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Plenary lectures

Free Radicals: Signaling and Damaging Functions in Aging. Is Harman's free radical theory of aging correct?

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ROS and hereditary disorders (defective genes) are rightly considered to be major causes of aging. However, up-to-date studies show that both factors can be mutually interconnected. Reactive oxygen species (ROS) superoxide and hydrogen peroxide perform important signaling functions in many physiological and pathophysiological processes, cell senescence and organismal age are being no exemptions. Aging-regulating genes *p66shc*, *Sirtuin*, *FOXO3a*, and *Klotho* are new important factors which are stimulated by ROS signaling. It has been shown that ROS participate in initiation and prolongation of gene-dependent aging development. ROS also participate in the activation of protein kinases Akt/PKB and extracellular signal-regulated kinase ERK, which by themselves or though gene activation stimulates or retard cell senescence. Different retarding/stimulating effects of ROS might depend on the nature of signaling species – superoxide or hydrogen peroxide. Importance of radical anion superoxide as a signaling molecule with "super-nucleophilic" properties points out at the possibility of the use of superoxide scavengers (SOD mimetics, ubiquinones, and flavonoids) for retarding the aging development.

Proteins as double agents for reactive oxygen species

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Proteins are generally seen as protecting agents under conditions of reactive oxygen species (ROS)-induced damage to other biologically important molecules (especially lipids and nucleic acids). Indeed, protective effects of proteins can be observed in many situations. Proteins have a major contribution to the total antioxidant capacity of blood plasma. However, they may be also seen as the principal target for the reactions of ROS as they are the main component of tissues in terms of concentration. Therefore, ROS react mainly with proteins and products of these reactions may act as intermediates in the transfer of ROS-induced damage to other targets. In the presence of oxygen, protein hydroperoxides are formed efficiently upon exposure to ROS. They are relatively stable species but under cellular conditions are subject to reduction which consumes antioxidants and reducing power of the cells. Examples can be found for such a damage-transmitting role of proteins. Another important role of proteins in reactions with ROS is that of redox signaling, based mainly on selective and reversible oxidation of protein thiol groups by ROS.

Factors affecting the size of cells

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Even a rough comparison of sizes of living cells points to the existence of considerable differences between them. However, some distinct regularities can be seen. Firstly, prokaryotic cells are usually smaller than eukaryotic ones although giant cells occur also in prokaryotes. In the 50's, an idea predominated that the small size of procaryotes is a simple consequence of the fact that the cell surface grows proportionally to the second power of the cell radius while their volume is proportional to the third power of the radius. Therefore, the surface to volume ratio decreases with increasing cell size which leads to problems with transport through membranes, performing also respiratory functions. The transfer of the respiratory chain to the inside of the cells and expansion of the membrane systems in eukaryotes dismissed this parameter as the key factor limiting the maximal size of the cells. The diffusion rate of macromolecules has been postulated as the factor limiting the maximal cell dimensions in eukaryotes. It suggested that cell size is limited by physical factors. Numerous exemptions from this rule enforced a revision of this view. Presently, it is thought that the fundamental factor determining the size of cells is their translation potential which, in turn, is significantly correlated with the amount of genetic information and ploidy. Arguments will be presented demonstrating that this view is only a next approximation since it does not take into account developmental strategies of various systematic groups, cell functions and the fact of competition of various species of unicellular organisms for food, which leads to some compromise between cell growth and the rate of cell repro-

duction. Changeable conditions in the natural environment impose additional limitations for the excessive volume growth. The lecture is based, to a significant extent, on results of studies of the author's team.

Mechanisms of sulfur radical cations stabilization relevant to oxidation of peptides and proteins containing methionine

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There is unambiguous theoretical and experimental evidence that sulfur radical cations ($R_2S^{+\bullet}$) can be stabilized through intramolecular complexation with nucleophiles that are present in neighboring groups. Reactions of this type are of special interest to biology when stabilization of $R_2S^{+\bullet}$ derived from methionine, Met(>$S^{+\bullet}$) occurs in peptides and proteins. The methionine (Met) residues in these biopolymers are susceptible to attack by Reactive Oxygen Species during oxidative stress and biological aging. Moreover, the pathogenesis of some neurodegenerative diseases (Alzheimer's or Jacob-Creutzfeld's) seems to be strongly linked to the presence in brain tissue either of β-amyloid peptide (β-AP) or human prion protein (hPrP), respectively. These macromolecules containing Met with β-AP having a Met^{35} residue in its C-terminal α-helical domain and hPrP having three out of nine Met-residues ($Met^{205,206,213}$) located within its α-helical segments. The progress of these reactions in real biological systems like proteins is rather difficult to unravel *in vivo* because of the complexity of the chemical environment. By investigating the time-development of radicals and radical-ions following pulse radiolysis coupled to time-resolved UV-Vis spectroscopy and conductivity detection in model compounds: two N-acetylated linear peptides (N-Ac-Gly-Met-Gly and N-Ac-Gly-$(Gly)_2$-Met-$(Gly)_3$) and two cyclic dipeptides (*c*-(L-Met-L-Met) and *c*-(D-Met-L-Met)), we were able to demonstrate *2c-3e* sulfur-oxygen and sulfur-nitrogen bonds formation between Met(>$S^{+\bullet}$) and heteroatoms located in the adjacent peptide bonds.

Age-related changes in photo-protective and antioxidant properties of retinal pigment epithelium melanin

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Due to high oxygen tension, chronic exposure to intense visible light and presence of peroxidizable substrates and potential photosensitizers, RPE and photoreceptor cells of the human eye are at elevated risk of oxidative stress. Melanin in the RPE is believed to play an important photo-protective role; however, the exact mechanism of this protection is not well understood and the nature of age-related changes of RPE melanosomes that may modify their photo-protective and antioxidant properties remains mostly unknown. We analyzed the ability of RPE melanosomes, obtained from human donors of different age, to photo-generate superoxide anion and photo-consume oxygen, and compared the corresponding results to those obtained with experimentally photoaged porcine and bovine RPE melanosomes in different model systems. Our data suggest that aging, as well as experimental photoaging, of RPE melanosomes is accompanied by distinct changes in physicochemical properties of both melanotic and non-melanotic components of the pigment granules, which make them more photoreactive and potentially more phototoxic. Using high frequency (W-band) electron paramagnetic resonance (EPR) spectroscopy, we observed characteristic changes in magnetic parameters of EPR spectra of animal RPE melanosomes that correlate with the extent of their photoaging, suggesting that this experimental approach could be used for detection of early signs of melanosome aging.

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Redox controlled activation of integrin receptors

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Activation of integrins, transmembrane receptors that mediate cell adhesion and cell migration, is accompanied by a series of conformational rearrangements resulting in changes in affinity (i.e. ligand binding) and avidity (i.e. receptor clustering). The divalent cation-dependent binding of physiological ligands to integrins is triggered allosterically by 'inside-out' activation signals that are propagated across the plasma membrane to induce ligand competency of the ectodomain. Ligand-occupied integrins in turn initiate signals that

travel 'outside-in' to modify cell behavior. In addition to inside-out signaling, integrins can be activated directly from the outside by (a) divalent cations, which bind to the ectodomain, (b) after stimulation with activating mAbs and (c) low molecular weight ligands.

Recently a number of observations showed that the induction of the high-affinity state of integrins involves the thiol-dependent step, which is clearly associated with activation of the integrin and precedes its ligation. Our data proved that this process depends upon association of protein disulfide isomerase (PDI) with the integrin, resulting in disulfide exchange occurring in the integrin molecule, and is necessary for the step of conformational change that enables sustained ligand binding. Because PDI alone is not sufficient to isomerize disulfide bonds and needs to be reoxidized, it requires oxidoreductase that controls and/or cooperates with PDI in close vicinity to integrin molecule. We provide evidence that Ero1α is bound to platelet membranes and colocalizes with αIIbβ3 and PDI. Its surface expression increases upon platelet activation, and it binds more efficiently to the activated αIIbβ3 conformer. Thus, Ero1α can support PDI activity toward αIIbβ3 providing oxidative equivalents to PDI, which in turn reduces or rearranges disulfide bonds in the cargo protein.

EPR biodosimetry – fundamentals, applications and perspectives

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Biodosimetry refers to methods of quantitative characterization of various chemical or physical factors by means of analysis of effects of their interactions with biological systems. Most commonly this term is used in relation to dosimetry of ionizing radiation. The issue of radiation biodosimetry has returned recently to interests of many research laboratories worldwide due to increased concern about potential scenarios of radiation exposures due to terrorists attacks resulting in dispersal of radioactive materials (commonly referred to as a "dirty bomb") and in potentially large numbers of casualties. In such situations effective triage based on dose assessments allowing to identify those, who are at risk is crucial for making life-saving medical treatment decisions. Dose estimations in individuals cannot be based on any existing dosimetry system, because, obviously, accidental civil victims are not equipped with personal dosimeters – and if, their readings might be useless due to accompanying mechanical damage or overdosing (as, for example, in technical personnel of Chernobyl power plant). In such situations the accidental dosimetry can be based only on analysis of physical, chemical or biological effects in irradiated individuals. Depending on the type of effects, analysis of which is used for dose estimations, the biodosimetry can be divided to: (1) symptomatic – based on intensity and time-course of clinical symptoms like acute radiation syndrome, hematopoietic symptoms, (2) cytogenetic – based on counting of changes induced in genetic material like dicentrics, translocations, micronuclei, (3) EPR dosimetry – based on detection of free radicals generated by the radiation and stabilized inside biological structures, particularly in those with low water content. The stability of those radicals strongly varies in different tissues and ranges from tens of hours (hair, fingernails) up to millions of years (tooth enamel), what allows for their quantitative analysis and assessment of the absorbed dose. For many years this method has been used for identification of radiation-processed food, where doses within kGy range produce detectable radicals not only in hard tissues but also in flesh of vegetables and fruits. In the range of much lower doses, typical for actions such as radiation protection or medical treatment of sufferers, the most important and helpful is EPR biodosimetry in bones and particularly in enamel, in which the free radical yield is the highest. Minimal detectable dose for enamel is about 20–40 mGy. Under conditions of controlled, *in vitro* exposures the (–13,+19) mGy accuracy of EPR dosimetry was reported [1]. The range of linear response to dose is about 40 Gy [1, 2]. Some of the most spectacular achievements of EPR biodosimetry were determinations of doses in victims of atomic bombs in Hiroshima and Nagasaki, done almost 40 years after exposures, or a large contribution of EPR/teeth dosimetry to documentation of individual doses received by populations of Ukraine and Belarus after Chernobyl disaster. The high sensitivity of EPR dosimetry at present is possible only in *ex vivo* measurements in X-band of microwaves, which is considered as a main shortcoming of this technique. However, a small sample mass (10–20 mg [3]) enables measurements on a fragment of a tooth crown, what allows for dosimetry on healthy teeth and not only on removed teeth. Crucial for performance of the *ex vivo* measurements are: details of the preparative technique, optimization of EPR signal registration procedure and method of separation of the dosimetric EPR signal from background signals and numerical analysis of its intensity.

A key issue for EPR biodosimetry is stability of the radiation induced radicals responsible for the dosimetric signal. Generally, kinetics of their decay is affected by metabolic processes in tissues and environmental factors – mainly humidity and presence of light. Processes of remodeling shorten the lifetime of radicals and accurate dosimetry requires a knowledge of kinetics of their decay. Rate of remodeling processes in bones undergoes significant variations between individuals, being dependent on sex, age, hormonal activity, pathologies – therefore reliable dosimetry on bone samples is possible within few months after exposure. In case of nails or hair, the decay of dosimetric EPR signal is mainly due to humidity and light. These factors limit the time elapse for dosimetry to only hours

after exposure. Lack of remodeling in teeth enamel causes an extremely high stability of radicals generated in its hydroxyapatite component and accurate dosimetry based on EPR in enamel is possible even after many tens of years after exposure. In practice, the dosimetric signal in enamel *in vivo* can be only affected by light, but this does not apply to bicuspid and molar teeth.

EPR biodosimetry allows to measure local dose, in contrary to cytogenic biodosimetry, often based on analysis in lymphocytes in peripheral blood, what gives information about a mean dose level after “dilution” of the local dose over whole body volume. The generalized body’s response to ionizing radiation (severity of acute radiation syndrome, rate of stochastic effects) depends mainly on effective dose absorbed in soft tissues, which, due to differences in elemental composition, differ in their absorption properties from hydroxyaptite. The two effects, i.e. local character of dose measurement and nonequivalence in absorption (the latter particularly at lower radiation energies) have to be taken into account in estimation of health effects of the doses measured by EPR method. Conversion factors relating the “EPR dose” to a specific organ dose or to the effective dose can be obtained from Monte Carlo calculations. Their values strongly vary for different assumed anthropomorphic models and are sensitive to geometry of the radiation field: for photons below 100 keV they can differ several times, above 1 MeV the differences can be tens of percent, when comparing different calculation models. However, this problem is not specific only to EPR dosimetry but applies to all dosimetry methods measuring local dose, as discussed by Regulla [4].

Nowadays EPR biodosimetry is facing a new *in vivo* era. Intensive research is carried on by leading laboratories on *in vivo* – L-band version of EPR dosimetry. The lower frequency allows for extension of dimensions of the active volume of the measuring system. In parallel to reduced sensitivity to dielectric losses, the increased size of measurement volume in L-band offers a possibility of simultaneous acquisition of EPR signal from several neighboring teeth in mouth. This partially compensate a loss of sensitivity inherent in the lower frequency band. Despite of this, threshold of detection of the *in-vivo* dosimetric techniques is still about 2 orders of magnitude worse then in the *ex vivo* – X-band measurements; at present, practical detection of doses *in vivo* is possible above 1–2 Gy [5, 6].

Accurate, retrospective dose reconstruction in enamel, feasible even after years from exposure, can be a valuable tool not only in cases of radiation accidents. It also makes possible verification of therapeutic doses in radiotherapy patients. Thus, besides its contribution to improvement of treatment planning algorithms, EPR dosimetry can be applied as reliable tool helpful in court trials in cases of a suspected medical malpractice or medical errors regarding a use of ionizing radiations.

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Ion channels in mitochondria – the matter of life and death

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After heart attack oxygen deprived cells die. The extension of heart damage may be reduced if the episode of anoxia is preceded by short periods of reduced oxygen supply. This phenomenon is called ischemic preconditioning. Similar effect can be obtained by using some chemical substances which has one thing in common – they are potassium channel openers (KCOs). Mitochondria are likely suspect to play a role in ischemic preconditioning. They use oxygen, produce ATP, have potassium channels in the inner membrane and are involved in apoptosis.

There are two major potassium channels present in the inner mitochondrial membrane: mito-K_{ATP} (mitochondrial potassium channel blocked by high concentration of ATP) and mito-BK_{Ca} (mitochondrial big conductance calcium activated potassium channel). Both are present in mitochondria of all cells studied. But the mitochondrial mechanism of ischemic preconditioning remains obscure.

The simplest explanation of KCOs action is the hypothesis that potassium channels are involved in controlling mitochondrial potential. The higher mitochondrial potential the higher is the rate on reactive oxygen species (ROS) production. Opening of the potassium channels, especially during reperfusion phase reduces ROS production protecting cells after ischemia.

But how ischemic preconditioning works? Does it increase the number of ion channels present in the inner membrane of mitochondria? Does it reduce the number of mitochondria producing high doses of ROSes? And what are the answers suggested by mitochondrial evolutionary history?

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Structural, geometrical and magneto-optical features of iron-dextran complex

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Measurements of refractive index increment, static light scattering and the Cotton-Mouton effect in NaCl water solution of the iron-dextran complex (Sigma-Aldrich) were used to determine the molecular mass of this complex, M_w, iron core radius of its particles, R_{Fe-D}, and the content of iron ions in the complex core, N_{Fe}. Knowing the number of iron ions permitted the estimation of the permanent magnetic dipole moment value, μ_{Fe}, and the anisotropy of linear magneto-optical polarizabilities components, $\Delta\chi$. Having both values and the value of the mean linear optical polarizability, α, it was shown that the total measured CM effect was due to the reorientation of the permanent and the induced magnetic dipole moments of the complex. Analysis of the measured magneto-optical birefringence indicated very small optical anisotropy of linear optical polarizability components, κ_a, which suggested a homogeneous structure of particles of spherical symmetry.

Spectroscopy of photosynthetic antenna complex LHCII

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Light-Harvesting Pigment-Protein Complex of Photosystem II (LHCII) is a largest photosynthetic antenna complex and most abundant membrane protein in biosphere. The crystallographic structure of the protein reveals that it binds 14 chlorophylls (8 molecules of chlorophyll *a* and 6 molecules of chlorophyll *b*) and 4 xanthophylls (2 molecules of lutein, 1 molecule of neoxanthin and 1 molecule of violaxanthin). Fluorescence excitation spectra of chlorophyll *a*, overlapping almost perfectly the one-minus-transmission spectra of LHCII in the Soret spectral region, demonstrate very efficient electronic excitation energy transfer from all the groups of pigments to chlorophyll *a*. Resonance Raman spectroscopy of the LHCII-bound xanthophylls, reveals the light-induced molecular configuration transformation of violaxanthin bound to the protein at the interface of the trimer-forming LHCII monomeric units. This suggests reorganization of the trimeric structures. On the other hand, analysis of the chlorophyll *a* fluorescence lifetimes in a single LHCII molecule and single LHCII trimeric structure, possible owing to the application of Fluorescence Lifetime Imaging Microscopy (FLIM), demonstrates that the trimer to monomer transition shortens the fluorescence lifetimes (from ca 3 ns to 1 ns) and therefore opens photophysical channels to dissipation of excess excitation energy under light stress conditions to plants.

Do lipid rafts mediate the drug actions?

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Different physical properties and/or specific interactions between particular molecules are the main reasons for the phase separation and the domain formation in lipid bilayers. Lipid rafts are special type of microdomains, consisting mainly of sphingolipids and cholesterol. In fluid biological membranes, due to the interactions between the raft constituents, these domains are more rigid (liquid ordered, L_o) than the surrounding bilayer (liquid disordered, L_d). Lipid rafts play a key role in the cellular signaling, sorting and transport processes. The mechanisms of the domain formation as well as the factors governing their stability and behavior are often studied in model lipid systems. Ternary lipid mixtures containing sphingolipid, phospholipid and cholesterol at different molar ratios are the compositions often used to mimic the behavior of rafts present in biological membranes. Direct visualization of membrane domains was facilitated by the introduction of giant unilamellar vesicle (GUV) experimental technique.

Phenothiazine derivatives interact with lipid bilayers and affect their biophysical properties. Trifluoperazine (TFP) alters bilayer fluidity in a way similar to cholesterol but it was also found to induce phase separation in phosphatidylcholine membranes. Recently we studied the influence of three phenothiazine drugs – trifluoperazine, chlorpromazine (CPZ) and thioridazine (TDZ) on the GUV formation processes. Among these compounds TFP most effectively, and in the concentration-dependent manner, increased the number of domains, decreased their average area and elevated the total length of the domain borders in GUVs. CPZ and TDZ were much less efficient modifiers of domain formation processes. Data analysis supported the hypothesis that the presence of drugs altered the domain interactions due to changes of the total domain dipole moment. TFP molecules trapped in the domain border region increase the domain repulsion and thus affect the domain coalescence process.

Bacterial membranes (unlike higher organisms) contain relatively high percentage of cardiolipin. In unperturbed bacteria, depending on the life cycle phase, this lipid could be organized in domains. Using particular

changes of the spectral properties of nonyl acridine orange (NAO), occurring after binding of this fluorescent dye to cardiolipin, bacterial lipid domains can be visualized. Using this technique we followed the changes in the cardiolipin domains morphology in *Escherichia coli* cells treated with subinhibitory concentrations of ciprofloxacin and colistin (polymyxin E). The antibiotic choice was based on the contrast in the targets of their action: ciprofloxacin is known to act at the DNA level, while colistin affects the bacterial membrane structure and integrity. Unexpectedly, significant domain structure alterations were observed after ciprofloxacin treatment, colistin effects were minute. Unusual localization of the cardiolipin domains in filamentous *E. coli* cells that appear after ciprofloxacin application can be explained by the perturbation of cell components distribution. Inhibition of cell division affects the intracellular localization of DNA and proteins bound to it, what in turn is reflects on the cell surface by altered pattern of cardiolipin domains.

Both presented above examples of our studies lead to the conclusion that actions of different drugs might alter the pattern of lipid domains/rafts present in the treated cells. These changes could only witness the main effects exerted by the drugs but they might also themselves change the activity of domain-dependent proteins, leading thus to some additional drug effects.

The biological chemistry of HNO and its interactions with thiol-containing proteins

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Nitroxyl (HNO) is a compound related to nitric oxide (NO) by one-electron reduction and protonation. While the biology and biochemistry of NO has become better defined, our understanding of the chemistry, biochemistry and biology of HNO remains unclear. The basic chemistry of HNO makes biological study difficult as HNO dimerizes and dehydrates to produce nitrous oxide requiring the use of HNO donors and indirect detection methods. HNO exhibits robust reactivity with thiols resulting in disulfide and sulfinamide formation, which may provide control in biological systems. To date, no endogenous source of HNO formation has been clearly identified. To better understand the biology and chemistry of HNO, our laboratory has prepared and characterized new acyloxy nitroso compounds as sources of HNO and evaluated them for their ability to release this redox relative of nitric oxide. These compounds modify and inhibit various thiol-containing proteins including aldehyde dehydrogenase and glyceraldehye phosphate dehydrogenase as well as other structural proteins involved in muscle contractility. These results reveal pharmacological activity for HNO separate from NO and may suggest the basis of HNO-based pharmaceuticals and intriguing roles for endogenous HNO production.

Effects of electric fields upon cells

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The effects of electricity upon living organisms have been studied since 18th century. The investigations on cells started about hundred years ago. In the last ten years new possibilities of their practical significance for medicine became recognized. In my short lecture I will attempt to turn attention to the modern research concerning:

i. the role of galvanotaxis in embryology and regenerative medicine;
ii. effects of extracellular electric fields on orientation of cell divisions;
iii. stimulation of endocytosis and targeted drug delivery to cells;
iv. electrofusion and electroporation as a routine methods for gene transfection, drug delivery, and cloning of mammals;
v. “irreversible electroporation” as a new method for selective cell killing and non-thermal electric surgery, in particular in oncology.

Besides the review of the new publications some own, new results will be presented.

Molecular mechanism of xanthophyll cycle

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The role of lipids in the molecular mechanism and regulation of two types of the xanthophyll cycle is discussed. One of them is violaxanthin (Vx) cycle which involves interconversion between Vx, antheraxanthin (Ax) and zeaxanthin (Zx). In the second type, the diadinoxanthin (Ddx) cycle, interconversion between Ddx and diatoxanthin (Dtx) occurs. The dependence between the conversion of Vx into Ax and Zx as well as Ddx to Dtx and the molecular dynamics of hydrophobic fraction of aggregates formed by inverted micelles as well as thickness of the hydrophobic fraction of the aggregates, size of the inverted micelles, and solubility of Vx and Ddx in various kind of lipids is presented.

Additionally, the influence of thylakoid lipids on the de-epoxidation of Vx, associated with the light-harvesting complex of PSII (LHCII) is demonstrated. Analysis of different LHCII preparations showed that

the concentration of LHCII-associated Vx was correlated with the concentration of the main thylakoid lipid monogalactosyldiacylglycerol (MGDG) associated with this complex. Decrease in the MGDG content of the LHCII led to diminished Vx concentration, indicating that a part of the total Vx pool was located in the MGDG phase surrounding the LHCII, whereas another part was bound to the LHCII apoproteins. Besides, almost complete Vx de-epoxidation in the LHCII fractions containing high amounts of endogenous MGDG was observed in in-vitro assays with the use of isolated Vx de-epoxidase. LHCII preparations with low concentrations of MGDG exhibited a strongly reduced Vx de-epoxidation, which could be increased by addition of exogenous, pure MGDG.

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Causal Analysis of Molecular Dynamics Events a Novel Strategy to a Better Understanding of Biomolecular Systems

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Functions of complex biomolecular systems result from atomic and molecular motions, which can be simulated using molecular dynamics (MD) methods. MD simulations of typical systems provide thousands of trajectories in a configuration-momentum space. In case when simulations are carried out for quantum-classical biomolecular systems (e.g. enzymes), a set of classical trajectories can be supplemented with time-dependent quantum degrees of freedom, e.g. parameters of a quantum wave function describing protons motions – for a quantum-classical simulation of an enzymatic process see e.g. [1].

Quite often we are able to sufficiently precisely predict evolution of a system, however, it doesn't automatically mean that we understand functioning of this system. Such situation is similar to that one known from weather forecasts – based on physical principles we are capable to simulate evolution of fields characterizing the state of atmosphere, it doesn't mean, however, that we understand sufficiently well the weather changes. We assume that weather forecasts are sufficiently precise – from the point of view of this project, it is only a technical problem. A similar situation refers to complex systems in economy.

From a mathematical point of view simulation results are time-series. Understanding a given system depends on pointing out causal relations between simulated (or observed) events resulting from its time evolution. In 2003 C.W.J. Granger got the Nobel Prize for formulating a formalism capable to detect and to analyze causal relations in complex economical systems, see e.g. [2]. Similar relations are studied by biomedical physicists and neurophysiologists when analyzing electrical brain potentials, see e.g. [3].

We applied the mentioned above methodologies in the analysis of MD simulation results for a number of model molecular systems with proton(s) transfer and for HIV-1 protease. A very important question is how to reduce dimensionality of the original problem (large number of trajectories) to a low-dimensional representation. The proposed approaches are described in [4, 5]. One applies either a transformation to internal degrees of freedom, or one uses a Principal Component Analysis (PCA) and one applies a projection of trajectories on principal axes, resulting finally in the analysis of time-dependent projection amplitudes. The proposed strategy is sufficiently general and allows for a better understanding and description of the logic of functioning of complex biomolecular systems. Basics of the proposed formalism and examples of the analysis will be given. Problems which require further studies, in particular nonlinear couplings with a time-shift between the PCA modes, will be discussed.

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Organization of cell surface proteins: Distribution of HLA-I on lymphoid cells

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Distance relationships of cell surface proteins play a pivotal role in the process of transmembrane signaling. Changes in the distribution of receptors and their clustering are often determining factors in the ligand receptor interactions. HLA-I and II have a key role in the immune response since they display fragmented pieces of an antigen on the host cell's surface. Previous studies indicated a non-random distribution of HLA-I molecules on the cell surface. In present study we analyzed the role of individual molecules on the pattern formation of HLA-I.

Several techniques are available for determining the distribution of protein molecules. Fluorescence resonance energy transfer (FRET) is an indispensable tool

for determining distance relationships and supramolecular organization of cell surface molecules. A major limitation of the technique is that it does not provide information on the distribution of molecular clusters. Atomic force microscopy, near field scanning optical microscopy and electron microscopy fill this gap.

In the lecture we show the applicability of Ripley's K(t) function for analyzing the cell surface receptors patterns. Finally we give a brief summary of the biological importance of the non-random events on the cell surface.

Interaction of biologically active stilbenes and flavonoids with membranes

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In recent years a tremendous increase of interest in various biological effects of natural products was observed and many novel low-molecular weight compounds that could have potential future applications in clinical practice were described. Polyphenolic plant compounds like flavonoids and stilbenes due to their presence in human diet constituents are especially widely studied. Some of the compounds from these groups are able to reduce the drug resistance in cancer which is an important goal in cancer research.

We have focused on the interaction of resveratrol and its new synthetic derivatives with model lipid membrane and on the influence of these compounds on proliferation and anticancer drug accumulation in sensitive and drug resistant, P-glycoprotein expressing cancer cell lines of human colon cancer. The similar studies were also carried out using several flavonoids. The effect of polyphenols on model membrane domain structure was studied by simulation of the experimental ESR spectra and application of GHOST condensation method. The change of motional pattern of spin probes was also observed in cancer cell membranes. Modifications in membrane domain structure in cancer cells could influence the response of cancer to chemotherapy for example by increase of apoptosis via signaling through lipid rafts as it was documented in many recent studies.

The significance of different substituents present in polyphenolic ring structure of the studied compounds for the way of their interactions with lipid model membranes and for inhibition of multidrug transporters in resistant cancer cells were discussed.

Uracil in DNA – its biological significance

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Uracil may arise in small quantities in DNA as a result of spontaneous cytosine deamination and/or misincorporation of dUMP during DNA replication. However, recently uracil formation *via* enzymatic deamination of cytosine has been found as the mechanism underlying (i) diversification of genes encoding Igs and (ii) inhibition of retroviral infection. DNA deamination is the only known process in mammalian development in which the coding capacity of the genome is changed by targeted modification of deoxycitidine. In this lecture we review sources of the origin of uracil in DNA and some properties of the enzymes responsible for the excision of uracil and their role in Ig diversification process, which comprises somatic hypermutation and class switch recombination, and consequences of cytosine deamination in other than *Ig* loci, in cell types different than B lymphocytes. Furthermore, the issue concerning the basal level of uracil in DNA and consequences of the presence of U:A pairs for DNA stability and cell functions will be discussed. Finally, we broach here possible involvement of aberrantly expressed AID and presence of uracil in DNA in carcinogenesis.

It is concluded that a non-canonical base uracil may be present in small quantities in DNA mostly as U:A pairs. If not removed from the genome this "background level" (likely in quantities of 10^4 per genome) may be well tolerated and in certain cases may even play some physiological functions.

Mitochondrial actions of melatonin: Preserving an optimal redox balance

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Melatonin is a natural occurring compound with well-known antioxidant properties. Melatonin is ubiquitously distributed and because of its small size and amphiphilic nature, it is able to reach easily all cellular and subcellular compartments. The highest intracellular melatonin concentrations seem to be in mitochondria, raising the possibility of functional significance for this targeting with involvement *in situ* in mitochondrial activities. Mitochondria are important cellular organelles that contribute to degenerative processes mainly through respiratory chain dysfunction and formation of reactive oxygen species, leading to damage to mitochondrial protein, lipids and DNA. Therefore, protecting mitochondria from oxidative damage could be an effective therapeutic strategy against cellu-

lar degenerative processes. Many of the beneficial effects of melatonin administration may depend on its effect on mitochondrial physiology. During free radical-mediated, mitochondrial-dependent apoptosis melatonin prevents the release of cytochrome c, thereby reducing apoptosis. Cardiolipin, a phospholipid located at the level of inner mitochondrial membrane, is known to be intimately involved in several mitochondrial bioenergetic processes as well as in mitochondrial-dependent steps of apoptosis. Alterations to cardiolipin structure, content and acyl chain composition have been associated with mitochondrial dysfunction in multiple tissues in several physiopathological situations and aging. Recently, melatonin was reported to protect the mitochondria from oxidative damage by preventing cardiolipin oxidation and this may explain, at least in part, the beneficial effect of this molecule in mitochondrial physiopathology.

4-Hydroxynonenal (HNE) metabolism and its age dependency

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Mammalian cells possess highly active pathways of aldehyde including HNE metabolism (1, 2). As primary HNE products in hepatocytes and many other cell types the HNE-glutathione conjugate (HNE-GSH), the hydroxynonenoic acid (HNA) and the corresponding alcohol of HNE – the 1,4-dihydroxynonene (DHN) were identified (3). The pattern of HNE metabolites involves a lot of further intermediates. The rapid HNE metabolism underlines the role of HNE degrading pathways in mammalian cells as important part of the secondary antioxidative defense mechanisms in order to protect proteins from modification by aldehydic lipid peroxidation (LPO) products.

From analysis of blood plasma and various tissues of human beings and further mammalian species it is known, that HNE levels increase with increasing age. It is not clarified if that is due to whether accelerated formation of HNE or to diminished metabolism and, therefore, removal, of this compound. In the model of human skin fibroblasts from donors of different age the age dependency of the HNE degradation rate was analyzed. It was found, that the overall HNE degradation rate strongly decreases with age. Both the formation of HNE-GSH – due to reducing intracellular GSH – and the generation of the HNA are reduced with increasing age. It is concluded, that at least the drastically diminishing HNE metabolism contributes strongly to increasing HNE levels in plasma and tissues with age.

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P-chiral analogues of oligonucleotides

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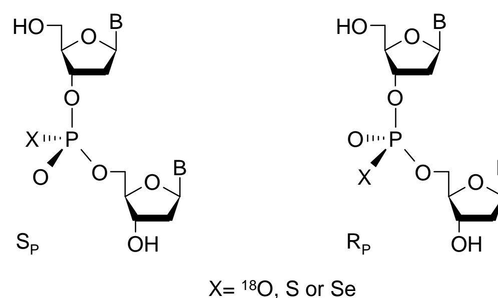
Replacement of non-bridging oxygen at the phosphorus atom of internucleotide phosphate by a stable oxygen isotope, sulfur or selenium atom, induces – by virtue of asymmetry – new centre/centers of chirality, providing stereochemical tools for studies of structure and function of nucleic acids, as well as their interactions with metal ions and proteins involved in their metabolic transformations. Isotopomeric substitution does not alter biological properties of oligonucleotide, while the presence of a sulfur atom (in PS-Oligos) affects these properties, mostly due to different steric requirements of sulfur atom, different affinity towards metal ions, and changes in negative charge distribution in the phosphorothioate anion. The same issues are related to phosphoroselenoate oligonucleotides (PSe-Oligos). It is important to note that PX-Oligos prepared via chemical non-stereocontrolled method exist as mixtures of hundreds of P-diastereomers of S_P or R_P absolute configuration.



X= ^{18}O, S or Se

Isotopomeric, phosphorothioate and phosphoroselenoate analogues of oligonucleotides were obtained in stereodefined forms by methods originally developed in this laboratory. Synthesized probes were used for mechanistic studies of the B-Z DNA conformational change and for research on unusual thermal stability of PX-DNA/RNA complexes. The obtained data confirm that the negative charge is localized mostly on S (Se) atom in the O-P-X groups. Moreover, the results suggest that a sulfur or selenium atom present in a P-X$^-$ moiety is able to form a charge assisted hydrogen bond that is stronger than that formed by the P-O$^-$ function. This conclusion is contradictory to the common belief

that the strength of the hydrogen bond depends predominantly on the electronegativity of the acceptor atom.

Reactivity between gold nanoparticles and bioactive molecules

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The interest of nanomaterials, especially gold nanoparticles (AuNPs), has recently grown in diverse areas of biology and medicine (1). The ease of AuNPs synthesis and their affinity for binding many bioactive molecules such as proteins, DNA, RNA, drugs and antioxidants, make them attractive candidates for various biomedical applications. Interestingly, AuNPs have been demonstrated to exhibit high reactivity for free radicals (2, 3).

In the present work, we synthesized two kinds of AuNPs: either stabilized with citrate (Au@citrate) or capped with a dithiol, i.e. dihydrolipoic acid (Au@DHLA). These latter AuNPs were varied according to their capping density. We fully defined physicochemical characteristics of the obtained AuNPs. Next, we studied the interaction between AuNPs and various compounds: 1,1-diphenyl-2-picryl-hydrazyl radical ($DPPH^{\bullet}$), a dye commonly used in antioxidant assays, reduced and oxidized glutathione (GSH and GSSG), S-nitrosoglutathione (GSNO), and bovine serum albumin. Developed analytical methods devoted to the measurement of corresponding interactions rely upon either HPLC or fluorescence.

Resulting data permit some interesting conclusions on respective usefulness of the different studied probes to investigate the surface reactivity of AuNPs, to define the most efficient capping and to select inert nanosized platforms as good candidates for drug delivery.

Then, the different AuNPs were tested on cultured cells (rat alveolar macrophages-NR8383) using two different medium conditions: with and without proteins. The AuNPs with the highest density of capping also exhibit the highest cellular uptake, probably because interaction with proteins in culture medium is minimized, thus cell interaction becomes more favorable.

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Kinetic chemiluminescence as a tool to study the mechanisms of free radical reactions and antioxidant effects

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Free radicals participate in two important processes in the life bodies: cell signaling and cell injury. Meanwhile, their extreme chemical reactivity makes impossible to determine directly their content and investigate their reaction mechanisms by routine biochemical technique, such as substrate isolation, purification, analysis etc. Fortunately, we can follow many important radical-mediated reactions by measuring *chemiluminescence*, accompanying those reactions.

We have elaborated an instrument *SmartLum*, specially adopted for measuring the non-stationary reaction kinetics, very sensitive and supplied with devices for thermostating, solution adding during the reaction, stirring and on-line data processing and storage by using a software *PowerGraph* (http://www.powergraph.ru/). The chemiluminescence kinetic curves were measured in different experimental conditions and compared with the curves calculated with a computer program *Kinetic Analyzer*, specially designed in collaboration with Dr. Dmitry Izmailov in our lab for complex reaction kinetic simulation. By using the initial concentrations of all reaction substrates and varying the reaction rates of all the reactions proposed, we compared the experimental and calculated curves. The coincidence of these curves is considered as an evidence that the reaction scheme and the rate constants are valid. Recently a student Leonid Batov has invented a software to fit automatically the reaction rates in the assumption that the reaction scheme is known.

The reaction schemes of the chain lipid peroxidation reactions in the presence of Fe^{2+} ions, antioxidants reactions in this system, reactions of luminol oxidation by H_2O_2 catalyzed by peroxidases and cytochrome c/ cardiolipin complex, and antioxidant reactions in the TRAP method were investigated by using the kinetic chemiluminescence method and the results are discussed, along with the mechanism of the antioxidant reactions and the antioxidants' antiradical activities.

Biophysics supports development in biomaterials engineering

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Since many years biomaterials have been successfully used in medicine for supporting, restoration or replacement of a wide range of tissues and organs, but medical implants or instruments can cause numerous undesirable effects in the human body. A very early stage of artificial surface contact with body fluids is a protein adsorption and in turn the attached proteins mediate contact with cells. As a result changes in genes expression and proteins profile of cells are observed.

SPR biosensors, light, fluorescence and electron microscopy, as well as RNA-microarray and 2D electrophoresis techniques were employed in our research for characterization of protein adsorption and cellular response to artificial surfaces of selected biomaterials, including metallic alloys, natural and synthetic polymers, hydroxyapatites, titanium dioxides and crystalline carbon materials. Obtained results support decisions concerning a proper choice of new materials for purpose of artificial heart and orthopedic implant production.

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Searching for the primary photoeffect of the light therapy

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The phototherapy is one of the oldest therapeutic method; initially as solar therapy, later light and at least low power laser therapy. Light therapy has been used for more than forty years to promote healing, reduce pain and inflammation, and prevent tissue death. Light irradiation as a local phototherapeutic modality is characterized by its ability to induce non-thermic, nondestructive photobiological processes in cells and tissues. A variety of studies both in vivo and in vitro showed significant influence of light irradiation on cell functional state. The response of light action is a biphasic after irradiation both *in vitro* and *in vivo*. The so-called Arndt-Schultz curve of energy dose versus response that is applied to light therapy states that low doses of energy stimulate cell and tissue processes, while high energy doses reverse the stimulation and lead to inhibition.

Results of experiments in vitro and in vivo on isolated cells as well as animals and humans are ambiguous. Despite many investigations on the subject, the therapy remains controversial largely due to uncertainties about the fundamental molecular and cellular mechanisms responsible for transducing signals from the photons that are incident on the cells to the biological effects that take place in the illuminated tissues. Some different explanations based on the light absorption by primary endogenous chromophores (mitochondrial enzymes, cytochromes, flavins, porphyrins) have been proposed to describe biological effects of laser light. However, the exact theory concerning the therapeutic effects of light biostimulation has not been developed.

The molecular mechanism of photochemical and therapeutic action of light from Near Infrared region *in vivo* and *in vitro* is discussed based on results of spectral and clinical studies of blood, plasma, platelets, leucocytes. We proposed the primary photochemical effect and primary photoacceptor common for very wide values of wavelengths and different materials. The proposed molecular mechanism is also common for such molecules like aminoacids, proteins and DNA.

Plasma membrane lipid-raft organization and cell cycle perturbation in osteosarcoma 143b cells exposed to natural and synthetic antioxidants

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Osteosarcoma were exposed to gallic acid and its lauryl derivative at concentrations of 1 and 10 μM respectively, and then labeled with 5-doxylstearic acid. EPR spectra show clearly two fractions of the label: strongly and weakly immobilized. This was reflected by two split lines in high field region of the EPR spectrum namely A and A'. The ratio of their amplitudes has been chosen as a measure of relative participation of membrane rigid domain and liquid regions. The ratio has been shown to significantly change in lauryl gallate treated cells and being dependent on its concentration. The S order parameter has been found to change from control value 0.827 to 0.816 and 0.806 after exposition to 1 and 10 μM gallic acid, respectively. 1 μM concentration of lauryl gallate change S to 0.760, while 10 μM of lauryl gallate decreased further S parameter more dramatically to 0.720. 1 μM and 10 μM of gallic acid change the rotational correlation time of the probe from a control value of 5.2 ns to 4.7 ns and 4.25 ns respectively. 1 μM and 10 μM of lauryl gallate exerted a profound effect on the rotational correlation time yielding values of 1.9 ns and 1.6 ns. While gallic acid has been found to influence cell

cycle at 1 and 10 μM concentrations, lauryl derivative at
10 μM inhibited cell cycle at G1 phase after 24 hours of incubation.

Qualitative and quantitative mapping of elements by energy-dispersive microanalysis in transmission electron microscopy

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X-ray microanalysis in electron microscopy gives a unique opportunity to combine the ultrastructural examination of the sample with its chemical composition. In TEM, however, it requires stringent methods of specimen preparation to avoid redistribution/loss of the elements. The newly installed equipment in our Lab JEM 1400 (JEOL Co., Japan) with EDS INCA Energy TEM (Oxford Instruments, Great Britain) has many advantages including the 126.8 eV spectral resolution of the detector and three times higher value of counting rate providing the same analytical results with a lower probe current, that is important for thin biological samples – used in the biology and medicine – to reduce radiation damage. Number of analyzed elements was widened due to a new type of beryllium window and the combination of EDS detector and STEM device allows to set precisely the area of measurements and to acquire a spectrum from a line, point or any rectangular region. Both the qualitative and quantitative analyses may be performed and mapping of elements may be superimposed over the image of the examined specimen at the nanometer scale. The software allows comparing spectra acquired from different sites/samples. Our experiments gave some hints: conditions of measurements depend both on sample durability (radiation damage, sample drift) and signal strength; the optimal number of counts for mapping is 500000–1500000 cps (acquisition time: 30–50 min) whereas for a point analysis 10000 cps (acquisition time: 2–6 min). Grids for EDS should be coated with formvar/carbon films for a better stability. Signal from this layer is very weak and benefits are higher than additional background signal (though coating is not recommended in search for the light elements).

Role of mitochondrial dysfunction in diabetes. Corrections of mitochondrial disorders in diabetes by melatonin and succinate

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Mitochondrial dysfunction may be a central cause of insulin resistance and associated complications in diabetes (Kim, et al., 2008). Metabolic regulation in the cell is largely dependent on mitochondria functional activity. Brownlee suggested that mitochondrial dysfunction occurs as a “unifying mechanism” for microvascular and macrovascular complications through increased ROS production (Brownlee, 2005). Genetic factors, oxidative stress, mitochondrial biogenesis impairments, and aging may affect mitochondrial function, leading to insulin resistance and diabetic complications. The main consequences of hyperglycemia under diabetes are formation, of advanced glycation end products (AGEs), their autooxidation and interaction with cell receptors, activation of isoforms of protein kinase C, induction of the polyol pathway and increased hexosamine pathway flux (Rosen, et al., 2001). Much of the hyperglycemia-induced damage is suggested to be a consequence of elevated production of ROS by the mitochondrial respiratory chain (Green, et al., 2004). Mitochondria are the major sites of ROS production in the cell and the main target of oxidative damage. Mechanisms that contribute to the formation of free radicals in mitochondria under diabetes may include metabolic stress resulting from changes in energy metabolism, increased levels of inflammatory mediators, non-enzymatic glycosylation, and glucose autooxidation. Excess ROS further stimulate various serine/threonine kinases and inflammatory signaling pathways that inhibit insulin signaling. Defects at multiple sites in insulin signaling pathway have been suggested as mechanisms underlying insulin resistance: increases serine phosphorylation of IRS proteins, increased degradation of IRS, increased activity of phosphatases (inositol 5’-phosphatase 2, phosphotyrosine phosphatase 1B) and decreased activation of insulin receptor downstream signaling molecules, including Akt and a typical PKC (Kim, et al., 2008).

We evaluated the mechanisms of liver mitochondria damage under diabetes and the metabolic effects of pharmacological doses of the respiratory chain substrate succinate and the known antioxidant melatonin. The experimental (30-days) streptozotocin-induced diabetes mellitus caused a considerable damage of respiratory activity in rat liver mitochondria. The basal (endogenous) respiration rate, V_1, decreased by 15 –20%. In the case of succinate as a respiratory substrate, the rate of oxygen consumption, V_2, and the

respiration rate after ADP consumption, V_4, did not change, but the ADP-stimulated respiration rate, V_3, considerably decreased (by 25%, $p < 0.05$) as well as the acceptor control ratio, (ACR) V_2/V_3, and the respiratory control ratio, (RCR) V_3/V_4, markedly diminished (by 25%, $p < 0.01$ and by 27%, $p < 0.01$, respectively). Similarly, we observed a decrease of the glutamate-dependent respiration rate V_2 by 20% and the ADP-stimulated respiration rate V_3 by 35% ($p < 0.05$), the respiration rate V_4 being unchanged. The ACR and RCR also decreased (by 20%, $p < 0.05$ and 25%, $p < 0.01$, respectively). Surprisingly, the phosphorylation coefficient ADP/O did not change under diabetic liver damage. The melatonin administration during diabetes (10 mg/kg BW, 30 days, daily) as well as succinate treatment (50 mg/kg BW, 30 days, daily) showed a considerable protective effect on the liver mitochondrial function, reversing the decreased respiration rate V_3 to the control values both for succinate-dependent respiration ($p < 0.05$ in comparison with diabetic animals) and for glutamate-dependent respiration ($p < 0.01$). Similarly, the melatonin or succinate treatment of diabetic rats reversed the effect of diabetes on the ACR and RCR values both for succinate-dependent respiration and for glutamate-dependent respiration.

The effects of melatonin might be due to both its radical scavenging properties, its metabolic effects and specific interaction with complexes of the respiratory chain. Succinate, the known bioenergetic substrate, is found to act as a specific ligand for orphan G-protein-coupled receptor (GPR91) and to have signaling functions (He, et al., 2004). Therefore, succinate can specifically regulate the physiological state of mitochondria. Our results suggest that the melatonin and succinate, while regulating the mitochondrial function, may have a therapeutic potential for correction of diabetic liver damage.

VO_2 kinetics during exercise – relationship with muscle fatigue

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The rest-to-work transition or an increase of generated power output by skeletal muscles requires a rapid adjustment in ATP supply in order to maintain a stable muscle ATP concentration. During the first seconds or the firsts tens of seconds of moderate intensity exercise the most ATP supply is generated from the phosphocreatine (PCr) splitting through the creatine kinase reaction, with some small contribution by anaerobic glycolysis. At high power outputs the contribution of anaerobic glycolysis to ATP production at the onset of exercise increases.

During rest-to-work transition the oxygen uptake (VO_2) increases much slowly than the rate of ATP usage in the working muscles. The VO_2 kinetics can be described as an exponential process. During moderate exercise intensity (below the lactate threshold – LT) VO_2 follows a mono-exponential process reaching steady state within about 3 minutes and its rate can be expressed by the time constant of the primary VO_2 component (τ_p). The τ_p in humans varies significantly, from < 10 s in highly trained athletes to > 60 s in patients with cardio-pulmonary insufficiency. During heavy exercise intensity (above the LT) no steady state in VO_2 but a progressive increase in VO_2 occurs. This effect is called the slow component of VO_2 kinetics.

During this lecture the relationship between the VO_2 kinetics and the muscle metabolic stability (including: PCr, ADP_{free}, IMP and ΔG_{ATP}), as well as its importance for muscle performance and fatigue, will be presented.

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