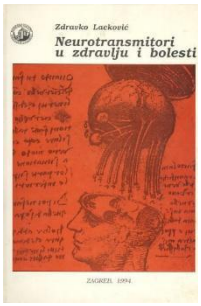
45 YEARS OF CONTINUOUS NEUROTRANSMITTERS RESEARCH IN CROATIA (1978 - 2023)

On the occasion of the 45th anniversary of the Laboratory for Molecular Neuropharmacology of the Department of Pharmacology of the University of Zagreb School of Medicine, with 20 years of cooperation with the Department of Pharmacology of the Faculty of Pharmacy and Biochemistry

Under the auspices of the Department of Medical Sciences Croatian Academy of Sciences and Arts

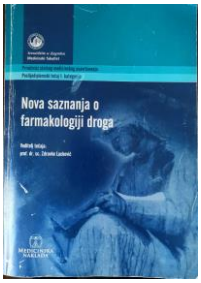
With the financial support of the Croatian Pharmacological Society

11 a.m. November 27, 2023
Library of the Croatian Academy of Science, Strossmayer Square 14, Zagreb



PROGRAM

- **11:00 -11:10 Acad. Prof. Vida Demarin**: Introduction and short overview of the relationship between basic research and clinical neurology
- **11:10-12:00** The laboratory through video clips and lectures about its past, present and future: **Prof. Zdravko Lacković** „From mummified frogs to invited lectures at European and world congresses"; **Prof. Lidija Bach Rojecky & Ivica Matak, PhD** „Botulinum toxin from our Laboratory to the CNS"
- **12:00-12:35 Prof. Melita Šalković-Petrišić** „How brain research in diabetes led to the development of the first model of sporadic Alzheimer's disease**"; Assoc. Prof. Jelena Osmanović Barilar** & **Jan Homolak, PhD** „Building on our legacy; moving forward with the sporadic Alzheimer's disease model towards metabolic dyshomeostasis and digestive tract alterations"



12:35-13:00 coffee break

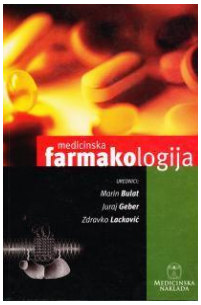
Invited lectures:

- **13:00-13:20 Assoc. Prof. Marco Pirazzini** (University of Padua) „From periphery to CNS: novel insights on tetanus pathophysiology and therapy"
- **13:20-13.40 Prof. Eugenio Barone** (University of Sapienza, Roma) „Insulin signalling in the brain: a matter of energy"
- **13:40-14:10 Acad. Prof. Dimitri Krainc** – by Zoom (National Academy of Medicine US /NAM – NASEM/, the President of The American Neurological Society) „The role of mitochondria-lysosome contacts in health and disease"
- **14:10-14:40 Acad. Prof. Peter Riederer** (German National Academy of Sciences – Leopoldina) „Oxidative stress: the unknown known in Alzheimer's disease"

14:40 lunch & poster exhibition

Posters of PhD students performing their research at the Laboratory for Molecular Neuropharmacology:

- **Petra Šoštarić Mužić** „Understanding the battle between botulinum toxin and hyperactive muscle"
- **Patrik Meglić** „Tetanus toxin: our arch-enemy turned into a tool to study movement disorders"
- **Giorgia Schiavone** „Novel natural source-based polysaccharide hydrogels as macromolecule delivery systems"
- **Davor Virag** „Phenotyping rat behaviour using MIROSLAV and VlaDiSlav, custom-made home cage monitoring devices"
- **Dalia Nemanić** „Botulinum Neurotoxin Type A Antinociceptive Activity in Spinal Cord and Trigeminal Sensory Nociceptive Nuclei Involves Central Trans-Synaptic Transport"



From periphery to CNS: novel insights on tetanus pathophysiology and treatment

F. Fabris[a], S. Varani[a], M. Tonellato[a], I. Matak[b], P. Šoštarić[b], P. Meglic[b], M. Caleo[a†], A. Megighian[a,c], O. Rossetto[a,d,e], C. Montecucco[a,d], and **Marco Pirazzini**[a,e]

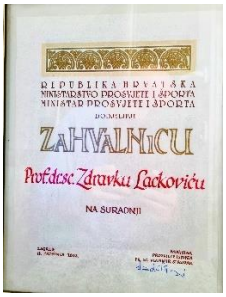
[a]Department of Biomedical Sciences, University of Padova, Italy, [b]Department of Pharmacology, University of Zagreb School of Medicine, Croatia, [c]Padova Neuroscience Center, University of Padova, Italy, [d]Institute of Neuroscience, Italian Research Council, University of Padova, Italy, [e]Interdepartmental Research Center of Myology, University of Padova, Italy

The clinical hallmark of tetanus is a spastic paralysis caused by the metalloprotease activity of tetanus neurotoxin within inhibitory interneurons in the spinal cord, which is among the first clinical conditions ever described in the history of medicine. At the same time, the most severe form of the disease known as cephalic tetanus, which follows head wounds and the intoxication of cranial nerves, starts with an idiopathic facial palsy. This initial symptom confounds the medical doctor and delays diagnosis thus favouring a rapid development of a deadly cardiorespiratory deficit occurring even without generalized spasticity. The molecular basis of this unexpected flaccid paralysis and rapid evolution of cephalic tetanus are described together with insights on the development of exceptionally potent human monoclonal antibodies that can open to the treatment of cephalic and generalized tetanus via the intrathecal route.

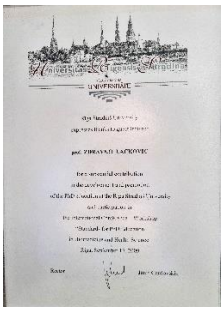
Insulin signaling in the brain: a matter of energy

Eugenio Barone

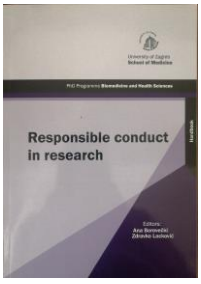
Department of Biochemical Sciences “A. Rossi-Fanelli”, Sapienza University of Rome, Italy



Brain insulin signaling acts as a key regulator for gene expression and cellular metabolism, both events sustaining neuronal activity and synaptic plasticity mechanisms. Alterations of this pathway, known as brain insulin resistance, are associated with a higher risk to develop age-related cognitive decline and neurodegeneration. Among the molecular mechanisms identified to promote brain insulin resistance, mitochondrial dysfunctions, failure of energy metabolism, and increased oxidative stress levels have been found to play a role. Studies from our group uncovered the role of the enzyme biliverdin reductase A (BVRA) that, beyond its activity in the degradation pathway of heme, is a novel regulator of the insulin signaling. BVRA regulates insulin signaling pathway by working either as a S/T/Y kinase or a scaffold protein. BVRA is required to promote the AKT-mediated inhibition of GSK3β in response to insulin, that promotes cell metabolism and survival. Findings from our group revealed that a reduction of BVRA protein levels is a key event driving brain insulin resistance development. Moreover, we identified a novel mechanism for which loss of BVRA is responsible for GSK3β hyper-activation that drives mitochondrial stress and bioenergetics failure in response to insulin in neuronal cells. These alterations accelerate the impairment of energy metabolism and the development of neurodegeneration. Conversely, rescuing BVRA functions reduces oxidative stress levels, ameliorates brain insulin signaling activation and cellular metabolism, finally contributing to improved cognitive functions in animal models of neurodegeneration. Overall, our data suggest that BVRA links insulin signaling activation and mitochondrial bioenergetics to preserve cellular homeostasis and preventing the development of neurodegeneration.



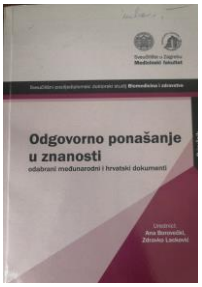
The role of mitochondria-lysosome contacts in health and disease



Dimitri Krainc

Department of Neurology, Feinberg School of Medicine, Northwestern University

Both mitochondria and lysosomes are multifunctional organelles that have been implicated in numerous human diseases. Mitochondria are necessary for cellular respiration and ATP synthesis, and they are also important storage sites for ions, lipids, and other



metabolites. Similarly, lysosomes act as storage sites for ions such as calcium, iron, nucleosides and play a critical role in degrading cellular contents such as proteins and lipids.

We have previously described the functional interplay of mitochondrial and lysosomal dysfunction in neurodegeneration (Burbulla et al, Science), whereas our recent data demonstrated direct contacts between mitochondria and lysosomes in various cell types (Wong et al., Nature). Importantly, the formation of mitochondria-lysosome contacts is independent from autophagosome biogenesis or mitophagy. We found that mitochondria-lysosome contacts are regulated by multiple proteins on the mitochondrial and lysosomal membranes. In its active GTP-bound and lysosome-localized state, Rab7 mediates the tethering between mitochondria and lysosomes. Rab7 GTP hydrolysis is stimulated by Rab GAPs (GTPase-activating proteins) such as TBC1D15, resulting in an inactive, cytosolic GDP-bound form of Rab7. Interestingly, TBC1D15 is recruited to the outer mitochondrial membrane by the mitochondrial transmembrane fission protein FIS1, and TBC1D15- mediated Rab7 GTP hydrolysis was found to promote untethering of mitochondria-lysosome contacts.

Our recent studies have suggested that mitochondria-lysosome contact sites regulate organelle dynamics and metabolite transfer. Mitochondrial dynamics also depend on contacts with lysosomes, which affect the rate of mitochondrial fission in a manner dependent on FIS1, TBC1D15, and Rab7 (Wong et al J. of Cell Biology). Beyond organelle dynamics, we found that mitochondria-lysosome contacts serve as sites of direct transfer of Ca2+ (Peng et al., PNAS) and amino acids (Peng et al Science Advances) from lysosomes to mitochondria.

Taken together, studies on the structure and function of mitochondria-lysosome contact sites reveal a clear role in cellular homeostasis, including in neurons. This raises the question of the importance of mitochondria-lysosome contacts to the development and progression of neurological disease. Indeed, we found that several proteins associated with contact sites have been implicated in the pathology of Parkinson's disease (Kim et al, Nature Communications, Peng et al Science Advances), Charcot-Marie-Tooth disease (Wong et a; Dev Cell), and other neurological disorders. We are currently pursuing further studies of the role of mitochondria-lysosome contacts in normal and diseased human neurons and glia. Thus, further insights into organelle contact site dynamics and regulation will shed important light on physiological and pathological cellular functions.

Oxidative stress: the unknown known in Alzheimer's disease

Peter Riederer

Clinic and Policlinic for Psychiatry, Psychosomatics and Psychotherapy, University Hospital Würzburg, University of Würzburg, Germany; Department of Psychiatry, University of South Denmark, Odense, Denmark

Research in Alzheimer's disease (AD) started around 1975, the year when Siegfried Hoyer reported on a deficit in blood flow and occurrence of disturbed oxidative metabolism and DM Bowen showed a massive deficit of acetylcholine transferase in brains of patients with AD.

Disturbed oxidative metabolism/especially glucose metabolism let researchers like Siegfried Hoyer assume a major role in their contributions to cognitive disturbances and even AD. Zdravko Lackovic and Melita Šalković-Petrišić developed the streptozotocin model of AD already 1989, and with this gave an enormous input to further elucidate the pathology of AD. Streptozotocin, like alloxan, when metabolized, is producing hydrogen peroxide, which is further converted to hydroxyl radicals in an iron-dependent reaction. This mechanism may explain the potency of toxicity released by these toxins. Although the role of iron in AD is not fully understood, disturbed iron uptake may lead to iron mediated upregulation of ß-amyloid, thus contributing to enhanced plaque formation. Indeed, oxidative stress parameters have been detected in post mortem brain regions of AD.

Oxidative stress can be released by other triggers of cognitive impairment, e.g. viruses, which contribute to neuroinflammatory-mediated degenerative mechanisms. Therefore, focus on oxidative stress related research combined with respective neuroprotective strategies is highly warranted.

On behalf of the organizers,

Prof. Zdravko Lacković *Prof. Melita Šalković-Petrišić*

Prof. Vladimir Trkulja *Assoc. Prof. Jelena Osmanović-Barilar*