Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training)*

1. Raziskovalna organizacija (*Research organisation)*:

Univerza v Ljubljani, Medicinska fakulteta

2. Ime, priimek in elektronski naslov mentorja (*Mentor's name, surname and email*):

Robert Zorec, robert.zorec@mf.uni-lj.si

3. Šifra in naziv raziskovalnega področja (*Research field*):

Nevrobiologija, 3.03 Medicina

4. Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training)*:

Navedite tudi morebitne druge zahteve, vezane na usposabljanje mladega raziskovalca (npr. znanje angleškega jezika, izkušnje z laboratorijskim delom, potrebne licence za usposabljanje…).

slo:

Astrociti so heterogene celice nevroglije, ki vzdržujejo homeostazo v osrednjem živčevju (CNS). Njihova anatomska pozicija in fiziološke lastnosti vplivajo na sinhroniziranje aktivnosti nevronskih mrež, ki vključuje mehanizme signalizacije astroglije s sinapsami in dostave energentov. Energetska podpora astrocitov temelji na aerobni glikolizi, kjer je končni metabolit L-laktat. Poleg sinteze ATP, v tem procesu nastajajo tudi itermediati za sintezo amino kislin, nukleinskih kislin in lipidov, ki so vsi potrebni za celično delitev in morfološke spremembe celice, ki predstavljajo sinaptično in nevroglijalno plastičnost v živčevju. Cilj predlaganega projekta je razumeti uravnavanje aerobne glikolize v astrogliji z noradrenalinom in L-laktatom, ki ni le energent oziroma gorivo, pač pa tudi zunajcelični signal. Pred kratkim smo odkrili, da v astrogliji agonisti, ki so selektivni za kanonični receptor GPR81, receptor za L-laktat v adipocitih, ojačajo astroglialno aerobno glikolizo, vendar neodvisno od tega receptorja, verjetno prek novega, še neodkritega receptorja (Vardjan et al. 2018). Cilj tega usposabljanja bo razviti postopke za identifikacijo tega novega receptorja in določiti, kako ta receptor uravnava aerobno glikolizo v astrocitih in tudi v nevronih. Pri tem bom še posebej študirali nevrone iz jedra Locus coeruleus, primarni izvor sinteze in izločanja noradrenalina v možganih. Za te poskuse bomo uporabili posebno metodologijo. Priprava primarnih celičnih kultur astrocitov in nevronov za študij subceličnih fizioloških značilnosti sekundarnih prenašalcev in metabolitov, na celični in subcelični ravni z napredno fluorescenčno mikroskopijo, kot npr. konfokalna mikroskopija in super-ločljivostna mirkoskopija SIM in STED. Poleg tega bomo študirali tudi celice v tkivnih rezinah. Dinamične spremembe citosolnih prenašalcev in metabolitov bomo določali s tehnologijo, ki jo omogočajo fluorescenčni nanosenzorji za cAMP, Ca^{2+} , D-glukoza, L-laktat. Ker astrociti vsebujejo glikogen, bomo študirali ali obstaja preferenčna subcelična lokalizacija tega skladišča energije, ki je vpleten kontinuirno v aerobno glikolizo. Poleg nanosenzorjev, bomo uporabili imunocitokemijo za označevanje specifičnih markerjev v astrocitih, nevronih in drugih celičnih tipih. Elektrofiziološke metode, vključno z meritvami membranske kapacitete, bomo uporabili za študij fluktuacij membranske celične površine in določili, kako na to vplivata noradrenalin in L-laktat, ki spodbudita spremembo oblike teh celic. Rezultati teh meritev bodo ustvarili novo znanje o vlogi metabolitov v celični signalizaciji, zelo kompetitivno področje v fiziologiji in patofiziologiji osrednjega živčnega sistema.

Vardjan N, Chowdhury HH, Horvat A, Velebit J, Malnar M, Muhic M, Kreft M, Krivec SG, Bobnar ST, Mis K and others. 2018. Enhancement of Astroglial Aerobic Glycolysis by Extracellular Lactate-Mediated Increase in cAMP. Frontiers in Molecular Neuroscience 11.

eng:

Astrocytes are heterogeneous neuroglial cells with homeostatic function in the central nervous system (CNS). Their anatomical position and functional properties synchronize the activity of neural networks where astroglial signaling mechanisms take place, linking synaptic activity with energy provision. The latter process depends on aerobic glycolysis where L-lactate is the main end-product. Besides providing ATP, aerobic glycolysis importantly contributes intemerdiates for the synthesis of amino acids, nucleic acids and lipids, key building blocks for dynamic cell shape changes, as present in synaptic and neuroglial plasticity. The aim of the proposed research is to understand the control of aerobic glycolysis in astroglia by noradrenaline and the end-product L-lactate, which is considered to be not only a fuel, but also an extracellular signal. Recently, we discovered that in astroglia, agonists selective for the canonical GPR81 receptor, a L-lactate receptor discovered orininally in adipocytes, enhance astroglial aerobic glycolysis, however independently of this receptor, indicating the presence of a novel, yet unidentified one (Vardjan et al., 2018). The aim of this research will be to develop assays to identify this new receptor and learn how it modulates second messengers and aerobic glycolysis in astrocytes and neurons. We will especially study neurons from the locus coeruleus, the prime site of noradrenaline production in the brain. For these experiments dedicated methodology will be used. Primary cell cultures of astrocytes and neurons will be prepared to study subcellular characteristics of second messenger and metabolites at cellular and subcellular levels by advanced flusoresence microscopy approaches, including confocal and super-resolution microscopies including SIM and STED. In addition, tissue slices will be used. Dynamic changes in nanosensor-dependent fluoresence signals, reporting cAMP, calcium, D-glucose, L-lactate will be monitored. Since astrocytes contain glycogen, we will study whether there is a preferential subcellular localization of this energy store, continuosly engaged in aerobic glycolysis. In addition to nanosensors, we will use immunocytochemistry to label specific markers for astroglia, neurons and other cells. Electrophysiological approaches, including membrane capacitance measurements, will be used to study membrane area fluctuations in these cells to complement the role of noradrenaline and L-lactate-mediated cell shape changes. The results of these experiments will provide key new knowlede about the role of metabolites in cell signaling, a very competitve topic in physiology and pathophysiology of the CNS.

Vardjan N, Chowdhury HH, Horvat A, Velebit J, Malnar M, Muhic M, Kreft M, Krivec SG, Bobnar ST, Mis K and others. 2018. Enhancement of Astroglial Aerobic Glycolysis by Extracellular Lactate-Mediated Increase in cAMP. Frontiers in Molecular Neuroscience 11.