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ULOGA PROCESA RAZVOJA I SMRTI ĆELIJA U NEUROHEMIJSKOJ ORGANIZACIJI I INTEGRATIVNIM FUNKCIJAMA MOZGA - NORMALNOJ I PATOLOŠKOJ

THE ROLE OF CELLULAR DEVELOPMENT AND CELL DEATH IN NEUROCHEMICAL ORGANIZATION AND INTEGRATIVE BRAIN FUNCTIONS - NORMAL AND PATHOLOGICAL

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Sažetak - Polazeći od sistemskog aspekta o zajedničkim molekularnim mehanizmima u razvoju, čiji važan deo je proces ćelijske smrti, i integrativne aktivnosti nervnog sistema, izvedena je kompleksna kliničko-eksperimentalna studija uticaja različitih neurotrofičnih i apoptotičkih faktora (proteini S100b, HLDF, moždani lektini CSL i R1) u učenju i pamćenju i ishemičnom šlogu. Dobijeni su podaci koji se odnose na specifično i heterohrono učešće ovih faktora u neurohemijskim mehanizmima učenja i memorije i njihove značajne uloge u mehanizmima nastanka ishemičnog šloga. Promene i dinamika sadržaja neurotrofičnih faktora i faktora rasta kao i auto antitela na iste u serumu pacijenata i likvoru, mogu se smatratii prognostičkim markerima moždane ishemije i efekata terapije.

Ključne reči: Neurohemija; Lektini + fiziologija; Smrt ćelija; Apoptoza; Učenje; Memorija; Cerebrovaskularni insult

Summary *- In terms of systemic aspects of common molecular mechanisms of development, important part of which is the process of cellular death and integrative activity of nervous system, a omplex clinical-experimental study of effects of various neurotrophic and apoptotic factors (proteins S100b, HLDF, brain lectins CSL and R1) on learning and memory and ishemic stroke was performed. Data concerning specific and heterochronic participation of these factors in neurochemical mechanisms of learning and memory in mechanisms of ishemic stroke formation were established. Changes of examined factors and their antibodies as well as the dynamics of changes in sera and cerebro spinal fluid can be considered as prognostic markers of ischemic stroke and efficiency of therapy.*

Key words: *Neurochemistry; Lectins + physiology; Cell Death; Apoptosis; Learning; Memory; Cerebrovascular Accident*

Razrada predstava o jedinstvu molekularne osnove procesa razvoja, čiji nerazdvojni deo su procesi smrti ćelija i specifične delatnosti mozga, izaziva sve veći interes neurobiologa. Danas takođe postoje dosta ubedljivi eksperimentalni rezultati o sličnosti između molekularnih mehanizama razvoja mozga i konsolidacije dugotrajnog pamćenja na nivou regulacije transkripcije [1-4].

Međutim, analogna istraživanja na nivou translacije i postranslacionom nivou, a takođe izučavanja drugih stadijuma pamćenja nisu tako uspešna. Ona se ograničavaju, po pravilu, prikazom učešća jednih istih molekularnih faktora u različitim spoznajnim procesima. Ideja o "sličnosti" ili "jedinstvu" molekularne osnove rasta i razvoja, učenja i pamćenja, po našem mišljenju, nije potpuno odgovarajuća za eksperimentalnu razradu. Nove aspekte problema razotkriva koncepcija sistemogeneze P.K. Anohina i hipoteza geneze akta ponašanja. Navedena koncepcija razmatra razvoj organizma kao genezu funkcionalnih sistema koja protiče po principu

The integrity of molecular basis of the developmental process, including cellular death and specific brain functions, is becoming a more and more interesting subject for neurobiologists. Today, there are very convincing experimental results about the similarity between molecular mechanisms of brain development and consolidation of long term memory on the level of transcription regulation [1-4].

On the other hand, analogous studies on the translational and posttranslational levels, and also studies of other stages of memory process are not that successful. They are regularly limited by presenting the role of the same molecular factors in different cognitive processes. The idea of "similarity" or "integrity" of molecular basis of growth and development, learning and memory, in our opinion, is not completely adequate for experimental studies. New aspects of the problem have been revealed by concept of systemogenesis by P. K. Anokhin. This recent concept considers organism development as a

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Skraćenice

CSL	- lektini rastvorljivi u vodi
RI	- lektini vezani za membrane neurona
S100b	- kalcijum-vezujući protein
HLDF	- specifični proteini neurona
ASR	- akustična start-reakcija

Abbreviations

CSL	- hydrophilic lectins
RI	- membrane binding
S100b	- calcium-binding protein
HLDF	- specific neuron protein
ASR	- acoustic starting reaction

heterohronije, ne samo u ontogenezi, nego i u zrelom organizmu [5,6].

U razvoju navedenih sistemskih predstava formulisan je stav o heterohroniji neurohemijske organizacije akta ponašanja, što pretpostavlja kvantitativnu, kvalitativnu, vremensku i strukturnu specifičnost molekularnih mehanizama, koji obezbeđuju procese učenja i pamćenja u svim etapama života individue. Molekularni markeri formiranja funkcionalnih sistema, posebno ponašanja, mogu da budu, po našem mišljenju, neurotrofični i apoptotički faktori. Oni su tesno povezani sa regulacijom razvoja i preživljavanja ćelija, sazrevanjem struktura i funkcija mozga, obezbeđenjem integrativne delatnosti nervnog sistema [7,8].

Posebnu pažnju privlače molekuli, koji kontrolišu procese sinapso- i neuritogeneze, koje su ne samo važne etape diferencijacije nervnih ćelija u ontogenezi, nego se nalaze u osnovi dugotrajnih plastičnih prestrojavanja u zrelom mozgu. Tako su, molekuli ćelijske adhezije - u vodi rastvorljivi lektini (CSL) i membranski vezani R1 lektini - uvučeni u regulaciju procesa preživljavanja i razvoja nervnih i glijalnih ćelija, a time u procese sinapso- i neuritogeneze. Belančevina S100b, koja pripada grupi kalcijum-vezujućih belančevina, poseduje trofička svojstva i povezana je sa formiranjem neurita i sinapsi takođe [9,10].

U našim eksperimentima, sprovedenim na polno zrelim životinjama, dobijeni su rezultati, koji svedoče o heterohroniji uvlačenja različitih neurotrofičnih faktora u molekularne procese učenja i pamćenja. Posebno je istaknuto da antitela protiv belančevine S100b, lektina CSL i R1 pri aplikaciji na vermis cerebeluma ili hipokampa ispoljavaju selektivno i doznozavisno dejstvo na vremenski različite procese pamćenja različitih oblika odbrambenog ponašanja (privikavanje u start-reakcijama i uslovnom strahu) kod polno zrelih pacova. Pri izgradnji dugotrajnog privikavanja na akustičnu start-reakciju (ASR), nađene su individualne razlike dinamike nivoa belančevine S100b, lektina CSL i R1 u različitim strukturama mozga životinja [11,12].

Povećani interes izazivaju i regulatorni faktori, neposredno uključeni u obezbeđenje integrativnih funkcija nervnog sistema, koji mogu kako da inhibišu, tako i da aktiviraju programiranu smrt (apoptozu) ćelija [13,14]. Tu spadaju belančevine S100b i HLDF. Nađeno je, da Ca^{2+} i Zn^{2+}- vezujući specifičnu belančevinu mozga S100b, koja poseduje neurotrofičnu i neurotropnu aktivnost u visokim koncetracijama ispoljava proapoptotičko dejstvo.

genesis of functional systems, which is part of the principal of heterochrony, not only in ontogenesis, but also in adults [5,6].

In development of these systemic performances, an attitude has been formed towards heterochromy of neurochemical organization of behavior, with quantitative, qualitative, structural and time specifity of molecular mechanisms that enable processes of learning and memorizing at all life stages. Molecular markers in forming functional systems, especially behavior, can be, in our opinion, neurotrophic and apoptotic factors. They are closely related to regulation of cellular development and survival, development of brain structures and functions by providing integrative functions of nervous system [7,8].

Special attention has been paid to molecules which control processes of synapse- and neuritogenesis, important not only as stages of nerve cell differentiation in ontogenesis, but also as a base of long term plastic modifications in adult brain. For example, cellular adhesion molecules - hydrophilic lectins (CSL) and membrane binding R1 lectins - are included in regulation of survival and development of nerve and glial cells, and thus also included in processes of synapse- and neuro-genesis. Protein S100b, which belongs to the group of calcium-binding proteins, possesses trophic characteristics and it is related to the process of forming neurits and synapses [9,10].

Our experiments, conducted in adult, sexually active animals, revealed that heterochromy of different neurotrophic factors is involved in molecular processes of learning and memorizing. It is underlined that antibodies against protein S100b, and also lectin CSL and R1, when applied on cerebellar vermis or hippocampus, present with selective and dosage dependent impact on different memory processes in various kinds of defensive behavior (adaptation in start-reactions and conditioned fear) in sexually active rats. In development of long-term adaptation to acoustic start-reactions (ASR), individual differences were found in dynamics of protein S100b, lectin CSL and R1 levels in different brain structures of animals [11,12].

Particular interest is paid to regulatory factors, indirectly involved in securing integrative functions of nervous system, which can either inhibit or activate programed death of cells (apoptosis) [13,14]. Protein S100b and HLDF belong to this group. It has been revealed that ions of Ca and Zn, binding with brain specific protein S100b, present with neurotrophic activity in high conce-

Belančevina HLDF, može da indukuje diferencijaciju ćelija, nalazi se u nervnom sistemu sisara i sadrži u svojoj strukturi aktivne peptidne fragmente-inhibitore i aktivatore apoptoze ćelija nervnog tkiva [15-17].

Našli smo da belančevina S100b u dozi koja indukuje procese apoptoze u kulturi nervnog tkiva, pri aplikaciji na vermis cerebeluma izaziva značajno smanjenje amplitude akustične start-reakcije, a isto tako narušava kratkotrajno i dugotrajno gašenje te reakcije i formiranje uslovnog straha. Na procese konsolidacije i reprodukovanja oba oblika odbrambenog ponašanja S100b u navedenoj dozi nije uticala. S100b u dozi koja izaziva neurotrofične efekte stimuliše izgradnju uslovnog straha, ne utičući na procese gašenja start-reakcije. U uslovima intraperitonealnog i intranazalnog davanja sintetičkog analoga peptidnog fragmenta faktora HLDF-peptida HLDFG - inhibirajući apoptotičko umiranje ćelija, stimuliše dugotrajno prostorno pamćenje u vodenom labirintu kod pacova i miševa, a pri centralnom unošenju, selektivno stimuliše ponašanje tipa izbegavanja. Na taj način, rezultati ogleda su pokazali da faktori koji stimulišu i inhibišu apoptozu ćelija u strukturama zrelog mozga, ispoljavaju različito usmereno selektivno dejstvo na različite oblike učenja i pamćenja.

Svestrano izučavanje procesa razvoja i smrti je principijelno važno ne samo za razumevanje fundametalnih problema neurobiologije, nego i razradu akutnih medicinsko-socijalnih problema, posebno, ishemične bolesti mozga. U osnovi naših istraživanja neuroimunohemijskih mehanizama patogeneze ishemične bolesti mozga, smatramo da je jedna od univerzalnih komponenti razvoja ishemičnog oštećenja nervnog sistema poremećaj trofične regulacije, te i procesa razvoja i smrti ćelija. U formiranju trofične disfunkcije i oštećenja nervnog tkiva važnu ulogu imaju poremećaj propustljivosti histohemijskih barijera i autoimune reakcije usmerene prema antigenima mozga, posebno onim koji poseduju trofičnu i apoptotičnu aktivnost [18,19].

Pri kliničko-imunohemijskom ispitivanju, praktično kod svih bolesnika sa hroničnom ishemičnom bolešću mozga, našli smo u krvnom serumu povišeni nivo primarnih antitela prema belančevini S100b i osnovnoj belančevini mijelina, a takođe sekundarnih antitela prema S100b. Nakon davanja aminokiseline glicina kod pacijenata je zapaženo poboljšanje kliničkog stanja i normalizacija titra antitela prema S100b i osnovnoj belančevini mijelina.

Kliničko-imunološka analiza ukazala je na značajne razlike titra serumskih antitela prema S100b u zavisnosti od patogenetske varijante razvoja akutnog poremećaja krvotoka mozga. Pri aterotrombotičkom inzultu titar primarnih antitela bio je signifikantno viši nego pri kardioemboličnom inzultu. Kod bolesnika sa aterotrombotičnim inzultom ustanovljene su jasne vremenske zakonitosti pri-

ntrations, or proapoptotic effect. Protein HLDF is believed to induce differentiation of cells in central nervous system of mammals. This protein carries active peptide fragments, inhibitors or activators of apoptosis in brain cells [15-17].

Our findings suggest that S100b induces apoptosis in the nerve tissue. During its application on cerebellar vermis significant decrease of amplitude of acoustic start-reaction was found. As such, this reaction induces both short and long-term disappearance of this reaction and modulates development of conditioned fear. S100b in neurotrophic doses, stimulates development of fear without impact on start-reactions. During intraperitoneal and intranasal application of synthetic analog of peptide fragment HLDF, which inhibits apoptosis, it stimulates prolonged spatial memory in water labyrinth in rats and mice. However, central application induced reaction of avoidance. These results indicate that factors which stimulate or inhibit apoptosis in mature brain cells also selectively induce different forms of learning and memorizing.

It is of great significance to study about processes of cellular developing and death in neurobiology, not only for the sake of science, but also for medico-social aspects of ischemic brain disease. Dysfunction of trophic regulation and thus the process of cell development is the basic part of our investigations of neuroimmunochemical mechanisms of ischemic brain disease pathogenesis and one of universal components of ischemic nervous system injuries. In development of trophic dysfunction and nervous tissue damage a great role belongs to malfunction in permeability of histochemical barriers and autoimmune reactions directed to antigens of the brain, especially those that express trophic and apoptotic activity.

Clinical-biochemical tests of almost all patients with chronic ischemic brain disease revealed high levels of primary serum antibodies against S100s protein and basic protein of myelin, as well as secondary antibodies against S100b. After administering amino acid glycine, patients exhibited an improvement of clinical status and normalization of antibody concentrations against S100b and basic myelin protein.

Clinical-immunological analysis showed a significant difference in concentrations of serum antibodies against S100b in regard to pathogenetic ways of acute brain blood flow damage development. In atherothrombotic strokes the concentration of primary antibodies was significantly higher than in cardioembolic strokes. In patients with atherothrombotic stroke, a clear timescale of primary and secondary antibodies formation could be established. A connection between the level of basic

marnog i sekundarnog obrazovanja antitela. Nađena je zavisnost stepena povećanja količine osnovne belančevine mijelina u cerebrospinalnoj tečnosti bolesnika od lokalizacije i dimenzija žarišta ishemije. Pored toga, kod pacijenata u čijoj cerebrospinalnoj tečnosti je zapaženo povećanje lektina R1 u trećem danu oboljenja, dolazilo je do značajnog obnavljanja poremećenih funkcija dok se pri nepovoljnom ishodu zapaža odsustvo ili negativna dinamika dotičnog neurotrofičnog faktora [20].

Kod bolesnika sa aterotrombotičnim inzultom, kod kojih je u prvom danu oboljenja registrovan pulsni pritisak veći od 80 mmHg stuba, količina belančevina HLDF u serumu bila je značajno snižena, dok je nivo autoantitela prema HLDF prevazilazio uzrastnu normu za 50-100 puta. Pri tome, kod ovih bolesnika sa prvobitno niskom koncenrtacijom HLDF zapaža se značajno povećanje žarišta ishemije mozga. Sprovedena bazična terapija ne dovodi do promena količine S100b u cerebrospinalnoj tečnosti i HLDF u serumu i likvoru ovih pacijenata.

Dobijeni rezultati navedenim istraživanjima svedoče o specifičnosti i heterohroniji učešća neurotrofičnih i apoptotičkih faktora u neurohemijskim mehanizmima učenja i pamćenja, a isto tako o važnoj ulozi tih molekula i autoimunih procesa usmerenjem prema njima u razvoju akutne i hronične ishemične bolesti mozga. Nađene promene količine i dinamike ispitivanih faktora i autoantitela prema njima u krvnom serumu i cerebrospinalnoj tečnosti pacijenata mogu da se razmatraju kao prognostički markeri ishemične bolesti mozga i efikasnosti sprovedene terapije.

myelin protein in the cerebrospinal fluid of patients and the localization and dimensions of the ischemic focus was established. Apart from this, in patients with an increase of lecitin R1 in the liquor three days after accident, a significant recurrence of damaged functions occurred, whereas at an unwanted turn of events absence or a negative dynamics of mentioned neurotrophic factors could be registered [20].

In patients with atherothrombotic stroke where pulse pressure was above 80 mmHg during the first day, the level of HLDF protein in the serum was significantly low, whereas the level of autoantibodies against HLDF reached levels of 50 to 100 times higher than age norms. Meanwhile, these patients with originally low concentration of HLDF presented significant increase of focal brain ischemia. Basic therapy did not change the amount of S100b in liquor, and HLDF in serum of treated patients.

Research findings suggest that neurotrophic and apoptotic factors are involved in processes of learning and memorizing. These factors play an important role in autoimmune processes and development of acute and chronic ishemic brain disease. Changes regarding their quantity and immune response to them in blood serum and liquor of patients can be used as prognostic markers in ishemic brain disease and efficacy of administered therapy.

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