatorne (glutamat koji djeluje preko kainatnih i N-metil D-aspartat; NMDA receptora), inhibitorne receptore γ-aminomaslačne kiseline (GABA) te neuromodulatorne koji su ujedno monoaminergički (dopamin, serotonin, noradrenalin, adrenalin) i kolinergički. Ovi neuromodulatorni neurotransmiteri mogu u danom trenutku biti i ekscitatorni i inhibitorni, ovisno o tome koliko i kako su stimulirani od strane glutamata. Drugim riječima, u poremećajima raspoloženja smanjenje ili povišenje (ili njihovo pojačano ili smanjeno djelovanje) monoamina može zaista imati za posljedicu poboljšanje ili pogoršanje raspoloženja (13-15), ali njihova razina prvenstveno ovisi o koncentraciji i ekscitatornom djelovanju glutamata preko NMDA receptora ili inhibitornom djelovanju GABE (16).

Nadalje, bitno je napomenuti da neuromodulatorni neurotransmiteri svoj učinak ostvaruju nakon što se vežu za stanični receptor povezan s G proteinom koji aktivira enzime sustava drugog glasnika (16). Ekscitatorni neurotransmiter glutamat ili inhibitorni neurotransmiter GABA djeluju na ionske kanale. Drugim riječima, glutamat i GABA djeluju trenutačno, dok se monoamninergički učinak može očekivati i u dužem vremenskom razdoblju (16).

**Neurotransmiteri i signalni putevi**

Bilo bi jednostavno objašnjavati psihijatrijske poremećaje, ali i „normalno" funkcioniranje, isključivo promjenama neurotransmitera ili njihovih receptora. Naime, kao što je u prethodnom odjeljku navedeno, konačni rezultat djelovanja neurotransmitera ostvaruje se tek aktivacijom ili inhibicijom drugih glasnika koji naposljetku mijenjaju ekspresiju gena, a time i fenotip! Na slici 1. nalazi se prikaz aktivacije drugog glasnika, gdje neurotransmiter smatramo „prvim glasnikom" koji preko svog ciljnog receptora djeluje na G-proteine koji se nalaze u staničnoj membrani. G-proteini prenose poruku od neurotransmiterskog receptora do „drugih glasnika" ili ionskih kanala. G-proteini sastoje se od 3 podjedinice: α, β i γ (15). G-proteini djeluju na čitav niz „drugih glasnika" od kojih su najpoznatiji sus-

diversified findings in the studies of neurotransmitters in mood disorders.

Furthermore, if the concentrations of neurotransmitters or their metabolites are within the normal limits but there is a deficit or excess of neurotransmitter receptors, their action will be variedly manifested (15). Recent studies of the action of antidepressants show their therapeutic effect to be eventually manifested by down-regulation of monoamine receptors (15). Besides a number of classifications, for this purpose neurotransmitters can be classified into excitatory (glutamate acting *via* kainate and N-methyl-D-aspartate (NMDA) receptors), inhibitory receptors of γ-aminobutyric acid (GABA), and neuromodulatory that are both monoaminergic (dopamine, serotonin, norepinephrine, epinephrine) and cholinergic neurotransmitters. These neuromodulatory neurotransmitters may assume both excitatory and inhibitory action at a given moment, depending on the level and mode of glutamate stimulation. In other words, in mood disorders a decrease or increase of monoamines (or their enhanced or suppressed action) may result in mood elevation or depression (13-15). Yet, their level primarily depends on the concentration and excitatory action of glutamate *via* NMDA receptors or on the inhibitory GABA action (16).

It should also be noted that neuromodulatory neurotransmitters exert their action upon having bound to the cell receptor *via* G protein and second messenger system (16). The excitatory neurotransmitter glutamate or the inhibitory neurotransmitter GABA act upon ion channels, i.e. glutamate and GABA act immediately, whereas monoaminergic effect can also be expected in a prolonged period of time.

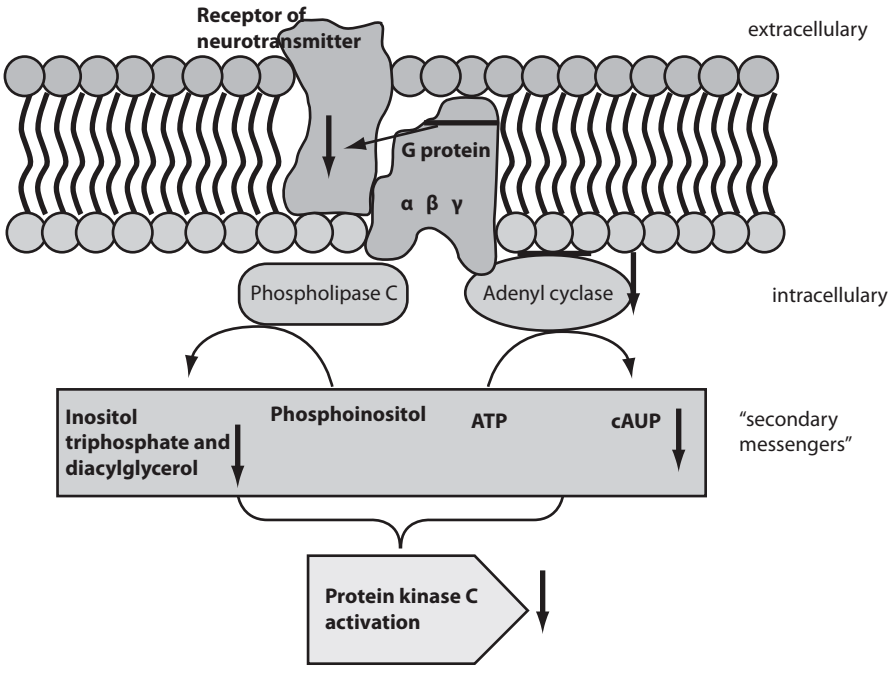
**Neurotransmitters and signal pathways**

It would be too simple to explain mental disorders or "normal" functioning exclusively by the modification of neurotransmitters or their receptors. As stated above, the result of the action of neurotransmitters is only achieved through the activation or inhibition of other messengers, which eventually change gene expression and thus also the phenotype. Figure 1 shows schematic presentation of the second messenger activation, where neurotransmitter is considered "first messenger" which acts, *via* its target receptor, upon G proteins found in the cell membrane. G proteins transfer the message from neurotransmitter receptor to particular enzymes serving as "second messengers" or to ion channels. G proteins are constituted of three subunits, α, β and γ (15). G proteins act upon a series of "second messengers", best known of which are the system of adenylate cyclase that converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), or the phospholipase C linked system that converts inositol lipids and phosphoinositol from the cell membrane to inositol triphosphate (IP3) and

tav adenilat-ciklaze koja pretvara adenozin-trifosfat (ATP) u ciklički adenozin-monofosfat (cAMP) ili sustav vezan uz fosfolipazu C koja pretvara inozitolske lipide i fosfoinozitol stanične membrane u inozitol-trifosfat (IP3) i diacilglicerol (DG) (13-16). IP3 i DG djeluju na protein-kinazu C ili receptore endoplazmatske mrežice što dovodi do oslobađanja kalcijevih iona u citoplazmu. Slijed aktivacije drugih glasnika prikazan je na slici 1.

Svakako treba napomenuti da neurotransmisija i učinci neurotransmisije ne završavaju oslobađanjem neurotransmitera, djelovanjem istog na receptor i aktivacijom drugog glasnika što se dešava u milisekundama (15). Krajnja je posljedica neurotransmisije regulacija genske ekspresije, sinteza proteina i preoblikovanja funkcije, ali i morfologije neurona ili glija stanica novim uvjetima. Te promjene najčešće znače ojačavanje ili uništavanje te preoblikovanje sinapsi i dendrita, povlačenje ili produženje aksona, ili čak i poticanje stanice na smrt (17). Aktiviran

diacylglycerol (DG) (13-16). IP3 and DG act upon protein kinase C or endoplasmic reticulum receptors, thus leading to the release of calcium ions to the cytoplasm. The cascade of second messenger activation is illustrated in Figure 1. It should be noted that neurotransmission and its effects are not terminated by the release of a neurotransmitter, its action upon the receptor and second messenger activation, which occurs in milliseconds (15). The final consequence of neurotransmission is the regulation of gene expression, protein synthesis, and modulation of the neuron or glial cell function and morphology according to the new conditions. These changes mostly imply strengthening or destruction as well as rearrangement of the synapses and dendrites, axon withdrawal or elongation, or even cell stimulation to death (17). The activated system of second messenger eventually activates transcription factors, which in turn activate regulatory region of the target gene stimulating the encoding gene sec-




**Slika 1.** Prikaz aktivacije drugih glasnika. Neurotransmitor kao „prvi glasnik" djeluje na receptor smješten na staničnoj membrani, a zatim preko G-proteina i fosfolipaze C ili adenilat-ciklaze dolazi do aktivacije „drugih glasnika" koji naposljetku potiču protein-kinazu C koja zatim djeluje u signalnoj kaskadi na gene ranog odgovora.

**Figure 1.** Schematic presentation of second messenger activation. Neurotransmitter as a "first messenger" acts upon receptor on the cell membrane, followed by the G protein and phospholipase C or adenyl cyclase mediated activation of "second messengers" that eventually stimulate protein kinase C, which then acts through a signal cascade on the early response genes.

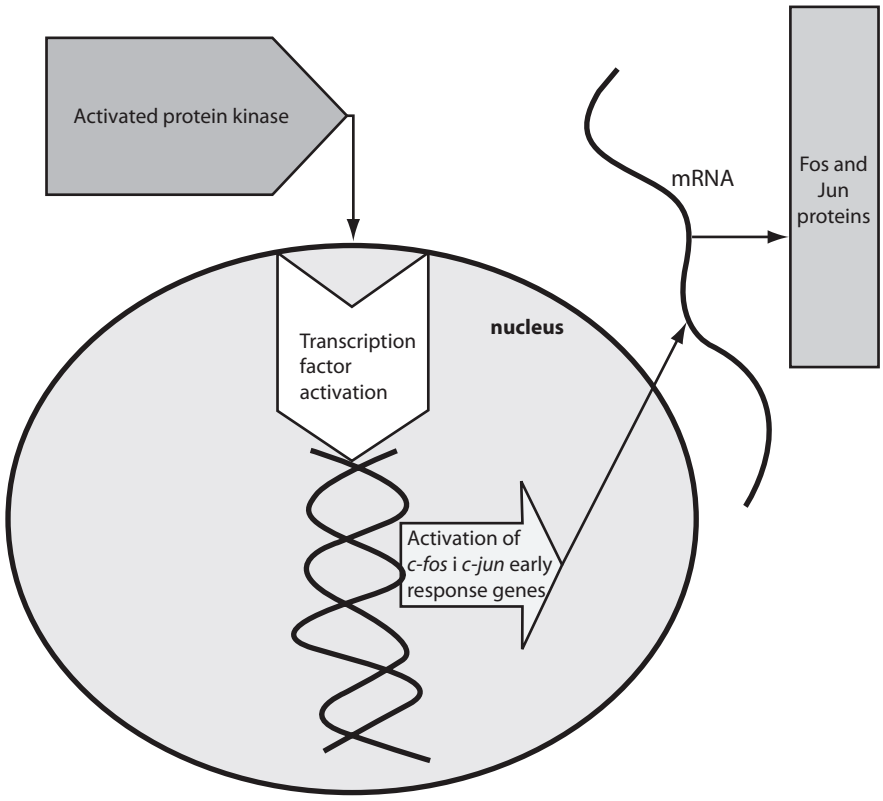
sustav „drugog glasnika" u konačnici aktivira čimbenike transkripcije koji aktiviraju regulatorno područje ciljnog gena, koja potiče kodirajući dio gena te se stvara ribonukleinska kiselina (RNK) i konačno ciljni protein (enzim, receptor, transportni protein, transkripcijski čimbenici i sl.) (16,17). U tablici 1. prikazana su neka dosadašnja istraživanja promjena sustava drugog glasnika u bolesnika s bipolarnim afektivnim poremećajem ili depresivnim poremećajem. Ipak, nedostatak ovih istraživanja je činjenica da su rađena na postmortalnim uzorcima mozga bolesnika s poremećajem raspoloženja ili na modelima kao što je trombocitni model. Svakako treba napomenuti da su, nakon aktivacije transkripcijskog čimbenika, prvi geni koji se aktiviraju su *c-Fos* i *c-Jun*, geni ranog odgovora. Nakon aktivacije drugog glasnika neurotransmiterom cFos i cJun su aktivni nakon 15 minuta, a djeluju do jednog sata. Produkti *c-Fos* i *c-Jun* gena su Fos i Jun proteini koji zajedno aktiviraju ili onemogućuju aktivaciju gena kasnog odgovora (18-20). Geni kasnog odgovora su zapravo specifični geni koji reguliraju specifični odgovor stanice na podražaj (Slika 2.). Cjelokupni živčani sustav je stoga vrlo prilagodljiv i mijenja se pod utjecajem brojnih vanjskih i unutrašnjih utjecaja, drugim riječima, „plastičan" je i u biokemijskom, ali i u morfološkom smislu (20). Naime, suprotno prijašnjem stavu, danas se smatra da su neuroni djeljivi i nakon poroda. Također, tijekom cijelog života mijenja se mijelinizacija, broj dendrita i sinapsi, dužine aksona, sve do nekroze ili apoptoze neurona pod utjecajem neurotransmitera, posebice glutamata ili djelovanjem hormona, glukokortikoida pod djelovanjem stresa (21).

tion, thus producing ribonucleic acid (RNA) and finally the target protein (enzyme, receptor, transport protein, transcription factors, etc.) (16,17). Table 1 shows some studies of the second messenger system changes in patients with bipolar affective disorder or depressive disorder. These studies have a drawback in common, i.e. they were performed in postmortem brain samples of patients with mood disorders or on models such as platelet model. It should be noted that upon transcription factor activation, the first genes to activate are *c-Fos* and *c-Jun*, the early response genes. Upon the neurotransmitter activation of second messenger, *cFos* and *cJun* genes become active in 15 minutes and act for one hour. The products of *c-Fos* and *c-Jun* genes are Fos and Jun proteins that jointly activate or prevent activation of the late response genes (18-20). The late response genes are specific genes that regulate specific cell response to the stimulus (Fig. 2). Therefore, the entire nervous system is highly adjustable and modifiable by numerous external and inner effects; it is flexible in biochemical as well as morphological terms (20). In contrast to previous beliefs, neurons are now considered also divisible after birth. Myelinization, the number of dendrites and synapses, and axon length are changing for life, up to the neuron necrosis or apoptosis under the influence of neurotransmitters, glutamate in particular, or of hormones such as glucocorticoids under the influence of stress (21).

**Tablica 1.** Prikaz nekih dosadašnjih istraživanja sustava „drugog glasnika" u bolesnika s poremećajem raspoloženja.

**Table 1.** Some studies of the "second messenger" system in patients with mood disorders

| Experimental model | Change |
|---|---|
| Postmortem on cerebral cortex or platelet model | ↑ G protein (α-subunit)<br>↓ G protein (α-subunit) in lithium treated patients<br>↓ G protein (α-subunit) in depressive patients<br>↑ G protein (α-subunit) in lithium treated depressive patients |
| Platelet or leukocyte model | ↑ phosphorylation of cyclic adenosine monophosphate (cAMP) dependent protein in bipolar patients<br>↓ cyclic adenosine monophosphate (cAMP) in lithium treated patients<br>↑ protein kinase in bipolar patients |
| Platelet or red blood cell model | ↑ inositol triphosphate (IP3) in manic patients<br>↓ inositol triphosphate (IP3) in lithium treated patients<br>↑ inositol-1-phosphatase in lithium treated patients |




**Slika 2.** Aktivacija *c-fos* i *c-jun* gena

**Figure 2.** Activation of *c-fos* and *c-jun* genes

## Neuroanatomske promjene u poremećajima raspoloženja

Neuroanatomska istraživanja poremećaja raspoloženja zasnovana su na postmortalnim istraživanjima, morfološkim promjenama praćenim kompjuteriziranom tomografijom (CT) kasnijih sedamdesetih i osamdesetih godina 20. stoljeća te istraživanjima nuklearnom magnetskom rezonancom (NMR) devedesetih godina 20. stoljeća. Napose treba spomenuti funkcionalno-morfološke metode istraživanja kao SPECT (engl. *Single Photon Computer Tomography*) i PET (engl. *Positron Emission Tomography*), koje su ukazale na ključne etiološke čimbenike poremećaja raspoloženja kao što su smanjena funkcija prečeonog korteksa ili smanjena aktivnost područja limbičkih struktura, posebno hipokampusa i amigdala, kod depresije ili obrnuto, povećana aktivnost istih područjima u maničnoj fazi bolesti (22). Studije NMR-om također pokazuju manji frontalni režanj u bolesnika s bipolarnim afektivnim poremećajem nego u ispitanika kontrolne skupine, istraživanja CT-om nisu dala veće rezultate što je dijelom i zbog ograničenja ove metode u ispitivanju finijih morfoloških promjena CNS-a.

## Neuroanatomical changes in mood disorders

Neuroanatomical investigation of mood disorders have been based on postmortem studies, morphological changes monitored by computed tomography (CT) in the late 1970s and 1980s, and nuclear magnetic resonance (NMR) studies in the 1990s. Also, mention should be made of the functional morphological methods such as single photon computerized tomography (SPECT) and positron emission tomography (PET), which have pointed to the key etiologic factors in mood disorders, e.g., diminished function of prefrontal cortex or decreased activity of the area of limbic structures, hippocampus and amygdala in particular, in depression, or contrary to this, an increased activity in the same regions in the manic phase of the disease (56-22). NMR studies also showed the frontal lobe to be smaller in patients with bipolar affective disorder than in the control group. CT studies failed to yield any significant results, in part due to the method limitations in the study of subtle morphological alterations in CNS.
In addition to the above mentioned macromorphological methods that have produced relatively modest findings

Osim nabrojenih makromorfoloških metoda koje su pokazale relativno skromne nalaze u bolesnika s poremećajima raspoloženja, postmortalne histološke metode dale su možda najkonzistentnije biologijske nalaze u psihijatriji uopće (23). Glija stanice su, suprotno očekivanjima, pokazale promjene (24). Naime, do današnjeg se vremena smatralo kako u funkcionalnom, ali i patofiziološkom smislu glija stanice, za razliku od neurona, u psihijatrijskim poremećajima nisu od većeg značaja (24). U bolesnika s bipolarnim afektivnim poremećajem, ali i onih s depresivnim poremećajem, nađen je manji broj stanica, s manjom gustoćom, ali i promijenjenom staničnom veličinom (25). Ove su promjene najizraženije u prečeonom korteksu, upravo u regiji u kojoj i SPECT i PET pokazuju promjene, ali i u amigdalama i hipokampusu (26). U prečeonom je korteksu broj glija stanica manji i do 41% prema kontrolnim uzorcima (27,28). Nadalje, suprotno prijašnjem mišljenju da su glija stanice samo potporno tkivo CNS-a, danas se zna da iste imaju i brojne neurotrasmiterske receptore te da imaju značajnu ulogu u koncentraciji serotonina, noradrenalina i dopamina u sinaptičkoj pukotini. Ipak, možda je najvažnija uloga glija stanica u regulaciji metabolizma glutamata, najvažnijeg eksicatornog neurotransmitera. Naime, glija stanice sadrže glutamatni transporter koji „kupi" višak glutamata iz sinaptičke pukotine i sprema ga u glavno spremište glutamata, glija stanice (15). Kao što je prije spomenuto, osim manjeg broja i manje gustoće glija stanica u bipolarnom afektivnom poremećaju i depresivnom poremećaju, nađeno je i povećanje volumena glija stanica (29). Također se ističe da promjene u strukturi čeonog korteksa, u smislu promjene strukturalne plastičnosti, nastaju prvenstveno zbog abnormalnosti glija stanica. Dosadašnja istraživanja zasnovana su na preparatima koji su bojani Nissl metodom, kojom nije moguće utvrditi koji je pod tip glija stanica (astrociti, oligodendrociti i mikroglija) zahvaćen patološkim promjenama u poremećajima raspoloženja (29). Važno je istaknuti da promjene u bipolarnom afektivnom poremećaju i depresivnom poremećaju nisu kao u ostalih klasičnih neurodegenerativnih bolesti, gdje je glioza ključan patomorfološki nalaz. Drugi istraživači idu dalje i ističu apoptozu kao ključan mehanizam u nastanku opisanih promjena u čeonom korteksu bolesnika s poremećajem raspoloženja.

## Apoptoza – mogući uzrok neurodegenerativnih promjena u poremećajima raspoloženja

Smrt svake stanice, pa i neurona i glija stanica može biti rezultat nekroze ili apoptoze. Nekroza ili akutna patološka smrt proces je koji nastaje nakon opsežnih kemijskih, biokemijskih, mehaničkih i sličnih oštećenja. Nekroza je okarakterizirana bubrenjem i puknućem stanične mem-

in patients with mood disorders, postmortem histology methods have provided perhaps the most consistent biological findings in psychiatry in general (23). Against expectations, glial cells have surprisingly exhibited alterations (24). To date, glial cells, unlike neurons, have been considered to be of little relevance in either functional or pathophysiological terms in psychiatric disorders (25). In patients with bipolar affective disorder as well as in those with depressive disorder, glial cells were found in lower number and density, also showing size changes (26). These changes were most pronounced in prefrontal cortex, in the region where changes are well visualized by SPECT and PET, and in amygdala and hippocampus (26). The number of glial cells in prefrontal cortex was by up to 41% lower in comparison with control samples (27,28). Furthermore, in contrast to the previous opinion that glial cells serve just as supportive tissue to CNS, now they are known to possess numerous neurotransmitter receptors and to play a major role in the concentrations of serotonin, norepinephrine and dopamine in the synaptic cleft. Yet, perhaps the most important role of glial cells is the regulation of the metabolism of glutamate, the crucial excitatory neurotransmitter. Glial cells contain glutamate transporter which "collects" excess glutamate from the synaptic cleft to deposit it in glial cells as the main glutamate storage (15). As mentioned above, besides the lower number and lower density of glial cells in bipolar affective disorder and depressive disorder, an increase in the glial cell volume has also been reported (29). It is also emphasized that alterations in the frontal cortex structure in terms of changed structural plasticity occur primarily due to glial cell abnormalities. To date, studies have been based on preparations stained by Nissl method, which cannot define the exact glial cell subtype (astrocytes, oligodendrocytes and microglia) involved by pathologic alteration in mood disorders (29). It should be noted that the changes observed in bipolar affective disorder and depressive disorder differ from those in other classic neurodegenerative diseases, where gliosis is the main pathomorphological finding. Other researchers go even beyond this concept and indicate apoptosis as the key mechanism in the occurrence of these alterations in the frontal cortex of patients with mood disorder.

## Apoptosis – the possible cause of neurodegenerative changes in mood disorders

Death of any cell, including neurons and glial cells, can be the result of necrosis or apoptosis. Necrosis or acute pathologic death is a process that occurs after extensive chemical, biochemical, mechanical or other similar damage. Necrosis is characterized by swelling and rupture of the

brane i organela, dolazi do izlijevanja staničnog sadržaja i na taj način do onečišćenja i upalnih promjena okolnog tkiva (31). Apoptoza je proces fiziološke programirane smrti stanice – staničnog samoubojstva (32). Hoće li će stanica umrijeti apoptozom ili nekrozom ovisi o koncentraciji nekog štetnog čimbenika ili dužini trajanja njegovog djelovanja, ali i o tipu oštećenja uzrokovanim navedenim čimbenicima, te o vrsti stanica (33). Nadalje, apoptoza ovisi i o prisustvu, odnosno odsustvu molekula koje induciraju apoptozu. Apoptoza je prvi put opisana 1972. godine. Naziv joj dolazi od grčkih riječi *apo* (od) i *poptosis* (padati), koji opisuje jesensko padanje lišća, odnosno normalnu fiziološku ulogu fenomena programirane stanične smrti (34). Morfološki, u apoptozi dolazi do „skupljanja" i pupanja stanica, membrane ostaju uglavnom očuvane, jezgre su piknotične i fragmentirane. Stanica se podijeli u apoptotična tjelešca, okružena membranama, tzv. Kerijeva tjelešca, od kojih svako sadrži različite organele. Apoptotične stanice u tkivima, pa i *in vitro*, budu fagocitirane od njihovih vijabilnih susjednih stanica ili od strane specijaliziranih fagocita. Za razliku od nekroze, u apoptotičnoj smrti stanice ne dolazi do upalne reakcije okolnog tkiva (35). Apoptoza ima važnu fiziološku ulogu u normalnom razvoju i održavanju homeostaze tkiva npr. pri morfogenetskoj smrti stanica za vrijeme embriogeneze, selekciji neurona kod formiranja sinapsi, uklanjanju glija stanica nakon stresnih događaja posredovanih glutamatom i/ili kortikosteroidima, u živčanoj plastičnosti, uklanjanju stanica kojima nedostaju neophodni čimbenici rasta (npr. apoptoza neurona zbog nedostatka nervnog čimbenika rasta) (36). Kao što je navedeno, apoptoza se smatra jednim od vjerojatnih čimbenika u etiologiji neurodegenerativnih procesa u središnjem živčanom sustavu bolesnika koji boluju od poremećaja raspoloženja (30). Primjerice, istraživanje provedeno *in vivo* na bolesnicima koji su bolovali od poremećaja raspoloženja (bipolarnog afektivnog poremećaja i velike depresije) pokazivali su statistički značajno veći broj apoptotičnih stanica (leukocita) na protočnom citometru nego kod zdrave kontrolne skupine. Drugo istraživanje provedeno postmortalno, na bolesnicima koji su bolovali od velike unipolarne depresije, pokazalo je neprimjereno velik broj apoptotičnih stanica u središnjem živčanom sustavu, posebno u području hipokampusa. U tom istraživanju istraživači još dokazuju izravnu vezu velikog broja hipokampalnih apoptotičnih stanica i ekspresije kortikosteroidnih receptora, te smatraju da je apoptoza posljedica poremećene hipotalamičko-hipofizno-adrenokortikalne osi u bolesnika s poremećajima raspoloženja (37).

**Molekularni mehanizami apoptoze**

Kao što je prethodno naglašeno, apoptoza, stanični suicid ili programirana stanična smrt ključan je mehanizam u održavanju homeostaze organizma (35). Apoptotski me-

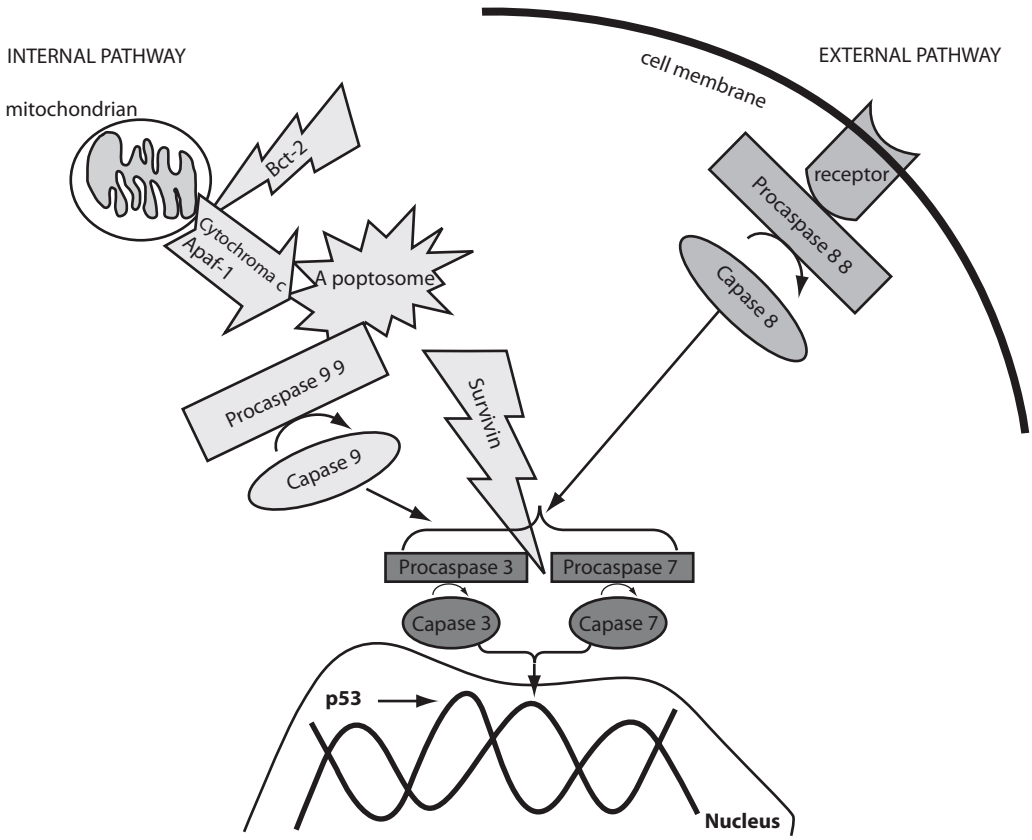
cell membrane and organelles, escape of the cellular content, and consequential contamination and inflammatory changes of the surrounding tissue (31). Apoptosis is a process of physiological programmed cell death, cell suicide (32). Should a cell undergo apoptosis or necrosis, it depends on the adverse factor concentration or length of its harmful effect, on the type of damage caused by the mentioned factors, and on the cell type (33). Furthermore, apoptosis also depends on the presence or absence of the molecules inducing apoptosis. Apoptosis was first described in 1972. The term apoptosis has been derived from the Greek words *apo* (off) and *poptosis* (falling down), describing the shedding of leaves in the autumn, i.e. the normal physiological role of the programmed cell death phenomenon (34). Morphologically, apoptosis implies shrinkage and budding of the cells, the membranes are generally preserved, and the nuclei are pyknotic and fragmented. The cell divides into membrane surrounded apoptotic corpuscles, so-called Keri bodies, each of them containing various organelles. Apoptotic cells in tissues, even *in vitro*, are being phagocytosed by the viable adjacent cells or specialized phagocytes. In contrast to necrosis, apoptotic cell death does not induce inflammatory reaction in the surrounding tissue (35). Apoptosis has an important physiological role in the normal development and maintenance of tissue homeostasis, e.g., in morphogenetic cell death during embryogenesis, neuron selection in the formation of synapses, glial cell removal following stressful events mediated by glutamate and/or corticosteroids, in neuronal plasticity, removal of cells lacking the necessary growth factors (e.g., neuron apoptosis due to the nervous growth factor deficit), etc. (36). As stated above, apoptosis is considered one of the likely factors in the etiology of neurodegenerative process in the CNS of patients suffering from mood disorders (30). For example, in a study conducted *in vivo*, flow cytometry revealed a statistically significantly greater number of apoptotic cells (leukocytes) in patients with mood disorders (bipolar affective disorder and major depression) than in the control group of healthy subjects. Another, postmortem study in patients with major unipolar depression showed an abnormally high number of apoptotic cells in the CNS, hippocampus in particular. In this study, the authors also demonstrated direct association between the great number of apoptotic cells in the hippocampus and the expression of corticosteroid receptors, considering apoptosis to occur consequentially to HPA axis impairment in patients with mood disorders (37).

**Molecular mechanisms of apoptosis**

As noted above, apoptosis, cell suicide or programmed cell death is the key mechanism in maintaining body homeostasis (35). Apoptotic mechanisms are of special importance in the formation of nervous system during the

hanizmi posebno su značajni u formiranju živčanog sustava u doba intrauterinog razdoblja, ali i kasnije tijekom razvoja CNS-a u djetinjstvu kao odgovor na učenje i u odrasloj dobi kao odgovor na različite podražaje iz društva, pri čemu se mozak plastično oblikuje prema zahtjevima okoline (37). Sama apoptoza je kompleksan molekularni mehanizam u kojem se isprepliću brojni unutarstanični putovi, dolazi do međudjelovanja brojnih proteina, enzima, lipida, mijenjanja ekspresije gena. Iz didaktičkih razloga apoptotski se mehanizam pojednostavljeno prikazuje i načelno se može opisati kao unutrašnji (mitohondrijski put koji vodi apoptozi) ili vanjski (receptorski put) (Slika 3.). Aktivacija unutarnjeg puta apoptoze posljedica je poremećaja u unutarstaničnoj homeostazi (38). U tom putu, kao što je navedeno, mitohondriji imaju središnju ulogu, pa se stoga ovaj put naziva i mitohondrijski put. Vanjski put apoptoze započinje vezanjem liganada na stanične membranske receptore koji se nazivaju „receptori smrti", pa se taj put naziva i „put receptora smrti" (38). Iako je način aktivacije ovih putova različit, između njih postoji povezanost, a na kraju, oba puta izazivaju karakteristične

intrauterine period as well as subsequently during the CNS development in childhood as a response to learning, and later in adult age as a response to various stimuli from the society, whereby the brain is being shaped in a plastic manner according to environmental requirements (35). Apoptosis itself is a complex molecular mechanism in which a number of intracellular pathways are intertwined, with interaction of numerous proteins, enzymes, lipids, and modification of gene expression. For didactic reasons, the mechanism of apoptosis is presented in a simplified manner and can generally be described as internal (mitochondrial pathway leading to apoptosis) or external (receptor pathway) (Figure 3). Activation of the apoptotic internal pathway occurs consequentially to intracellular homeostasis impairment (38). In this pathway, as mentioned above, mitochondria play a central role, hence it is termed mitochondrial pathway. The external pathway of apoptosis begins with ligand binding to cell membrane receptors called death receptors, hence it is termed death receptor pathway (38). Although differing in the mode of activation, these pathways are interrelated and even-




**Slika 3.** Prikaz vanjskog i unutrašnjeg puta apoptoze. Vanjski put apoptoze potaknut je preko receptora („receptori smrti") na površini stanice. Unutrašnji put apoptoze počinje unutar stanice u mitohondrijima.

**Figure 3.** Internal and external apoptosis pathways. External apoptosis pathway is initiated *via* receptors ("death receptors") on the cell surface. Internal apoptosis pathway begins within the cell, in mitochondria.

biokemijske i strukturalne promjene stanice svojstvene apoptozi. Kako je spomenuto, vanjski put aktiviraju ligandi vezanjem na receptore smrti (38). Ti receptori mogu biti različiti, a pripadaju receptorskoj porodici tumorskih nekrotizirajućih faktora (engl. *tumor necrosis factor,* TNF). Više je supstrata koji mogu izazvati aktivaciju vanjskog apoptotskog puta. U pogledu funkcioniranja CNS-a u fiziološkim ili u patofiziološkim uvjetima, sljedeći supstrati mogu imati značajan utjecaj na receptore smrti na staničnoj membrani: čimbenik rasta neurona (engl. *Neural Growth Factor*, NGF), moždani jezgrin čimbenik (engl. *Brain Derivated Nuclear Factor*, BDNF), interleukini, glutamat i neurotrofini (38). Nakon vezivanja liganada na receptore, na taj kompleks vežu se molekule prilagodbe i kaspaze pokretači (kaspaza-8 i kaspaza-10). To dovodi do aktivacije prokaspaza, do cijepanja izvršnih kaspaza 3 i 7, cijepanja DNK u jezgri na specifične dijelove, tzv. „ljestvice", te konačno do osobitih strukturalnih promjena. Mitohondrijski put započinje otvaranjem pora na mitohondrijskoj membrani, otpuštanjem citokroma c (i drugih proteina) iz mitohondrija, stvaranjem apoptosoma od citokroma c, prokaspaze-9, i Apaf-1 čimbenika (engl. *Apoptotic Protease Activating Factor*), aktivacije efektorskih kaspaza 3 i 7 i cijepanja DNK (38). Ključni u regulaciji apoptoze, odnosno preživljenja stanica, su članovi obitelji protein B limfoma tipa 2 (engl. *B cell limphom 2 protein*), (Bcl-2). Bcl-2 proteini obuhvaćaju proapoptotske (Bax, Bak, Bad, Bid, Bik) i antiapoptotske (Bcl-2, Bcl-$X_L$, Mcl-1) proteine (39). Ovi proteini mogu biti višedomenski (Bak i Bax) ili imati samo BH-3 domenu, tzv. *BH3-only* proteini (Bad, Noxa, PUMA). Više-domenske molekule mogu se aktivirati interakcijom s BH-3 proteinima. Bcl-2 proteini kontroliraju (direktno ili vezivanjem na proapoptotske proteine) propusnost mitohondrij oblikovanjem pora na vanjskoj membrani ili reguliranjem otvaranja i zatvaranja pora za propusnost (engl. *permeability pores*).

Mitohondrijski put može aktivirati nepopravljeno oštećenje u DNK. Središnju ulogu u ovom putu ima tumor supresorski gen *p53* (iako postoji i p53-neovisni put). P53, jezgrin fosfoprotein, ima središnje mjesto u signalnim putovima kojima stanica „osjeća" stres i na njega odgovara (40). Stabilnost genoma, stanični ciklus i apoptozu kontrolira p53 koji nije funkcionalan u 50% tumora. Uloga p53 u održavanju genomske stabilnosti obuhvaća: a) zadržavanje stanica u G1/S fazi staničnog ciklusa koje koči umnožavanje oštećene DNK i omogućuje popravak oštećenja DNK; b) poticanje apoptoze kako bi se uništile stanice s oštećenjem DNK; c) međudjelovanje s drugim molekulama koje sudjeluju u tim procesima. U apoptozi, p53 može mijenjati ekspresiju apoptotskih gena uključenih u unutrašnji ili vanjski signalni put: povećati ekspresiju proapoptotskih gena (Bax, Noxa, PUMA), smanjiti ekpresiju antiapoptotskih gena (Bcl-2), odnosno potaknuti izražajnost receptora smrti (kao Fas) i time potaknuti apoptozu.

tually both pathways give rise to characteristic biochemical and structural cell changes characteristic of apoptosis. As mentioned above, the external pathway is activated by ligands through their binding to death receptors (38). These receptors may vary, and they belong to the receptor family of tumor necrosis factors (TNF). A number of substrates can induce activation of the external apoptotic pathway. Considering CNS functioning in physiological or pathophysiological conditions, the following substrates may significantly influence death receptors on the cell membrane: neural growth factor (NGF), brain derived nuclear factor (BDNF), interleukins, glutamate, and neurotrophins (38). Upon ligand binding to receptors, adaptive molecules and effector caspases (caspase-8 and caspase-10) are bound to the complex. This leads to the activation of procaspases, cleavage of effector caspases 3 and 7, intranuclear DNA cleavage to specific segments, so-called "ladders", and eventually to specific structural changes. The mitochondrial pathway starts by opening of the mitochondrial membrane pores, release of cythochrome c (and other proteins) from the mitochondria, formation of apoptosomes from cytochrome c, procaspase-9 and apoptotic protease activating factor (Apaf-1), activation of effector caspases 3 and 7, and DNA cleavage (38). Members of the B cell lymphoma 2 protein family (Bcl-2) play a key role in the regulation of apoptosis and cell survival. Bcl-2 proteins include proapoptotic proteins (Bax, Bak, Bad, Bid, Bik) and antiapoptotic proteins (Bcl-2, Bcl-$X_L$, Mcl-1) (39). These proteins may have multiple domains (Bak and Bax) or only BH-3 domain, hence termed BH3-only proteins (Bad, Noxa, PUMA). Multiple domain molecules can be activated by interaction with BH-3 proteins. Bcl-2 proteins control (directly or by binding to proapoptotic proteins) mitochondrial permeability by modulating the outer membrane pores or regulating opening and closure of the permeability pores.

Mitochondrial pathway can be activated by an irreversible DNA damage. In this pathway, the central role is played by the tumor suppressor gene *p53* (although a p53-independent pathway also exists). The nuclear phosphoprotein p53 has a key role in the signal pathways through which the cell "feels" stress and responds to it (40). Genome stability, cell cycle and apoptosis are under the control of p53, which is out of function in 50% of tumors. The role of p53 in maintaining genome stability includes the following: (a) retaining cells in the G1/S phase of the cell cycle to hamper replication of damaged DNA and to enable repair of DNA damage; (b) stimulation of apoptosis to destroy cells with damaged DNA; and (c) interaction with other molecules involved in these processes. In apoptosis, p53 can modulate expression of apoptotic genes involved in the internal or external pathway, enhance expression of proapoptotic genes (Bax, Noxa, PUMA), diminish expression of antiapoptotic genes (Bcl-2), and sti-

Kaspaze (cistein aspartat specifične proteaze) središnji su izvršitelji apoptoze (41). U stanicama se nalaze kao neaktivne prokaspaze. Aktiviraju se proteolitički, oligomerizacijom ili cijepanjem od strane drugih kaspaza. Kaspaze mogu djelovati kao inicijatorske (kaspaze 2, 8, 9, i 10) ili efektorske (kaspaze 3 i 7). Zbog njihovog razornog djelovanja, aktivnost im mora biti vrlo precizno regulirana, kako bi se izbjegla nepotrebna smrt stanica. Inače, dosadašnja istraživanja na ljudima otkrila su četrnaest kaspaza. Supstrat na koji djeluju kaspaze, među ostalima, je PARP protein (engl. *Poly-(ADP-ribose)-polymerase*) koji se djelovanjem kaspaza cijepa. Cijepanje PARP proteina koristi se stoga i kao biljeg apoptoze. Aktivnost kaspaza nadziru inhibitori apoptotskih proteina (42). Među njima veliku ulogu ima survivin (43). Survivin se direktno veže za kaspaze 3 i 7 te tako inhibira njihovu aktivnost (44). Osim toga, njegova ekspresija ovisi o staničnom ciklusu, što upućuje na dvojnu ulogu ovog proteina, u kontroli staničnog ciklusa i u regulaciji apoptoze (44). Zanimljivo je spomenuti da se survivin eksprimira tijekom embriogeneze, ali ne i u odraslih osoba. Iznimka su stanice tumora kod kojih je ponovo zabilježena značajna ekspresija survivina (43, 44).

## Litijeve soli – stari stabilizatori raspoloženja, novi molekularno farmakološki aspekti djelovanja, fokus na apoptozu

Litijeve soli koriste se kao stabilizatori raspoloženja, odnosno najčešće su standardna psihofarmakoterapija bolesnicima koji boluju od bipolarnog afektivnog poremećaja (3). Litij se često dodaje antidepresivu kao „pojačivač" antidepresivnog djelovanja bolesnicima koji boluju od depresije. Litijeve soli imaju i antimanično djelovanje tako se taj lijek daje i bolesnicima s maničnom kliničkom slikom (6).

Litij je metal, atomskog broja 3 i atomske težine 6,94; pripada IA alkalnoj grupi metala periodnog sustava elemenata (45). Kao novi kemijski element otkrio ga je 1817. godine Johan Arfwedson. Već 1843. godine Alexander Ure uvodi litij u medicinu kao lijek za liječenje urične dijateze – gihta. Kasnije, u drugoj polovici 18. stoljeća bilo je uobičajeno tumačenje mnogih bolesti kroz poremećen metabolizam mokraćne kiseline. U tom su periodu Francuz A. Trosseani i Englez A. Haig uklopili tezu o uričnoj dijatezi i metabolizmu mokraćne kiseline u etiologiju manije i depresije. Zbog toga se litij davao različitim bolesnicima, a već 1886. Carl Lange, u Danskoj, opisuje prve rezultate uspješnosti liječenja depresija litijem. 1894. njegov brat Fritz opisuje prve rezultate liječenja litijem akutnih depresivnih bolesnika. 1873. u Sjedinjenim Američkim Državama, William Hammound opisuje svoje rezultate liječenja manija litijem (3,45). Početkom 20. stoljeća litij postaje praktički *lijek za sve*, pa se čak počinje stavljati i u pića. Tridesemulate expression of death receptors (such as Fas), thus stimulating apoptosis.

Caspases (cysteine aspartate specific proteases) are central executors of apoptosis (41). Inside the cells, they are found as inactive procaspases, and are activated in a proteolytic manner, by oligomerization or cleavage by other caspases. Caspases may act as initiating (caspase 2, 8, 9, 10) or effector (caspase 3 and 7) caspases. Because of their destructive action, their activity must be regulated very precisely in order to avoid unnecessary cell death. Otherwise, studies in humans have identified fourteen caspases. Among others, a substrate they act upon is the poly (ADP)-ribose polymerase (PARP) protein that undergoes cleavage under the action of caspases. Therefore, the PARP protein cleavage is also used as a marker of apoptosis. The activity of caspases is supervised by the apoptotic protein inhibitors (42), of which survivin has a major role (43). Survivin binds directly to caspases 3 and 7, thus inhibiting their activity (44). In addition, its expression depends on cell cycle, suggesting a dual role of this protein in the control of cell cycle and regulation of apoptosis (44). It should be noted that survivin is expressed during embryogenesis but not in adult individuals, with the exception of tumor cells where a significant re-expression of survivin has been recorded (43,44).

## Lithium salts – old mood stabilizers with new molecular pharmacological aspects of action, with special reference to apoptosis

Lithium salts are used as mood stabilizers and are the most widely used standard psychopharmacotherapy in patients suffering from bipolar affective disorder (15). Lithium is frequently added to antidepressants for enhancement of antidepressant action in depression patients. In addition, lithium salts have an antimanic action; therefore it is also administered to patients exhibiting a manic clinical picture (15).

Lithium is a metal, atomic number 3 and atomic weight 6.94; it is member of the IA alkaline group of metals in the periodic system of elements (45). Lithium as a new chemical element was discovered in 1817 by Johan Arfwedson. As early as 1843, Alexander Ure introduced lithium in medicine as an agent for the treatment of uric diathesis, i.e. gout. Later on, in the second half of the 18$^{th}$ century, many diseases used to be explained by the impaired metabolism of uric acid. At that time, A. Trosseani, a French, and A. Haig, an Englishman, integrated the concept of uric diathesis and uric acid metabolism in the etiology of mania and depression. Hence, lithium was administered to a wide variety of patients, and as early as 1886 Carl Lange described first results on the successful treatment of depression with lithium in Denmark; in 1894, his

tih godina 20. stoljeća počinju i prva testiranja litijevih soli u liječenju arterijske hipertenzije, a litijeve soli počinju se koristiti kao nadomjestak za natrijev klorid, kuhinjsku sol. 1949. godine prikazani su i prvi smrtni slučajevi koji su bili posljedica izuzetno toksičnog učinka litija, te se litij odlukom komisije za hranu i ljekove (engl. *Food and Drug Administration*, FDA) povlači s tržišta Sjedinjenih Američkih Država i zabranjuje svaka njegova uporaba kod ljudi (45). Istodobno, u Australiji Jon Cade ponovno, nakon već zaboravljenog povoljnog učinka litija u poremećajima raspoloženja, daje litij manično bolesnicima rezistentnim na dotadašnju terapiju s izuzetnim terapijskim uspjehom, ali ne zadugo, jer su nakon nekoliko mjeseci liječenja litijem, zbog toksičnih učinaka litija i njegovi bolesnici umrli. Tek šezdesetih godina 20. stoljeća litij ponovno postepeno ulazi „na mala vrata" u psihijatriju, ovaj put uz redovite kontrole razine litija u tjelesnim tekućinama i regulaciju doze prema nalazu koncentracije litija u serumu. 1974. godine litijeve soli ponovno su registrirane od strane FDA kao lijek za liječenje poremećaja raspoloženja (3,45).

**Farmakokinetika litijevih soli**

Litijeve soli najčešće se proizvode u obliku tableta od 300 mg. Litij se kompletno i potpuno apsorbira nakon osam sati, s najvećom serumskom razinom za 1 do 1,5 sati od unošenja pojedinačne doze. Poluživot litija u serumu je 15–30 sati. Nakon resorpcije u gastrointestinalnom traktu litij se postepeno distribuira u izvanstaničnoj tekućini s posebnim afinitetom prema tkivu štitnjače, kostima i bijeloj supstanci mozga. Ipak, prolaz litija kroz krvno-moždanu barijeru je spor, ali kada se postigne dinamička ravnoteža (engl. *steady state*) koncentracija litija u cerebrospinalnoj tekućini iznosi oko 40% od koncentracije u plazmi. Dinamička ravnoteža postiže se za 5–6 dana (46). Zapažena je i negativna korelacija između koncentracije litija u plazmi i tjelesne težine. Približno 95% pojedinačne doze litija izluči se putem bubrega, mokraćom. Oko dvije trećine pojedinačne doze izluči se tijekom 6–12 sati u početnoj fazi izlučivanja, a kasnije se ostatak pojedinačne doze izluči sporim izlučivanjem kroz slijedećih 10–14 dana.
Veći dio tubularne reapsorpcije litija odvija se većinom u proksimalnim tubulima i ne mijenja se diureticima koji djeluju na uzlazni dio Henleove petlje i distalni tubul. Litij se izlučuje u mlijeku te se ne preporučuje njegova primjena kod dojilja. Terapija litijem mora se kontinuirano nadzirati, određivanjem koncentracije litija u serumu za koju je preporučljivo da se kreće između 0,5 do 1,5 mmol/L (47).

**Mehanizmi djelovanja litija**

Do proteklog desetljeća o mehanizmu djelovanja litija praktički se nije ništa znalo. Glavni je razlog bio u tome što je litij lijek koji ne djeluje prvenstveno na koncentracije neurotransmitera ili na njihove receptore, čije je određivanje donedavno bilo moguće jedino u neuropsiho-

brother Fritz reported initial results on lithium therapy for acute depression. In 1873, William Hammound reported his results on lithium therapy for mania in the United States (15,45). At the beginning of the 20th century, lithium actually turned into a "panacea" and even used to be added to drinks. In the 1930s, initial testing of lithium salts in the treatment of arterial hypertension was launched, and lithium salts were used as a substitute for sodium chloride, i.e. table salt. In 1949, first fatal cases caused by the highly toxic action of lithium were reported, and lithium was withdrawn from the market in the United States by a decision issued by the Food and Drug Administration (FDA), placing ban on its use in humans (45). At the same time, after the favorable effect of lithium in mood disorders had been forgotten for quite a long time, Jon Cade in Australia reintroduced lithium administration to manic patients refractory to other therapeutic modalities with excellent therapeutic success, however, not for long because all his patients died within several months of lithium therapy due to its toxic effects. It was only in the 1960s that lithium gradually re-entered psychiatry by the side-door, this time with regular control measurement of lithium concentration in body fluids and dose regulation according to the serum lithium finding. In 1974, lithium salts were registered again by FDA as an agent for the treatment of mood disorders (45).

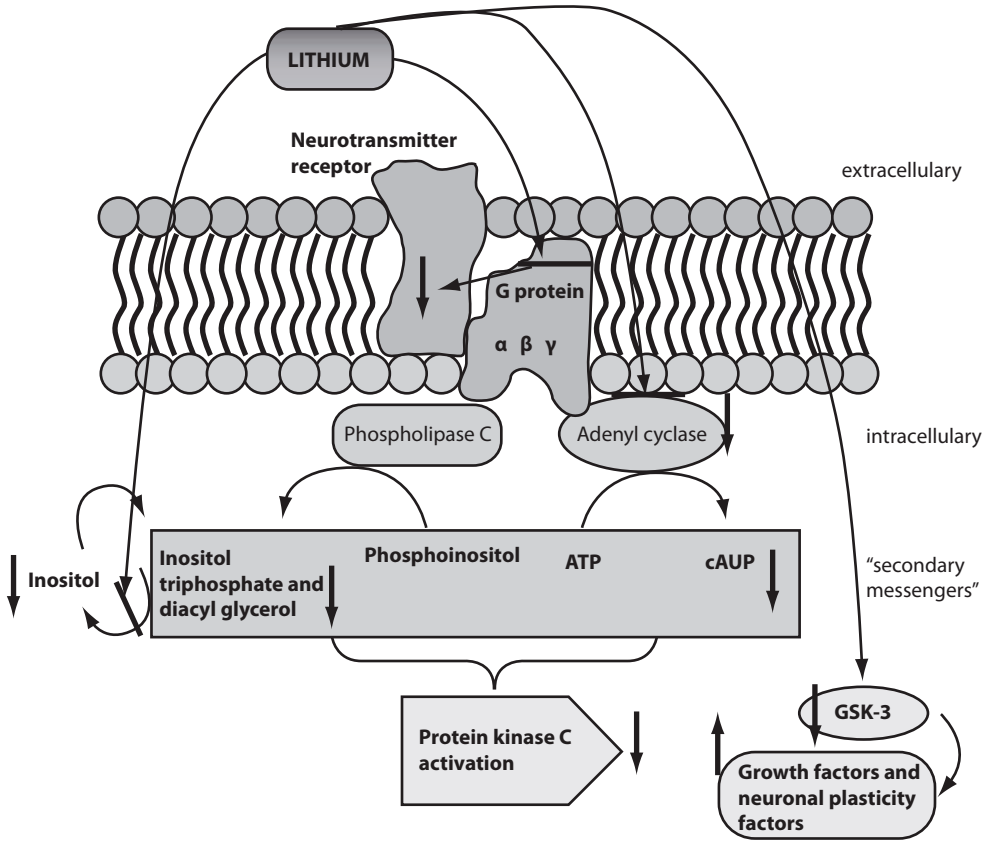
**The pharmacokinetics of lithium salts**

Lithium salts are generally available in the form of tablets á 300 mg. Lithium is completely absorbed within 8 h, reaching peak serum level in 1 to 1.5 h of individual dose administration. Lithium half-life in serum is 15–30 h. Upon resorption in the gastrointestinal system, lithium is gradually distributed *via* extracellular fluid, with special affinity for thyroid tissue, bone and substantia alba. The passage of lithium across the blood-brain barrier is slow; however, once the steady state has been achieved the CSF lithium concentration is about 40% of its plasma concentration. Steady state is achieved in 5–6 days (46). A negative correlation has been observed between plasma lithium concentration and body weight. Approximately 95% of individual lithium dose is eliminated by the kidneys, i.e. in urine. About two thirds of individual lithium dose are excreted over 6–12 h in the initial phase of elimination, followed by slow excretion of the remaining dose over 10–14 days.
Tubular reabsorption of lithium mostly occurs in proximal renal tubules and is not modified by diuretics, which act upon the ascending portion of Henle's loop and distal tubules. Lithium is excreted in human milk, therefore its use in lactation is discouraged. Lithium therapy requires close monitoring by regular determination of serum lithium concentration, which is recommended to be between 0.5 and 1.5 mM/L (47).

farmakološkim istraživanjima. Tek u proteklih nekoliko godina razvojem laboratorijskih tehnika i metoda, kojima se mogu registrirati promjene na staničnoj membrani i u unutarstaničnom prostoru i metoda molekularne biologije, moguće je dobiti neke spoznaje o djelovanju litija. Naime, litijeve soli svoj učinak u CNS-u ostvaruju preko reguliranja sustava drugog glasnika, odnosno posljedičnom regulacijom gena ranog odgovora, a time i regulacijom živčane plastičnosti. Rezultati dosadašnjih istraživanja mogu se iščitati iz slike 4. (48).

**The mechanisms of lithium action**

Until the past decade, practically nothing was known about the mechanism of lithium action, mainly because lithium is an agent that does not primarily act upon the concentration of neurotransmitters or their receptors, which until recently were the only measurable parameters in neuropsychopharmacological studies. Some information on the action of lithium could only be obtained in the last few years, with the development of laboratory methods and techniques recording changes in cell mem-




**Slika 4.** Mehanizam djelovanja litija. Litij svoj učinak ostvaruje na nekoliko razina. Prvenstveno kronično djeluje na podjedinice G-proteina tako da mijenja njihovu konformaciju čime G-protein postaje inaktivan i koči djelovanje neurotransmitera koji se veže za receptor. Nadalje, djeluje inhibitorno na adenilatciklazu te smanjuje koncentraciju cikličkog adenin-monofosfata (cAMP-a) i protein-kinaze C. Također, koči enzim koji razgrađuje inozitol-trifosfat te time smanjuje mogućnost stvaranja novog inozitol-trifosfata (koji također djeluje na aktivnosti protein-kinaze). Konačno, jedno od ključnih mjesta djelovanja litija je inhibicija enzima glikogen-sintaza kinaza (GSH-3) koji je inhibitor čimbenika rasta i živčane plastičnosti.

**Figure 4.** The mechanism of lithium action. Lithium exerts its action at several levels. Firstly, it exerts sustained action on G protein subunits modifying their conformation, resulting in G protein inactivation and inhibiting the action of neurotransmitter, which binds to the receptor. Furthermore, lithium has an inhibitory effect on adenyl cyclase and reduces the concentration of cyclic adenine monophosphate (cAMP-a) and protein kinase C. It also inhibits the enzyme for inositol triphosphate degradation, thus reducing the possibility of inositol triphosphate neogenesis (which also influences protein kinase activity). Finally, inhibition of the enzyme glycogen synthase kinase (GSH-3), a growth factor and neuronal plasticity factor inhibitor, is one of the crucial sites of lithium action.

Litij ostvaruje svoj učinak utjecajem na više elemenata glasničkih puteva: a) na G proteine, b) na protein-kinaze (PKC) i adenilat kinazu, c) na inozitol trifosfat i d) na protein kinazu koja fosforilira glikogen sintazu (49).

Kako je iz slike razvidno, litij smanjuje djelovanje protein-kinaze koja je zajednički supstrat oba puta aktivacije drugih glasnika, preko cAMP-a aktivacijom adenilat-ciklaze ili aktivacijom fosfolipaze C, koja iz fosfoinozitola stvara diacilglicerol i inozitol-trifosfat. Inozitol trifosfat se pomoću inozitol fosfataza cijepa na inozitol. Litij blokira ovu reakciju čime smanjuje mogućnost stvaranja inozitol-fosfata. Naime, inozitol, koji nastaje razgradnjom inozitol trifosfata, koristi se ponovno za izgradnju fosfo-inozitola (49).

Dugoročni učinak litija očituje se u manjoj podražljivosti stanice na neurotransmitere tako što mijenja konformaciju G-proteinskih podjedinica i na taj način onemogućuje aktivaciju drugih glasnika nakon poticanja G-proteina neurotransmitorskim receptorima.

Također, jedno od ključnih mjesta djelovanja litija jest inhibicija enzima glikogen-sintaze-3 (GSK-3), ključnog enzima koji je inhibitor mnogih čimbenika rasta stanica CNS-a i čimbenika živčane plastičnosti. Konačno, litij izaziva mnoge promjene na staničnoj razini trenutačno, a poboljšanja u kliničkoj slici nastaju nakon višednevnog uzimanja i djelovanja litija. Upravo sekundarni učinci, koji su rezultat poticanja promjena u drugim glasnicima, kao transkripcija gena za nove proteine ili čimbenike rasta primjerice BDNF, NGF predstavljaju dugotrajne učinke terapije litijem (50-52).

U vlastitom smo istraživanju došli do spoznaje da litij u terapijski relevantnom koncentracijskom području uzrokuje povećanje ekspresije gena survivina. Ovaj gen ključan je čimbenik u sprečavanju programirane stanične smrti. Drugim riječima, litij omogućuje preživljavanje stanica (u ovom eksperimentalnom modelu to su bile stanice glioblastoma) tako da putem molekularno-biokemijskih mehanizama mijenja ekspresiju gena (53). Nadalje, pokazali smo da predtretman litijem od 72 sata sprečava smanjenje ekspresije antiapoptotskog gena survivina nakon naknadnog tretmana glutamatom. Na taj se način može pretpostaviti da litijeve soli i djeluju u stabilizaciji raspoloženja i preveniraju ponovne mahove promijenjenog raspoloženja. U našem smo radu potvrdili, sukladno brojnim znanstveno-literarnim navodima, da litij u terapijski relevantnim koncentracijama nije bio citotoksičan za stanice, u ovom slučaju glioblastoma (54).

Do sada je objavljeno samo nekoliko istraživanja koja su ispitivala utjecaj litijevih soli na ekspresiju proapoptotskih ili antiapoptotskih gena. Kao istraživački *in vitro* model korištene su stanice neuroblastoma ili cereberalnih granuliranih stanica kao stanične kulture (55-65). Druga vrsta istraživanja bila su dizajnirana *in vivo* na eksperimentalnim životinjama, pretežito miševima (61-65). Dosadašnja su istraživanja također bila dizajnirana tako da se

brane and intracellular space, and the methods of molecular biology. Lithium salts exert their effect on the CNS through regulation of the second messenger system and consequential regulation of the early response genes, thus regulating neuronal plasticity. The respective studies are summarized in Figure 4 (48). Lithium influences a number of elements of the messenger pathways: (a) G proteins; (b) protein kinase (PKC) and adenylate kinase; (c) inositol triphosphate; and (d) glycogen synthetase kinase (49).

As shown in Figure 4, lithium suppresses the action of protein kinase, which is a common substrate for both pathways of the second messenger activation, *via* cAMP by activation of adenylate cyclase or phospholipase C that produces diacylglycerol and inositol triphosphate from phosphoinositol. Inositol triphosphate is cleaved to inositol by inositol phosphatase. This reaction is blocked by lithium, thus reducing the possibility of inositol phosphate formation, as the inositol produced by the inositol triphosphate breakdown is reused for the production of phosphoinositol (49). The long-term effect of lithium is manifested as lower cellular excitability to neurotransmitters through conformational modification of G protein subunits, thus preventing activation of other messengers upon G protein stimulation by neurotransmitter receptors.

One of the key sites of lithium action is inhibition of the enzyme glycogen-synthase-3 (GSK-3), the crucial enzyme acting as inhibitor of many CNS cell growth factors and neuronal plasticity factors. Finally, lithium immediately induces numerous changes at the cellular level, while improvement in clinical picture is observed after days of lithium administration and action. Thus, long-term effects of lithium therapy are secondary effects that result from changes in second messengers such as transcription of genes for new proteins or growth factors, e.g., BDNF or NGF (50-52).

In our study, we found lithium administered in a therapeutically relevant concentration range to enhance expression of the survivin gene. This gene is a crucial factor in the prevention of programmed cell death. In other words, lithium enables cell survival (glioblastoma cells in this experimental model) by changing the gene expression *via* molecular-biochemical mechanisms (53). Furthermore, we demonstrated that 72-h lithium pretreatment prevented the antiapoptotic gene survivin expression decrease following subsequent glutamate treatment. Thus, lithium salts could also be postulated to act in mood stabilization and to prevent recurrent episodes of mood alteration. In line with numerous literature reports, we confirmed that lithium in therapeutically relevant concentration had no cytotoxic effect, in this case on glioblastoma cells (54).

promatrao učinak litija na ekspresiju gena ili citotoksični učinak litija nakon tretmana samo litijem ili učinak litija nakon poticanja apoptotskih mehanizama nekim drugim agensom (55-66).

Učinak litija na stanice hipokampusa istraživao je Chen sa suradnicima (55). Istraživanje je provedeno na miševima tretiranim tijekom dva tjedna otopinom litijeva-karbonata. Koncentracija litija u serumu miša bila je oko 1 mmol/L (miševi su dobivali 2,4 g/kg litijeva karbonata dnevno). Životinje su nakon tretmana žrtvovane, te su uzorci hipokampusa analizirani mikroskopom nakon imunohistokemijske obrade ili je iz stanica hipokampusa izdvojen jedan dio proteina kako bi se Western-blottingom odredio sadržaj bcl-2. Morfološka analiza hipokampusa pokazala je da životinje tretirane litijem pokazuju veći volumen hipokampusa, gušće stanice hipokampusa i veći broj hipokampalnih stanica u odnosu na kontrolne uzorke koji nisu bili tretirani litijem.

Također u stanicama hipokampusa, životinja koje su bile tretirane litijem, nalazi se veća koncentracija antiapoptotskog gena Bcl-2 nego u kontrolnih netretiranih uzoraka.

U drugom istraživanju provedenom na eksperimentalnim životinjama, ispitivan je utjecaj litija na stanice čeonog korteksa (56). Ispitivan je utjecaj litija na ekspresiju antiapoptotskog gena *bcl-2*. Kao i u prethodnom istraživanju, ustanovljeno je da litij u koncentracijama oko 1 mmol/L tijekom 2 tjedna uzrokuje povećanje broja stanica i njihove gustoće, u ovom slučaju stanice čeonog korteksa. Također je u stanicama čeonog korteksa nađeno povećanje ekspresije antiapoptotskog gena, *bcl-2*.

Rena Li sa suradnicima je na stanicama neuroblastoma provela istraživanje u kojemu je ispitivala utjecaj litija na ekspresiju apoptotskog proteina kaspaze-3 (57). U ovom istraživanju cilj je bio usredotočen na mogući protektivni učinak litija. Pokazano je da litij smanjuje ekspresiju kaspaze-3 i to nakon apoptoze potaknute različitim citostaticima. U ovom eksperimentalnom modelu litij nije mijenjao ekspresiju kaspaze-3, što je možda posljedica različitog dizajna samog eksperimenta i korištenih staničnih linija. Konačni rezultat istraživanja Rene Li jest da litij djeluje neuroprotektivno i sprečava povećanje ekspresije apoptotskih gena.

Također na stanicama neuroblastoma, Hennon je sa suradnicima proveo istraživanje u kojem je ispitivao neuroprotektivni i antiapoptotski utjecaj litija nakon apoptoze izazivanom oubainom (58). Rezultati su također upućivali na neuroprotektivna i antiapoptotska svojstva litija.

Jedno od zanimljivijih i dobro dizajniranih istraživanja utjecaja litija na molekularne mehanizme apoptoze je ono autora Chena i Chanuanga koji su dokazali da litij povećava ekspresiju antiapoptotskog gena Bcl-2 i suprimira ekspresiju gena p53 (59). Istraživanje je provedeno tako da su u prvom dijelu pokusa stanice, primarne kulture cerebralnih mišjih stanica tretirane litijem u koncentraciji od 0,5

Only several studies investigating the effect of lithium salts on the expression of proapoptotic or antiapoptotic genes have been reported to date. The cells of neuroblastoma or cerebellar granulated cells as cell culture were used as *in vitro* experimental models (55-65). Other studies were of *in vivo* design in experimental animals, mostly mice (60-65). The studies published so far were so designed as to observe the effect of lithium on gene expression, or the cytotoxic effect of lithium following lithium only therapy, or the effect of lithium following apoptotic mechanism stimulation with some other agent (55-65).

The effect of lithium on the cells of hippocampus was investigated by Chen et al. (55). The study was performed on mice treated with lithium carbonate solution for two weeks. Lithium concentration in mouse serum was around 1 mM (the mice were administered 2.4 g/kg lithium carbonate *per* day). Upon treatment, the animals were sacrificed and hippocampus specimens were submitted to immunohistochemistry and microscopy, or a part of protein was isolated from hippocampal cells to determine the content of bcl-2 by Western blot. Morphological analysis of the hippocampus showed the lithium treated animals to have a greater volume of the hippocampus, higher density of hippocampus cells, and greater number of hippocampus cells than the control specimens from animals not administered lithium. In addition, the hippocampal cells of lithium treated animals showed a higher concentration of the antiapoptotic bcl-2 gene in comparison with control, untreated specimens.

Another study performed in experimental animals investigated the effect of lithium on the cells of frontal cortex (56). The effect of lithium on the antiapoptotic *bcl-2* gene expression was assessed. Like the previous study, lithium administration in concentrations of about 1 mM for two weeks led to an increase in the number and density of cells, in this case frontal cortex cells. An increase in the expression of the antiapoptotic bcl-2 gene was also recorded in the cells of frontal cortex.

Li and El-Mallahk investigated the effect of lithium on the expression of the apoptotic protein caspase-3 on neuroblastoma cells (57). This study was focused on the possible protective effect of lithium. Lithium has been demonstrated to decrease caspase-3 expression following apoptosis induced by various cytostatics. In this experimental model, lithium did not modify the expression of caspase-3, probably due to different experimental design and cell lines used. The conclusion reached in the study by Li and El-Mallahk is that lithium has a neuroprotective action and prevents increase in the expression of apoptotic genes (57).

In their study on neuroblastoma cells, Hennon et al. investigated the neuroprotective and antiapoptotic effect of lithium following ouabain induced apoptosis. Their re-

do 5,0 mM. U drugom dijelu istraživanja stanice su nakon predtretmana glutamatom koji je izazivao porast ekspresije gena p53 i Bax-a, te smanjenje ekspresije gena Bcl-2, tretirane litijem koji je ublažio navedene promjene izazvane glutamatom.

Španjolski istraživači Moroa i suradnici istraživali su sprečavanje apoptoze litijem i valproatom, kao stabilizatora raspoloženja, nakon izazivanja apoptoze niskim koncentracijama kalija (60). Istraživanje je provedeno na primarnim kulturama mišjih cerebralnih granuliranih stanica. U tom istraživanju poseban je naglasak stavljen na ispitivanje utjecaja litija i valproata na fosfo-inozitol i protein-kinazu, koje valproat povisuje, ali ne i litij. Konačni zaključak tog istraživanja bio je da iako valproat i litij imaju neuroprotektivno, odnosno antiapoptotsko djelovanje, taj učinak ostvaruju različitim mehanizmima. Drugim riječima, litij vjerojatno antiapoptotski učinak ostvaruje tako da djeluje na antiapoptotske gene. U sljedećem radu isti autori proširili su istraživanje te su na istovjetnim kulturama stanica kao i istom modelu dokazali da litij uzrokuje blokiranje aktivacije kaspaze 3 (61).

Također, na mišjim su cerebralnim granuliranim stanicama Nonaka i suradnici istraživali utjecaj litija na apoptozu izazvanu antiepilepticima fenitoinom ili karbamazepinom (62). U ovom istraživanju MTT metodom dokazano je veće preživljavanje stanica predtretiranih litijem. Prethodno navedeni antiepileptici izazivali su apoptozu na cerebralnim granuliranim stanicama tako da je uočena značajna fragmentacija DNK koju je litij mogao spriječiti. Osim nabrojenih *in vitro* istraživanja postoji i jedno *in vivo* istraživanje u kojem su laboratorijski miševi hranjeni uz uobičajenu prehranu i litijevim solima (63). Za razliku od grupe miševa koji su hranjeni samo aluminijevim pripravcima, koji su jaki neurotoksini, životinje hranjene i litijem pokazivale su manje apoptotskih stanica u hipokampusu, te manji pad ekspresije antiapoptotskog proteina Bcl-2 i manji porast ekspresije apoptotskog proteina Bax (58-65).

Navedeni znanstveni podaci iz literature jasno pokazuju da je ispitivanje molekularnog djelovanja litija započelo posljednjih godina. Noviji podaci različitim eksperimentalnim modelima pokušavaju objasniti dugotrajne učinke litija, te putem vrlo složene kaskade signalnih putova na kraju povezati početne biokemijske promjene koje on izaziva s promjenama raspoloženja tretiranih pacijenata.

Pretpostavlja se, da bi jedna od mogućih veza između djelovanja litija i stabilizacije raspoloženja bila sprečavanje indukcije apoptoze u glialnim ili neuronalnim stanicama koja je jedan od mogućih patofizioloških mehanizama u podlozi poremećaja raspoloženja (29,31).

U malobrojnim radovima koji istražuju mogući antiapoptotski učinak litija na apoptozu izazvanu različitim stimulusima, praćena je ekspresija tri-četiri apoptotska gena: *Bcl-2*, *p53, kaspaze-3* i *Bax* (66-70). No apoptoza je vrlo složen i precizno reguliran proces u kojemu sudjeluju brojni

sults also pointed to the neuroprotective and antiapoptotic properties of lithium (58).

One of the interesting and properly designed studies of the effect of lithium on molecular mechanisms of apoptosis is the study by Chen and Chuang, demonstrating that lithium enhances the antiapoptotic Bcl-2 gene expression and suppresses the p53 gene expression (59). In the first part of the study, primary cultures of the mouse cerebral cells were treated with lithium at a concentration of 0.5 to 5.0 mM. In the second part of the study, after pretreatment with glutamate, which increased the expression of p53 and Bax genes, and decreased the expression of Bcl-2 gene, the cells were treated with lithium, which was found to mitigate the changes induced by glutamate (59).

In Spain, Moro et al. investigated the prevention of apoptosis with lithium and valproate as mood stabilizers following induction of apoptosis with a low concentration of potassium (60). The study was performed on primary cultures of mouse cerebral granulated cells, and was focused on the effect of lithium and valproate on phosphoinositol and protein kinase, which are increased by valproate but not by lithium. The authors conclude that both valproate and lithium exert a neuroprotective and antiapoptotic effect but *via* different mechanisms of action. In other words, the antiapoptotic effect of lithium is most likely accomplished by its acting upon antiapoptotic genes. In their next study, the same authors extended their investigation, demonstrating on the same cell cultures that lithium induced caspase-3 activation (61).

In their study on mouse cerebral granulated cells, Nonaka et al. investigated the effect of lithium on apoptosis induced by the antiepileptics phenytoin and carbamazepine. Using the MTT method, they demonstrated a higher survival rate of lithium pretreated cells. In addition, the mentioned antiepileptics caused apoptosis on cerebral granulated cells, with significant DNA fragmentation that could not be prevented by lithium (62).

Besides these *in vitro* studies, there is one *in vivo* study in which mice were fed standard laboratory chow and lithium salts (63). In contrast to the group of mice fed aluminum preparations alone, which have strong neurotoxic effects, the animals fed lithium in addition to aluminum showed less apoptotic cells in the hippocampus, less suppressed expression of the antiapoptotic Bcl-2 protein, and less increase in the expression of the apoptotic Bax protein (55-65).

All these literature data clearly indicate that the investigation of the molecular action of lithium has been initiated in the past few years. Even more recent are data attempting to explain the long-term effects of lithium in various experimental models, and to eventually associate, by the very complex cascade of signal pathways, the initial biochemical changes caused by lithium with mood alterations in the treated patients. Prevention of the induc-

geni (32-36), pa najvjerojatnije ima i drugih apoptotskih gena na čiju bi ekspresiju litij mogao djelovati. Potrebna su daljnja istraživanja koja bi iste mehanizme trebala potvrditi i na *in vivo* istraživanjima.

tion of apoptosis in glial or neuronal cells, which is one of the possible pathophysiological mechanisms underlying mood disorders, has been postulated as a possible association between the action of lithium and mood stabilization (29-31). In the few studies investigating the possible antiapoptotic effect of lithium on the apoptosis induced by various stimuli, the expression of several apoptotic genes, Bcl-2, p53, caspase-3 and Bax was observed (66-70). However, apoptosis is a very complex and precisely regulated process in which numerous genes are involved (32-36), thus there probably are some other apoptotic genes the expression of which could be influenced by lithium. Therefore, additional studies are needed to confirm these mechanisms in *in vivo* experiments.

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