

## Recent Development of Biomedical Research at the Institute of Physiology of the Czech Academy of Sciences in Prague

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Since its establishment in 1954, the Institute of Physiology of the Czech Academy of Sciences (till 1992, Czechoslovak Academy of Sciences) focused on normal physiology and pathophysiology, namely on neurophysiology, specific aspects of metabolism and cardiovascular research. This complex yet well-defined approach provided a synergism in studies of specific problems, while most of the research projects could benefit from intramural collaborations of scientists specialized in different fields. In this respect, the Institute of Physiology represents a unique center of biomedical research in the Czech Republic. Two major goals are a) to uncover and characterize basic biological mechanisms and b) to help public health by improving prevention, diagnostics and treatment of serious disease states. These mainly include cardiovascular diseases, obesity and diabetes mellitus, inherited metabolic disorders, neurological and psychiatric disorders, neurodegenerative diseases such as Alzheimer's, and several others.

Scientists at the Institute of Physiology contributed significantly to the above described areas of research, as summarized in the special issue of this journal published in 2014, at the occasion of the 60-year's anniversary of the Institute of Physiology (*Physiological Research* 63: Suppl. 1, 2014) [http://www.biomed.cas.cz/physiolres/2014/S1\\_14.htm](http://www.biomed.cas.cz/physiolres/2014/S1_14.htm)

The aim of this report is to provide a condensed outline of the major achievements of research conducted at the Institute during the last five years.

### Neurophysiology

A significant progress has been made by several research groups in this rapidly expanding field. Recent

evidence suggests that abnormal regulation of membrane receptors plays a fundamental role in the development of many neurological and psychiatric disorders, including Parkinson's, Alzheimer's, and Huntington's diseases, epilepsy, anxiety, depression, bipolar disorder, schizophrenia, lupus erythematosus and ischemia. Proper understanding of the fundamental mechanisms regulating the membrane receptors in the mammalian nervous system is thus essential if novel approaches for treating these disorders are to be successfully developed. The progress in this field may have implications for the development of new compounds for treating cognitive disorders and pain, and improving learning and memory.

Research into the mechanisms underlying the effects of so-called allosteric modulators affecting the activity of a receptor at a site different from the receptor's active site represents a topic of shared interest in the field of ion channel and G-protein coupled receptor research, in both peripheral and central nervous systems. Specifically, studies on the N-methyl-D-aspartic acid (NMDA) subgroup of ionotropic glutamate receptors have focused on the identification of clinically relevant antagonists capable of preferentially blocking the excitotoxic receptor activation, without interfering with its functions essential for a normal synaptic transmission and neural plasticity.

Studies at the *Department of Cellular Neurophysiology* (Head: Ladislav Vyklický) have considerably extended the knowledge on function, structure, trafficking, molecular genetics, and pharmacology of ligand-gated ion channels including glutamate, acetylcholine, and pain-related transient receptor potential channels (Boukalova *et al.* 2010, 2014,

Balik *et al.* 2013, Korinek *et al.* 2015, Lichnerova *et al.* 2015, Vyklicky V *et al.* 2015).

The *Department of Neurochemistry* (Head: Vladimír Doležal) has contributed to the understanding of the allosteric pharmacology and signal transduction of muscarinic receptors (Jakubík *et al.* 2011a,b, 2014a,b) and of adverse effects of  $\beta$ -amyloid on muscarinic transmission at cellular level (Janičková *et al.* 2013) and in animal model of Alzheimer's disease (Machová *et al.* 2010).

The research of the *Department of Cellular and Molecular Neuroendocrinology* (Head: Hana Zemková) was focused on the role of purinergic P2X receptors in secretion of pituitary and hypothalamic hormones. High expression level of P2X2, P2X4 and P2X7 receptors was observed in neurons secreting oxytocin and vasopressin that control parturition, lactation and exhibit antidiuretic properties, but also influence social behaviors, stress-related responses and mental state (Vavra *et al.* 2011). Low expression of P2X receptor-channels was found in neurons of suprachiasmatic nuclei that orchestrate circadian rhythm in endocrine functions (Bhattacharya *et al.* 2013). Combined electrophysiological and molecular biology methods were used to identify the molecular mechanisms underlying the action of ivermectin, a positive allosteric regulator of the P2X4 subtype, (Jelinkova *et al.* 2008) and cell-surface expression of the P2X7 receptors, that are crucial for the capability of extracellular ATP to induce apoptosis (Jindrichova *et al.* 2012, 2015). The progress in this field may reveal risk mutations whose presence could significantly affect human health and development.

The *Department of Functional Morphology* (Head: Jiří Paleček) has demonstrated that transient receptor potential vanilloid receptors (TRPV1) located on presynaptic endings of primary afferents in the spinal cord dorsal horn play a significant role in the development of acute and chronic pain states. Their modulatory role is potentiated under pathological conditions when their activity may be affected by a number of cytokines, and their sensitivity to endogenous agonists is increased. Modulation of their function may provide analgesic effects and new pain treatments (Spicarova and Palecek 2009, 2010, Spicarova *et al.* 2011, 2014a,b, Uchytlova *et al.* 2014, Li *et al.* 2015).

The *Department of Biochemistry of Membrane Receptors* (Head: Petr Svoboda) has reported that plasma membranes isolated from forebrain cortex of rats exposed

to morphine for 10 days contained the largely increased amount of adenylyl cyclase ACI and ACII. The increase of these crucial signaling molecules of cAMP-dependent signaling cascades in brain faded away 20 days after the last dose of morphine (Ujcikova *et al.* 2011, 2014). Degradation of membrane domains and plasma (cell) membrane integrity by cholesterol depletion resulted in a decrease in affinity of functional coupling between trimeric G proteins and  $\delta$ -opioid receptor. Agonist binding site of  $\delta$ -opioid receptor was unchanged (Brejchova *et al.* 2011). Our data indicated the high ability of living organism to oppose the drastic, morphine-induced change with the aim to return to the physiological norm after complete withdrawal of the drug.

In cognitive neuroscience, the *Department of Neurophysiology of Memory* (Head: Aleš Stuchlík) reported several internationally-excellent findings from the field of basic and oriented research of learning, memory and behavior. Scientists convincingly demonstrated a crucial role of the hippocampus in a continuous updating of changing information in a novel robot-avoidance task (Telensky *et al.* 2011). It was shown that the hippocampus is critical for recognition of positions of objects projected in an inaccessible space (Levcik *et al.* 2013). Spatial cognition has been studied not only in laboratory rats but also in non-human primates, where the ability to process abstract spatial information was demonstrated (Nekovarova *et al.* 2007). Amnesic mild cognitive impairment patients, with a high probability to develop Alzheimer's disease, were shown to have impaired selectively the allocentric spatial memory (Hort *et al.* 2007). In multi-laboratory collaboration with other research groups, a novel neuroprotective steroids have been successfully patented (Rambousek *et al.* 2011). A representative series of more than 10 papers on the cognitive deficits in animal models of brain disorders and in clinical conditions was published in 2014 in *Frontiers in Behavioral Neuroscience*, a leading journal in behavioral sciences. These papers have included original works, hypotheses and reviews.

In 2014 the *Department of Molecular Neurobiology* (Head: M Balašík) was established with the focus on molecular mechanisms in neural development (particularly axon growth and guidance) and neurodegeneration (Alzheimer's disease). Recent work of the department demonstrated that cooperative action of protein phosphorylation and isomerization plays an

essential role in axon guidance. In multiple in vitro and in vivo systems (primary neuron cultures, mice and zebrafish) it was shown that prolyl-isomerase Pin1 and CDK5 kinase control axon growth by changing conformation of CRMP2 (collapsing response mediator protein 2) and consequently axon guidance in Semaphorin3A gradients (Balastik *et al.* 2015).

The research at the *Department of Computational Neuroscience* (Head: Lubomír Košťál; Petr Lánský until the end of 2013) has been focused on advanced statistical methods for electrophysiological data analysis and on the fundamental problems of neuronal information processing. Several novel methods for neuronal spiking data classification and characterization were proposed (Kostal *et al.* 2007, 2013). It was shown that noncompetitive interactions play a major role in the neural coding of natural odorants (Rospars *et al.* 2008), hence pointing to the key aspect of the artificial nose design. Furthermore, optimal stimulus-decoding strategies display catastrophic loss of performance below a certain size of the encoding neural population (Kostal *et al.* 2015), thus determining the minimal sensory array size in artificial neuronal implants and systems.

Using a complex approach, the *Department of Developmental Epileptology* (Head: Hana Kubová) has demonstrated that an intense epileptic activity leads to both acute and long-lasting morphological and functional alterations, often of progressive nature, in rats younger than two weeks (Kubová and Mareš 2013). Mechanisms responsible for the damage in immature brain include oxidative stress and mitochondrial dysfunction (Folbergrová *et al.* 2012). Postictal refractoriness is absent in the infantile brain and appears in the third postnatal week (Mares and Kubova, 2015a). In juvenile animals, GABA<sub>B</sub> receptor blockade partially abolishes postictal refractoriness in the sensorimotor cortex (Mareš and Kubová 2015a,b). Changes in neuronal dynamics preceding the onset of seizures and highly specific type of epileptic activity, which can be found only in the areas of brain involved in seizure genesis, were identified. These results bring new insight into the mechanisms involved in seizure initiation and open prospects for improved presurgical diagnosis and outcomes of epilepsy surgery (Cho *et al.* 2014).

Recent progress in chronobiology is having far-reaching consequences in other fields of physiology. The scope of the field has broadened due to the discovery of the temporal regulation of cellular processes and its vital importance for cell survival. Using the molecular biology

tools, the *Department of Neurohumoral Regulations* (Head: Alena Sumová) discovered how the circadian system and its individual components develop during ontogenesis (Sumová *et al.* 2012, Houdek and Sumová 2014). Studies in humans have revealed that neuropsychiatric disorders are associated with malfunctioning of the circadian system at the level of molecular clock regulation (Nováková *et al.* 2012, Nováková *et al.* 2015). Studies on the circadian system in animals spontaneously developing cardiovascular and metabolic disease (SHR) have demonstrated significant aberrancies that might be related with development of the pathology (Sládek *et al.* 2012, Polidarová *et al.* 2013). In collaboration with the *Department of Epithelial Physiology*, the studies on the circadian regulation in the gastrointestinal system revealed a specific function of the circadian clock in the colonic epithelial cells, and the mechanisms entraining these clocks with the external environment (Sládek *et al.* 2007, Polidarová *et al.* 2011). Importantly, malfunction of the colonic clock has been associated with colorectal cancer development (Soták *et al.* 2013, 2014). While studying the physiology and pathophysiology of the gastrointestinal tract, the *Department of Epithelial Physiology* (Head: Jiří Pácha) focused on the local metabolism of glucocorticoids and on the analysis of diurnal profiles of clock genes, clock-controlled genes regulating cell cycle and intestinal transporters during inflammatory bowel disease (IBD) and colitis-associated cancer. Based on studies of bioptic samples of patients with IBD and experimental colitis or arthritis, a novel model of regulation of glucocorticoid metabolism during inflammation was proposed. The model is based on positive regulatory role of pro-inflammatory cytokines in increased supply of local glucocorticoid hormones, which contribute to the feedback regulation of inflammation (Ergang *et al.* 2010, 2011). It has been also shown that social stress has similar effect on glucocorticoid metabolism as inflammatory stress (Vodička *et al.* 2014). In collaboration with other groups the metabolism of glucocorticoids (Moravec *et al.* 2014) and circadian rhythmicity during neoplastic transformation was described in colon cancer (Soták *et al.* 2013) together with the circadian changes of colonic transport (Soták *et al.* 2011). The above findings may contribute to new chronotherapeutic approaches to various diseases.

## Metabolism

Studies are focused on basic mechanisms of

transport across biological membranes and of energy conversion, as well as on the impact of disorders of these processes on health. The metabolic basis of obesity-associated diseases, and the use of nutritional manipulations in prevention and treatment of these morbidities, represent another key topic. The obesity-associated diseases are causally linked with low-grade inflammation appears. This concerns not only peripheral tissues but also the brain, hence inflammation represents an emerging field of interest at the Institute. Studies in the metabolic field interlink research conducted by most of the groups at the Institute of Physiology, reflecting the common use of modern biochemical, cell biology and analytical approaches.

While studying the role of membrane transporters in cell cation and pH homeostases, a number of novel results have been obtained in the *Department of Membrane Transport* (Head: Hana Sychrová). A combination of molecular biology (cloning of genes and their heterologous expression), biophysics (measurements of relative membrane potential or intracellular pH and their changes) and chemistry (estimation of cation content and fluxes) made it possible to identify, clone and characterize 14 new transporters involved in the regulation of cation homeostasis, response to osmotic stress and sugar uptake in yeast cells (Duskova *et al.* 2015, Kinclova-Zimmermannova *et al.* 2015, Leandro *et al.* 2014, Leandro *et al.* 2013, Zimmermannová *et al.* 2015) and to describe the structural properties of cation/proton antiporters (Kinclova-Zimmermannova *et al.* 2015).

Gaining molecular and structural insights into the mechanisms of physiologically relevant processes is the long term research goal of the *Department of Protein Structure* (Head: Veronika Obšilová). The research of this department is focused on studying molecular mechanisms by which protein function can be regulated. In particular, main attention is given to 14-3-3 proteins and their complexes with proteins involved in apoptosis, cancer, G-protein and calcium-triggered signaling pathways. By employing both biophysical (fluorescence spectroscopy, small angle X-ray scattering, analytical ultracentrifugation, protein structure modeling) and biochemical (site-directed mutagenesis, enzyme activity measurements) approaches the important details about the 14-3-3 protein-dependent regulation of several important 14-3-3 binding partners were obtained. Studied binding partners included tyrosine hydroxylase (Obšilova *et al.* 2008), forkhead transcription factor FOXO4 (Silhan *et al.*

2009), the regulator of G-protein signaling 3 (RGS3) (Rezabkova *et al.* 2011) and the yeast neutral trehalase Nth1 (Veisova *et al.* 2012, Kopeccka *et al.* 2014).

In the field of energy metabolism, mechanistic studies on specific aspects of mitochondrial energy conversion, mitochondrial reactive oxygen species (ROS) formation and concomitant redox regulations and other physiological roles and pathophysiological aspects of mitochondria in the cell have been extended to a research more strongly focused on either integrative cell biology or direct links to medicine, namely to mitochondrial diseases as well as obesity and diseases associated with it.

The recent studies of mitochondrial uncoupling protein synergy with phospholipase iPLA<sub>2γ</sub> (PNPLA8) in the *Department of Membrane Transport Biophysics* (Head: Petr Ježek) revealed this cytoprotection preventing oxidative stress in tissues such as lung, and lipotoxicity in pancreatic β-cells, where iPLA<sub>2γ</sub> participates in fatty acid-induced insulin secretion (Jabůrek *et al.* 2013, Ježek J *et al.* 2015). The role of mitochondria and redox regulations in glucose-stimulated insulin secretion (Dlasková *et al.* 2010) upon cell hypoxic adaptation and in tumorigenesis is also subject to investigation (Smolková *et al.* 2015) likewise physiology and pathophysiology of nucleoids of mitochondrial DNA (Tauber *et al.* 2013).

Mitochondrial diseases result from insufficient mitochondrial energy provision due to altered function of oxidative phosphorylation system and belong to the most severe inborn metabolic disorders. Identification of disease causing genes and associated molecular pathogenic mechanisms has therefore direct clinical relevance, but can also significantly improve our understanding of mammalian mitochondrial biology. The main focus of the *Department of Bioenergetics* (Head: Tomáš Mráček; Josef Houšťek until the end of 2013) is on characterization of biogenesis of multi-subunit enzyme complexes of the mitochondrial oxidative phosphorylation system, namely ATP synthase and cytochrome c oxidase (COX). It has significantly contributed to the description of SURF1 protein function in COX biogenesis (Kovarova *et al.* 2012) and made major impact by the identification of two new genes responsible for nuclear genetic defects of the ATP synthase – assembly factor TMEM70 (Cizkova *et al.* 2008, Hejzlarová *et al.* 2011, 2014) and structural subunit ε (Mayr *et al.* 2010). Furthermore, establishment of non-invasive diagnostic protocol using peripheral blood cells became important for frontline screening for suspected

mitochondrial patients (Pecina *et al.* 2014).

Obesity-associated diseases like diabetes mellitus and cardiovascular diseases represent a major problem for health care system in affluent societies. With the aim to improve prevention and treatment of obesity-associated diseases, the *Department of Adipose Tissue Biology* (Head: Jan Kopecký) has focused on the mechanisms behind beneficial effects omega-3 fatty acids (omega-3) (Flachs *et al.* 2014). For example, omega-3 supplementation could result in reduction of hepatic lipid accumulation in dietary-obese mice. This effect was stronger when omega-3 were supplied as phospholipids rather than triacylglycerols (Rossmesl *et al.* 2014) and depended on AMP-activated protein kinase (Jelenik *et al.* 2010). Omega-3 and calorie restriction could induce healthy phenotype of white adipocytes, reflecting stimulation of triglyceride/fatty acid cycling and mitochondrial oxidative phosphorylation in adipocytes (Flachs *et al.* 2013); adipocyte-macrophage cross-talk could be involved (Massoodi *et al.* 2015). It was also shown that omega-3 could augment lipid-lowering and other effects of antidiabetic pharmaceuticals both in mice (Horakova *et al.* 2012) and in human patients with diabetes mellitus.

### Cardiovascular research

The last years have witnessed a considerable progress in studies focused on multifactorial polygenic diseases such as hypertension, ischemic heart disease, atherosclerosis, arrhythmias, heart failure or vascular disease. New approaches were also developed for the replacement of damaged blood vessels. Thus, results of these studies are relevant for prevention and treatment of a cluster of diseases representing a cause of death of about a half of population in the developed countries.

A great progress in the genetic analyses of complex pathophysiological traits in the BXH/HXB recombinant inbred (RI) strains developed from spontaneously hypertensive rat (SHR) and Brown Norway (BN) progenitors in the *Department of Genetics of Model Diseases* (Head: Michal Pravenec), has been achieved, in collaboration with several international rat genomic centers. The recombinant inbred (BXH/HXB RI) strains represent a model system for genetic and correlation analyses of human metabolic syndrome. Recent technical advances, including the next-generation sequencing technology, transcriptome analysis, genomic variation analysis, methylation-sequencing and ChIP-seq, quantitative proteomics, metabolomics, advanced

bioinformatics as well as new tools for manipulating the rat genome, made it possible to identify the molecular basis of the first genetic determinants predisposing the SHR to disturbances of lipid and glucose metabolism (Pravenec *et al.* 2001), hypertension (Pravenec *et al.* 2008), left ventricular hypertrophy (McDermott-Roe *et al.* 2011) or cardiac fibrosis (Liška *et al.* 2014). An increasing attention has been paid not only to the expression of genetic abnormalities but also to pathophysiological alterations responsible for abnormal vascular tone and high blood pressure (BP).

The *Department of Experimental Hypertension* (Head: Josef Zicha) investigated the mechanisms of hypertension development during the ontogeny as well as clinically relevant topics such as the treatment of chronic kidney disease. The contribution of altered calcium influx and/or calcium sensitization to BP maintenance was performed in several experimental models including SHR, Dahl salt hypertensive rats and Ren-2 transgenic rats (Pintérová *et al.* 2010, Behuliak *et al.* 2013, Zicha *et al.* 2014a,b). The latter strain was used in studies exploring end-organ damage following kidney disease and its alleviation after the combination treatment with renin-angiotensin system blocker and endothelin receptor A blocker (Vaněčková *et al.* 2012, Čertíková Chábová *et al.* 2014).

Considering high blood pressure as a major risk factor of the ischemic heart disease, the alterations of myocardial tolerance to ischemia-reperfusion injury in distinct forms of systemic hypertension (Neckář *et al.* 2012) have been studied in the *Department of Developmental Cardiology* (Head: František Kolář). In addition, a significant progress has been made in delineating the molecular mechanisms underlying high ischemic tolerance of immature and chronically hypoxic hearts, with a particular attention paid to the role of various mitochondrial proteins (Milerová *et al.* 2010, Borchert *et al.* 2011), pro-inflammatory cytokines (Chytilová *et al.* 2015) and redox-dependent protective signaling pathways (Neckář *et al.* 2013).

The *Department of Functional Morphology* (Head: Jiří Paleček) studied whether a chronic administration of red palm oil (RPO, rich in antioxidants) and omega-3 can ameliorate pathophysiological changes including cardiac arrhythmias and lipid level alterations induced by thyroid hormones. Using hyperthyroid and hypothyroid rats as “models of a diseased organism”, it has been established that RPO and omega-3 can partly ameliorate changes in cardiac tissue remodeling, cell-to-

cell communication, expression of Cx43 and protein kinases or in myosin heavy chain composition induced by thyroid hormone level alterations (Radosinska *et al.* 2013, Rauchová *et al.* 2013, Soukup 2014).

Developmental arrhythmology is a dynamically expanding field, in part owing to the advent of new tools such as high-speed cameras for optical mapping, as well as new markers of the developing cardiac conduction system. The analysis of several mutant mouse strains in the *Department of Cardiovascular Morphogenesis* (Head: David Sedmera) shed new light on the function of the cardiac conduction system during embryonic development. Connexin40, a dominant connexin expressed in the His bundle, bundle branches and Purkinje fibers, has been found to be crucial for the right bundle branch conductivity only during later embryonic and postnatal stages (Sankova *et al.* 2012). An analysis of mice with myocardial-specific deletion of *Pitx2C* has demonstrated that their hearts have dual sinoatrial nodes, both acting as functional cardiac pacemakers (Ammirabile *et al.* 2012). Parallel studies in the chick embryonic model focused on safety of commonly used cardiac anti-arrhythmic drugs to the developing fetus (Kockova *et al.* 2013) revealed that beta-blockers possess a considerable margin of safety at clinically relevant dosage potentially used to treat pregnant mother. On the contrary, ivabradine used to reduce heart rate caused profound bradycardia, decreased cardiac output and embryonic lethality.

The *Department of Biomaterials and Tissue Engineering* (Head: Lucie Bačáková) concentrates on the reconstruction of irreversibly damaged blood vessels. This is achieved by either innovating synthetic polymeric vascular prostheses currently used in clinical practice or by constructing new bioartificial vascular replacements using tissue engineering methods. The existing vascular prostheses are innovated mainly by coating their inner surface with specific protein layers, particularly fibrin-based ones, which is then followed by *in vitro* endothelialization (Chlupáč *et al.* 2014). The construction of novel bioartificial blood vessels utilizes synthetic or natural polymers functionalized with nanoparticles or various ligands for cell adhesion receptors as well as nanofibrous or nanoporous scaffolds as carriers for the attachment, growth and phenotypic maturation of vascular endothelial and smooth muscle cells (Novotná *et al.* 2013, Musílková *et al.* 2015). A special attention has also been paid to the development of periadventitial drug delivery system into blood vessels (Filová *et al.* 2011).

## New technologies and methodological approaches

Techniques of whole-body phenotyping in rodents have been systematically introduced providing now a comprehensive panel of approaches encompassing characterization of behavior, telemetry of physical activity and blood pressure, energy metabolism and fuel partitioning, glucose homeostasis, and whole body composition. This is complemented by non-invasive techniques to characterize organ functions and metabolism *in vivo*, and by various methodologies at cellular, organelle and biochemical level, including targeted metabolomics. Several Departments of the Institute contribute to the above methodological repertoire. Most of the research studies depended on our animal facilities, providing a possibility to conduct animal experiments under standard conditions while observing all the rules for proper handling and treatment of laboratory animals. Various rat and mouse models are available for studying both the basic mechanisms in the context of normal physiological conditions and under disease state. In spite of the general trend to use of mice rather than rats for the research, unique rat models (obtained also with the help of advanced techniques for transgenesis), which allow for fast dissection of the role of genetic background, are becoming increasingly important (see above, the *Department of Genetics of Model Diseases*).

Recent imaging and tissue preparation techniques providing high-resolution 3D image data were introduced by the *Department of Biomathematics* (Head: Jiří Janáček). The mission of this department is to develop new microscopic visualization, data analysis and modeling methods, and to apply them to various organs such as nerves (Cvetko *et al.* 2015) and placenta (Jirkovská *et al.* 2012). New procedure for evaluation of diffusion coefficient of fluorescent molecules on plasma membrane revealed debilitating effect of low cholesterol on metabotropic receptors (Brejchová *et al.* 2015) suggesting that cholesterol lowering drugs shall be prescribed with caution. Comparative study on length measurement of tubular structures in 3D (Kubínová *et al.* 2013) present original state of the art methods.

Analytical separation methods and their application to physiologically important compounds (e.g., steroids or pigments) were developed at the *Department of Analysis of Biologically Important Compounds* (Head: Ivan Mikšík). Besides these methods this department serves as Proteomic facility of our Institute. In the area of biomedical research this department studies factors of

teeth decay when proteomes of dentin (289 proteins) and pulp (342 proteins) were described (Eckhardt *et al.* 2014, Jágr *et al.* 2014). Another approach is connected with study of aging (as well as diseases like diabetes mellitus) of proteins, when some new modifications of proteins were described (Pataridis *et al.* 2013).

### Future directions

As up to now, research to be conducted at the Institute of Physiology should focus on both characterization of basic biological mechanisms and studies aimed to help in prevention, diagnostics and treatment of selected human diseases. It would be too ambitious to judge, which lines of research may bring the most exciting and important results in the years to come. However, several fields of research will certainly continue, reflecting the common interests at the Institute as well as the need to counteract the still increasing negative impacts of sedentary lifestyle, stress and aging on public health. Sedentary lifestyle and the whole modern societal environment are the key triggers of obesity and associated diseases. Diabetes mellitus is causally linked to obesity, with about 10 % of the Czech population suffering from this disease and imposing an increased risk for the cardiovascular diseases, which, in turn, results in about a half of death in this country. Similarly, social and environmental factors contribute to development of psychiatric and neurological disorders. Thus e.g. the incidence of Alzheimer's disease, with 45 % of people older than 85 years suffering from this disease, will increase even further with increasing mean age of population.

In the field of neurophysiology, the studies of selected receptors engaged in neural transmission will continue with the goal to understand the function of nervous system and brain, with important projections to aging-associated disorders of central nervous system functions; role of these receptors in sensation of pain represents another key aspect of this research. The mechanistic studies will include testing of new classes of small molecules capable to modulate receptor functions; on the other hand, new approaches in cognitive neuroscience will represent a complementary bridge to clinical setting. On a special track, the epileptology, reflecting the need to help to about 80 000 patients with epilepsy in the Czech Republic alone, will continue to represent an important research direction with prospective clinical impact. Chronobiology research will link the studies of central regulatory mechanisms with

those at the periphery to further characterize the complex interplay between the circadian clock and metabolism, with the projections to both severe neuropsychiatric disorders and life-style evoked changes in metabolism.

It is advantageous that the long-time research experience in the field of energy metabolism and adipose tissues biology is matched with an equal expertise in experimental cardiology and in the studies on the mechanisms engaged in blood pressure control. This provides a unique possibility for further characterization of the basic mechanisms, which are essential for human health. Further understanding of the role of these mechanisms if disturbed under obesity, diabetes or hypoxia settings will benefit from the complex approach, which is increasingly available at the Institute. Moreover, the studies on the genetic basis of the disorders in the field of obesity-associated diseases will benefit from recombinant inbred mapping in rats, which is well established at the Institute. Mitochondrial research represents yet another angle, with important implications for improvement in diagnostics of severe metabolic diseases; discoveries of new inherited mitochondrial disorders in human patients will provide novel models for deciphering the molecular basis of structure and function of the key organelles of energy provision. Characterization of the various transporters across cell membranes will benefit from application of still advancing biophysical techniques while heterologous expression of the human transporters in yeast will enable to find novel low-molecular weight compounds capable to modulate activity of these proteins with potential impact of treatment of several diseases.

The overall strategy of research at the Institute of Physiology should focus on themes encompassing more research areas such as e.g. low-grade inflammation, which is involved in disorders of brain functions, pain sensation and metabolism in peripheral tissues in obesity. From the methodological point of view, the existing comprehensive platform for whole-body phenotyping in rodents, as well as targeted metabolomics, should be developed even further, to enhance the possibilities for complex characterization of the effects of various treatments and the potential to implement the results from animals to human medicine. Advanced bioinformatic approach to the detailed analysis of data obtained will be required for their successful translation into modern medicine. Further deepening of collaborations with both academic and clinical research centers will be required.

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