2nd Collaborative Workshop IOCB – IPHYS

November 19, 2019

IOCB Lecture Hall (Building A, 2nd floor, A2.01)

ÚOCHB AV ČR
IOCB PRAGUE

INSTITUTE OF PHYSIOLOGY CAS

Programme at a Glance

Time	Speaker	Presentation
9:30- 9:35	Z. Hostomský	Workshop opening
9:35- 9:45	A. Stuchlík (IPHYS)	Neurobehavioral phenotyping in fast and reliable way
9:45- 9:55	M. Balaštík (IPHYS)	Molecular regulation of neural development using primary neuron cultures and mouse models
9:55- 10:05	H. Zemková (IPHYS)	Allosteric modulation of purinergic P2X receptor channels
10:05- 10:15	J. Jakubík (IPHYS)	Muscarinic receptors and other GPCRs
10:15- 10:25	E. Kudová (IOCB)	Neurosteroids as therapeutics for CNS diseases versus steroids as hormones
10:25- 10:35	P. Šácha (IOCB)	IOCB compound library
10:35- 10:45	M. Sládek (IPHYS)	Screening for novel small-molecule modulators of circadian clock
10:45- 10:55	K. Smolková (IPHYS)	Regulation of isocitrate dehydrogenace-2 function by sirtuin-3 deacetylase: identification of acetylated sites
10:55- 11:05	O. Zimmermannová (IPHYS)	Comprehensive approach to study structure and function of membrane transporters
11:05- 11:35 – COFFEE BREAK		
11:35- 11:45	M. Rossmeisl (IPHYS)	Phenotyping of energy metabolism and insulin sensitisity in mice
11:45- 11:55	J. Jiráček (IOCB)	Analogues of insulin, IGF-1 and IGF-2 and their applications
11:55- 12:05	F. Kolář (IPHYS)	In vivo and ex vivo evaluation of heart function
12:05- 12:15	I. Vaněčková (IPHYS)	The analysis of cardiovascular parameters (blood pressure, heart rate and analysis of blood pressure variability) using telemetry monitoring in unique models of hypertension and chronic kidney disease
12:15- 12:25	M. Vrábel (IOCB)	Bioorthogonal reactions for bioimaging and beyond
12:25- 12:35	M. Hocek (IOCB)	Medicinal chemistry of base-modified nucleosides, nucleotides and nucleic acid
12:35- 12:45	R. Nencka (IOCB)	Design of novel enzyme inhibitors – PI4KB and nSMase2 case studies
12:45- 12:55	H. Mertlíková - Kaiserová (IOCB)	Biological tools to promote early drug discovery at IOCB
12:55- 13:05	O. Kuda, T. Čajka (IPHYS)	Lipidomics and metabolomics - profiling and pathway analysis
13:05- 13:10	Z. Hostomský, J.Kopecký	Concluding remarks
13:10- 14:00 - LUNCH		

ABSTRACTS

Aleš Stuchlík
9:35 - 9:45 AM

NEUROBEHAVIORAL PHENOTYPING IN FAST AND RELIABLE WAY

Department of Neurophysiology of memory is a top-tier Czech laboratory for neurophysiological and behavioral phenotyping. We can conduct studies aimed at evaluation of various new drugs on most types of behavior and neural circuit- a cell-activity. We have a pipeline of assessment of anti-psychotic, anti-anxiety, anti-dementia and procognitive drugs. We offer equipment and expertise for most types of behavior, including basic and spontaneous activities, anxiety and compulsive-like behaviors, all types of learning and memory, cognition, and social behavior. Simultaneously we can investigate effect of drugs of interest on adult neurogenesis, brain structure and cell and circuit activity with molecular imaging, immunohistochemistry and optogenetics.

Martin Balaštík
9:45 - 9:55 AM

MOLECULAR REGULATION OF NEURAL DEVELOPMENT USING PRIMARY NEURON CULTURES AND MOUSE MODELS

The department of Molecular Neurobiology focuses on characterization of signaling cascades and genes regulating neuron migration, neuron polarization, axon guidance and synapse formation during the process of neural development and their defects related to various neurodevelopmental disorders. For the purpose we use combination of in vitro model systems as primary neuron cultures, compartmentalized cultures etc, as well as in vivo models, e.g. transgenic mice generated using in utero electroporation and their detailed molecular, histological and microscopic analysis.

Hana Zemková
9:55 - 10:05 AM

ALLOSTERIC MODULATION OF PURINERGIC P2X RECEPTOR CHANNELS

P2X receptors, ATP-gated ion channels, have been documented in many physiological and pathological processes including inflammation, pain and cancer, and are potential therapeutic targets for treatment of these pathologies. This perspective is closely related to understanding the molecular physiology and pharmacology of P2X receptors that offer multiple binding sites for positive or negative allosteric modulators enhancing or blocking receptor function.

Jan Jakubík
10:05 - 10:15 AM

MUSCARINIC RECEPTORS AND OTHER GPCRS

GPCRs are target for more than 30% of marketed pharmaceutics. We study signaling via class A GPCRs primarily focusing on muscarinic acetylcholine receptors. We are able and open to test functional activity of compounds of interest at muscarinic and other GPCRs.

Eva Kudová
10:15 - 10:25 AM

NEUROSTEROIDS AS THERAPEUTICS FOR CNS DISEASES VERSUS STEROIDS AS HORMONES

Neurosteroids developed at the department of Neurosteroids have been originally developed as inhibitors of N-methyl-D-aspartate receptors. These compounds have been demonstrated as neuroprotectives in several models of CNS-indications (epilepsy, neuropathic pain, ischemie,etc.). However, considering the pleiotropic nature of our in vivo results, it becomes obvious that the sticky issue is their mechanism of action.

Pavel Šácha
10:25 - 10:35 AM

IOCB COMPOUND LIBRARY

The IOCB Compound Library preserves original compounds synthesized at IOCB Prague. The library is in 384-well plate format ready for testing with the emphasis on a low expenditure of substances. The library is screened internally but also by external collaborators.

Martin Sládek
10:35 - 10:45 AM

SCREENING FOR NOVEL SMALL-MOLECULE MODULATORS OF CIRCADIAN CLOCK

Circadian clock regulates most aspects of physiology and metabolism. In humans, dangers of endogenous misalignment with external time due to jet-lag or shift-work make pharmacological manipulation of circadian clock a tempting prospect. We offer high-throughput cell-based screening + detailed ex vivo and in vivo assays of novel and repurposed compounds for clock-modulating effects, as well as chronopharmacological testing of drug efficacy and toxicity.

Katarína Smolková
10:45 - 10:55 AM

REGULATION OF ISOCITRATE DEHYDROGENACE-2 FUNCTION BY SIRTUIN-3 DEACETYLASE: IDENTIFICATION OF ACETYLATED SITES

Mitochondrial Isocitrate dehydrogenase 2 (IDH2)has been implicated in production of oncometabolite 2-hydroxyglutarate (2HG). We have studied possible regulatory role of

mitochondrial deacetylase SIRT3 in regulation of IDH2 function. In collaboration with IOCB we identified lysine residues of IDH2 which are plausible substrates of SIRT3 deacetylation and might be involved in 2HG production. Our lab has also recently demonstrated role of redox signaling in insulin secretion using several genetic mouse models. As future perspective we intend to study physiological posttranslational modifications of mitochondrial proteins, including ATP-synthase, thioredoxins, and phospholipase A2ɣ, which could be relevant in regulation of insulin secretion by pancreatic beta-cells.

Olga Zimmermannová
10:55 - 11:05 AM

COMPREHENSIVE APPROACH TO STUDY STRUCTURE AND FUNCTION OF MEMBRANE TRANSPORTERS

Transporters of Na+, K+ and H+ are crucial for all living cells as they ensure optimal intracellular concentrations of alkali-metal cations and protons. We use the yeast Saccharomyces cerevisiae as a model organism to study structure and mechanisms of regulation of monovalent cation transporters. Set of experiments in vivo as well as in vitro revealed that transport activity of the plasma-membrane Na+, K+/H+ antiporter Nha1 is negatively regulated by binding of 14-3-3 proteins. 14-3-3 binding induced a disorder-to-order transition of the C-terminus of Nha1 in vitro. Polarization microscopy of living cells expressing Nha1 tagged with GFP confirmed that the Nha1 C-terminus lacking the 14-3-3-binding site exhibits less ordered structure. Presented comprehensive approach combining various in vivo and in vitro methods can be useful for the characterization of regulation of any plasma-membrane protein.

Martin Rossmeisl
11:35 - 11:45 AM

PHENOTYPING OF ENERGY METABOLISM AND INSULIN SENSITISITY IN MICE

Obesity is associated with various metabolic disorders commonly referred to as metabolic syndrome. The underlying feature of metabolic changes in obesity is the loss of insulin sensitivity in peripheral tissues accompanied by impaired metabolic flexibility, i.e. the ability to respond or adapt to conditional changes in metabolic demand. In mice, we use the technique of indirect calorimetry and hyperinsulinemic-euglycemic clamps to assess in vivo changes in energy metabolism and insulin sensitivity caused by experimental diets, pharmaceuticals, or changes in environmental conditions.

Jiří Jiráček
11:45 - 11:55 AM

ANALOGUES OF INSULIN, IGF-1 AND IGF-2 AND THEIR APPLICATIONS

Insulin/IGF system is a complex biological network that consists of insulin, insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), their membrane receptors (IR-A, IR-B, IGF-1R and IGF-2R) and IGF-binding proteins. Insulin, IGF-1 and IGF-2 cross-bind to the receptors with different binding affinities and elicit different biological effects that are mainly metabolic for insulin and mainly growth-inducing for both IGFs. Imbalanced activation of IGF-1R and IR-A can result in development of cancer. We are interested in deciphering which structural determinants in the

hormones are behind their different biological effects and in the development of more receptor-specific insulin and IGF analogues that could be useful for safer treatment of diabetes, growth-related disorders or cancer.

František Kolář
11:55 - 12:05 PM

IN VIVO AND EX VIVO EVALUATION OF HEART FUNCTION

We are able to measure heart function in rats and mice non-invasively by transthoracic echocardiography or by catheterization (pressure-volume analysis). Tests of ischemic tolerance and drug cardiotoxicity are available also on isolated perfused hearts or isolated cardiomyocytes. We are interested in collaboration focused on new therapeutic approaches to myocardial infarction and chronic heart failure.

Ivana Vaněčková
12:05 - 12:15 PM

THE ANALYSIS OF CARDIOVASCULAR PARAMETERS (BLOOD PRESSURE, HEART RATE AND ANALYSIS OF BLOOD PRESSURE VARIABILITY) USING TELEMETRY MONITORING IN UNIQUE MODELS OF HYPERTENSION AND CHRONIC KIDNEY DISEASE

Several interesting models of hypertension (Dahl salt-sensitive, NO-deficient L-NAME hypertension, Ren-2 transgenic rats) and chronic kidney disease (partial nephrectomy, 2-kidney-1-clip) are studied in the Department of Experimental hypertension of IPHYS. Telemetry blood pressure monitoring is the most accurate method how to measure and analyze BP parameters in conscious freely moving animals (systolic, diastolic, pulse, and mean BP and heart rate) together with the analysis of its sympathetic and vagal component (low frequency vs. high frequency) which reflect cardiovascular status of the animals.

Milan Vrábel
12:15 - 12:25 PM

BIOORTHOGONAL REACTIONS FOR BIOIMAGING AND BEYOND

Bioorthogonal reactions are a set of chemical reactions that can proceed selectively under stringent biological conditions. We have developed series of such reactions that can be efficiently applied for visualization of biomolecules and small organic molecules inside living cells. In addition, we are now able to perform these reactions at the subcellular level. By modulating the reagents, we can release small molecules inside the organelle, which should enable modulation and control of biological processes selectively in e.g. mitochondria

Michal Hocek
12:25 - 12:35 PM

MEDICINAL CHEMISTRY OF BASE-MODIFIED NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACID

Short summary of most important results in design, synthesis and biological profiling of substituted or fused deazapurine nucleosides and recent results in synthesis of base-modified nucleotides and their use in enzymatic synthesis of modified nucleic acids for applications in selection of aptamers.

Radim Nencka
12:35 - 12:45 PM

DESIGN OF NOVEL ENZYME INHIBITORS – PI4KB AND nSMase2 CASE STUDIES

The lecture will be focused on the comparison of structure-based and ligand-based approaches towards modern drug design. These approaches will be demonstrated on two distinct examples from our lab. The structure-based discovery of novel inhibitors of phosphatidylinositol 4-kinase β (PI4KB) will be discussed. Numerous positive-sense single-stranded RNA (+RNA) viruses highjack this enzyme, one of four different isoforms of PI4K present in human cells, in order to alter membranes and set up bases for functional replication machinery. Therefore, PI4KB is one of the potential interesting targets for antiviral drug discovery. In contrast, novel human neutral sphingomyelinase 2 (nSMase 2) inhibitors, which might be useful for the treatment of neurodegenerative disorders and brain injuries, were strictly developed via ligand-based drug discovery.

Helena Mertlíková-Kaiserová
12:45 - 12:55 PM

BIOLOGICAL TOOLS TO PROMOTE EARLY DRUG DISCOVERY AT IOCB

The department of Biochemical Pharmacology provides a unique platform facilitating the earliest phases of drug discovery projects at IOCB. Traditionally, we maintain a close cooperation with medicinal chemistry groups. We focus on small molecules screening towards pharmacologically relevant targets, assay development (both cell-free and cell-based), target identification and basic ADME/Tox characterization of the compounds. Our flow cytometry core provides user support in multicolor analysis and cell sorting. We also run a small animal facility intended for short-term experiments in mice or rats such as pharmacokinetic experiments or primary cell isolation. The projects we actively pursue in our group are all collaborative with IOCB partners and largely head towards oncology, immunology and virology.

Ondřej Kuda, Tomáš Čajka
12:55 - 13:05 PM

LIPIDOMICS AND METABOLOMICS - PROFILING AND PATHWAY ANALYSIS

We are able to perform lipidomics and metabolomics profiling on various types of samples including plasma and tissues. Current LIMeX 6D LC-MS/MS workflow covers hundreds of polar and non-polar metabolites. We are interested in collaborations, where we could apply metabolic flux analysis using substrates labeled with stable isotopes 2H, 13C or 15N.