Session 10. Electrophysiology and Electrochemical Sensors

Lectures

L10.1

Stress-induced plasticity of excitatory and inhibitory postsynaptic currents in rat central neurons

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Corticotropin-releasing hormone (CRH)-synthesizing neurons of the hypothalamic paraventricular nucleus (PVN) play a key role in the activation of the hypothalamic-pituitary-adrenal axis (HPA). We used whole-cell patch-clamping to record spontaneous and miniature excitatory and inhibitory postsynaptic currents (sEPSCs/mEPSCs and sIPSCs/mIPSCs) from parvocellular neuroendocrine neurons of the rat PVN in *ex vivo* brain slices. Repeated restraint stress (10 min; twice daily for 3 days) resulted in an increase in the mean frequency of sEPSCs/mIPSCs and in a decrease in the rise time and the decay time constant of sEPSCs/mEPSCs. There was no change in the mean amplitude of sEPSCs/mIPSCs. The parameters characterizing sIPSCs/mIPSCs remained unaltered. We also studied the effects of repeated injections of corticosterone, as a model of a chronic stress, on sEPSCs and sIPSCs recorded from layer II/III pyramidal neurons in ex vivo slices of the rat frontal cortex. Corticosterone administered repeatedly for 7, 14 and 21 days induced an increase in the frequency but not the amplitude of sEPSCs. Collectively, these studies suggest that repeated stress appears to selectively affect glutamatergic, but not GABAergic transmission in the rat hypothalamus and frontal cortex, and suggest that repeated stress induces an enhancement of spontaneous glutamate release from presynaptic terminals.

L10.2

Muscarinic and adrenergic receptor control of pyramidal neuron membrane potential in the medial prefrontal cortex

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Impairment of prefrontal cortex neuron activity occurs in widespread neuropsychiatric disorders such as schizophrenia, senile dementia, depression, ADHD, and PSD. The key neurotransmitters, i.e. dopamine, serotonin, acetylcholine (Ach) or norepinephrine (NE), control PFC neuron activity by activation of the metabotropic receptors, activation of the cellular transduction system and alteration of ionic channel activity. In order to correct this disordered neuronal activity we first have to understand how these neurotransmitters control prefrontal cortex neurons in healthy subjects. The purpose of our study was to clarify the mechanisms responsible for control of prefrontal cortex pyramidal neuron activity by muscarinic receptors and adrenergic receptors in rats. It was found that pyramidal neuron membrane potential is controlled by M1 muscarinic receptors which open Nav1.9-like Na^+ channels. The transduction system occurs in a membrane-delimited fashion and involves $\beta\gamma$ subunits. NE controls the membrane potential mainly by activation of α_2 adrenergic receptors which lead to the closing of Ih channels. Transduction from the receptors to the effectors also occurred in a membrane-delimited fashion.

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L10.3

Investigation of oxidative stress biomarkers using electrochemical quartz crystal nanobalance and other methods

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Oxidative stress biomarker, glutathione (GSH), plays the fundamental role in redox homeostasis and signaling processes associated with apoptosis. GSH maintains the redox potential level in cells and body fluids and protects organisms against oxidative damage to their DNA, proteins, and lipids caused by reactive oxygen species (ROS) [1-3]. Homocysteine (Hcys), another sulfur containing compound, is an amino acid biomarker of oxidative stress [4-5] which plays a critical role in many diseases such as stroke, cardiovascular diseases, diabetes, and Alzheimer. In view of the increasing occurrence of these common diseases, the analysis of biomarkers of oxidative stress becomes a new pressing necessity for diagnosis and treatment [1-5].
We have investigated physicochemical aspects of the interactions of oxidative stress thiol biomarkers with functional self-assembled monolayers (SAMs) to gain further insights into the nature of small molecule biorecognition phenomena that could be utilized in the designs of novel biosensors for rapid and inexpensive detection of these biomarkers. The interactions of GSH with gold nanoparticles (AuNP) using plasmonic absorption, resonance elastic light scattering (RELS) spectroscopy, and electrochemical quartz crystal nanogravimetry (EQCN) have been investigated in detail. The GSH- and Hcys-induced citrate-capped AuNP assembly has been evaluated as a function of pH and the importance of charge state and interparticle hydrogen bonding have been elucidated. The ligand exchange mechanisms for citrate-capped AuNPs, with competitive adsorption and 2D island nucleation and growth, have been proposed and tested using GSH moderator and Hcys exchanger ligands. The GSH-capped $AuNP_{5nm}$ particles have been applied in the designs of buried potential barrier biosensors with an anti-GSH antibody immobilized on a piezoelectric quartz monocrystal resonator. The EQCN transduction has also been employed in the design of a conductive polymer-based molecularly-templated biomimetic biosensor able to recognize GSH-capped AuNPs as the analyte. We have also investigated the interactions of biothiols with fluorosurfactant Zonyl coatings on piezoelectrodes and on AuNPs to study small biomolecule-induced interparticle cross-linking. Using EQCN and RELS, we have found that minor differences in permeation and interactions with the fluorosurfactant molecules can be used to measure differences in responses to structurally similar biomolecules like cysteine and homocysteine. Finally, the mechanisms of oxidative stress development under hypoxia and reperfusion conditions have been studied to gain control and diminish the mitochondrial damage caused by ROS.

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L10.4

Elucidation of the epithelial ion transport mechanism

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Ion transport is responsible for maintenance of transmembrane and transcellular electric potentials, fluid transport and cellular volume. Disturbed cell homeostasis caused by anomalous ion transport is related to various diseases. Cystic fibrosis (CF) is the most common genetic disorder caused by disturbed ion transport. In CF abnormal ion transport leads to increased viscosity of the mucus secretion in airway epithelium and chronic lung infections leading to patient death. There are many contradictory hypotheses of the ion transport through airway epithelium, but reliable mechanism of epithelial ion transport is still unknown. Available measuring techniques give only the information about the total current flowing across epithelial cell monolayer, without possibility of distinguishing particular ion (K^+, Na^+, Cl^-, HCO_3^-) contribution.
In our group the novel potentiometric system for simultaneous determination of K^+, Cl^-, Na^+ ions and pH in cell monolayer was designed. The designed system allows for real-time monitoring of ion transport within cell layers or tissues. The constructed miniaturized electrodes were integrated with reference electrode in one probe allowing direct observation of changes in the concentration of ions at the surface layer of cells. The ISE-based system for real time measurements was successfully applied for *in vitro* study of ion fluxes in human bronchial cell monolayer. Early experiments show that in human bronchial epithelium sodium ions are transported *via* paracellular and transcellular routes whereas chloride ions are transported only transcellularly.

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Oral presentations

O10.1

Romanian clinoptilolite as a sensing probe for conductometric sensors and biosensors

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Ammonium as a major constituent of deep ground waters (NH_4-N up to 39 mg/L) has received a tremendous amount of attention, due to effects of N loading in part on health and potentially on ecosystems. Determination of urea, a compound of the diagnostic significance for variety of the metabolic disorders, has become possible due to just determination of ammonium, generated in the enzymatic decomposition of urea, by the electrochemical biosensors. Search of the selective probes for ammonium is of great importance for development of the highly sensitive and reliable sensors for determination of ammonium and improvement of the enzyme biosensors for urea.

The scope of the study is investigation of the ammonium exchange sorption on the raw form of the natural zeolite, Romanian clinoptilolite (RClt), for the material application in the ammonium conductometric sensor and the conductometric biosensor for urea.

The receptor membrane of the conductometric sensor for ammonium was prepared by adhesion of the zeolite particles to the gold surface of the working region of a pair of the interdigitated electrodes. The coefficients of selectivity of a RClt-modified pair of electrodes were determined in the aqueous solutions of NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Al^{3+} ions and in phosphate buffer solution (according to the *Fixed Interference Method*). The sensitivity of the RClt-modified pair of electrodes in phosphate buffer, Tris-HCl and HEPES-NaOH buffer solutions were studied and compared with the sensitivity of the non-modified pair in the same buffer solutions.

To find the most efficient immobilization technique for preparation of the zeolite-based urease membrane, four configurations of the biomembrane were studied. The most significant improvements in analytical characteristics of urea biosensors were achieved when urease and zeolite were immobilized in a single layer deposited on the uniform zeolitic adlayer (the sensitivity and LOD of the developed biosensor were 20.36±2.78 μS/mM and 1.0×10^{-6} M, respectively compared to the corresponding values of 5.63±0.6 μS/mM and 1.0×10^{-5} M, obtained for the non-modified biosensor). The RClt-based biosensors for urea demonstrated short response time and high operational stability (a coefficient of the signal variation reached 0.74%); their lifetime exceeded five months.

Therefore, highly sensitive (bio)sensors based on clinoptilolite for ammonium and urea can be considered as promising devices for their further application in the biomedical and environmental tasks.

O10.2

New nonspecific modulators of mechanosensitive channels MscS and MscL from *Escherichia coli*

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Mechanosensitive (MS) channels are widely distributed among all organisms and fulfill plethora of functions including osmoregulation, touch and hearing sensation. Several genetic diseases are connected with mutations in eukaryotic MS channels. Bacterial MS channels MscS and MscL open upon osmotic down-shocks releasing small molecules from cytoplasm and thereby protect cells from lysis. MscL and MscS are proteins of distinct structure. MscL is a homopentamer in which single subunit has two transmembrane helices TM1 and TM2. MscS is a homoheptamer with three TM helices (TM1-3). MscS channel, in contrast to MscL, exhibits inactivation, which is attributed to transient interaction of peripheral helices with pore forming TM3. Mechanisms of MscL and MscS channel activation were studied in details. It is well established that both channels are activated directly by tension within the membrane and no accessory proteins are required for channel activity. In spite of the extensive biophysical knowledge no specific modulators of these channels are known. However, consistently with the role of physical state of the membrane in functioning of both MS channels, a number of membrane modifying agents was shown to modulate their activity. Lysophosphatidylcholine (LPC) and chlorpromazine (CPZ), molecules of inverted conical shape, activate both channels. On the other hand, Gd^{3+} blocks MS channels by altering the packing and lateral pressure of anionic lipids. In our attempt to identify new MscL and MscS modulators, we discovered small molecules that exert opposite effects leading to increase and decrease of activities of MscL and MscS, respectively. These molecules act on both sides of the membrane in the same fashion but share no apparent chemical similarities. However, the conical shape of all these molecules indicates for common mechanism of action which is associated with the membrane modification.

O10.3

Characteristic of photo-electrochemical interactions of Light Harvesting Complex II supported by screen-printed graphite electrode

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Light Harvesting Complex of photo system II (LHCII) complex is an antenna pigment-protein complex that harvests light energy and transfer it to the PSII reaction centers, where charge separation occurs. This complex in thylakoid membranes is present in the trimeric form (LHCII3) associated in LHCII-PSII supercomplexes as well as in aggregated form of $LHCII_3$ disconnected from PSII reaction centers. The ability of $LHCII_3$ to reversible formation of the trimmers or aggregates allows to increase the energy transfer or protect photosystems against photo-damage [1]. Property of the isolated LHCII gives the opportunity for its immobilization in a simple matrix on the surface of a screen-printed graphite electrode (GE) to measure specific interactions in protein-pigment-electrode hybrid. The characterization of redox properties and organization of LHCII complexes and the accumulation of charge on the electrode surface were performed by differential pulse voltammetry (DPV) and chronoamoperometry (CA) [2]. Effect of light and potential applied to the electrode on excitation transfers in LHCII complexes was examined by low temperature (77K) as well as lifetime chlorophyll fluorescence [3]. Both voltammetry and fluorescence data provide information about LHCII-GE layers and electron transfer between electrode and pigment-protein complexes. Characteristics of this system will contribute to construct disposable photo-biosensor for environmental analysis based on natural or artificial pigment-protein bioreceptor.

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O10.4

Droplet microfluidic system for generation of lipid bilayers and screening of membrane proteins activity

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This paper reports a microfluidic system, that automates the formation of lipid bilayers at the interface of nanoliter water droplets submerged in oil [1, 2]. We introduce a new microfluidic architecture — a trap [3] designed to localize the droplets with respect to each other and with respect to the electrodes. Recently, few microfluidic platforms [4-7] utilizing Droplet Interface Bilayers (DIBs) technique were developed for formation of artificial lipid bilayers. However, they do not allow for both high-throughput, automated generation of bilayers and electrophysiological measurements at the same time. For efficient and repetitive creation of functional lipid bilayers we exploit the Droplet on Demand system [8], which provides high degree of control over composition and location of each droplet. Exclusion of manual handling of droplets is an important feature that increases the reproducibility of the protocol.
The microfluidic device comprises 2 polycarbonate plates, milled with CNC milling station and subsequently thermally bonded in a hydraulic press. The device consists of i) a microfluidic trap (N) in which droplets were contacted to form a DIB ii) two microfluidic T-junctions (D and G) with additional sample-ports and iii) an inlet port for a sequence of droplets containing various inhibitors (F). Pair of silver wires coated with AgCl serve as electrodes inserted into each of the two chambers of the trap. The accuracy of our approach was tested in the screening experiment of activity of various concentrations of γ-cyclodextrin on α-hemolysin pore. We were able to perform several subsequent repetitions of screening of 6 concentrations (1–50 μM) of inhibitor. Using the traces obtained for 10–50 μM we calculated dissociation constant K_d value for binding of γ-cyclodextrin to α-hemolysin to be equal 60.70 ± 7.29 μM. Up to now, droplet microfluidics was used to generate Droplet Interface Bilayers at a high yield [4] but without the ability to perform electrophysiological measurements on samples encapsulated in droplets. The technique that we report here allows for automation of these measurements and for additional operation, such as washing and exchange of both the inhibitor solution and of the protein.

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Posters

P10.1

Assembly of ATP-capped gold nanoparticles induced by metal ions studied using RELS and EQCN

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Adenosine triphosphate (ATP) is the main energy source in cells and an important biomolecule participating in many cellular reactions in living organisms. It is formed in many biological processes, such as cellular respiration, fermentation, and phosphorylation. The deviation from normal intracellular ATP concentration level has been attributed to various diseases, including ischemia, hypoxia, hypoglycemia, angiocardiopathy, and certain malignant neoplasia. Therefore, monitoring of ATP levels has become an important element in the evaluation of cellular bioenergetics, abnormalities in cell metabolism, and disease control. Thus, a sensitive analytical method for the determination of ATP is needed and highly desired. The current methods that have been developed for ATP detection, including high performance liquid chromatography, mass spectrometry, electrochemistry, chemiluminescence, and aptameric biosensors, contribute to our understanding of ATP role in life processes. However, most of these methods require specialized equipment, suffer from complicated laboratory procedures, or provide low selectivity and sensitivity. In this work, we have explored the utilization of plasmonic nanoparticles with functionalized interfaces to develop very sensitive and inexpensive sensing platforms for the determination of ATP, based on the recent progress in the development of oxidative stress biosensors [1-3]. The optical and electronic properties of plasmonic gold nanoparticles (AuNP) have incited their widespread utilization in biosensor designs, nanotechnology applications, and medical diagnostics [1-4]. The assembly processes of gold nanoparticles are important in understanding physicochemical aspects of small biomolecule interactions with metal nanoparticles for new nanomedical applications. In this work, the formation of network of surfactant-capped gold nanoparticles by ATP and ATP-capped AuNP by metal ions have been investigated by resonance elastic light scattering spectroscopy (RELS) and electrochemical quartz crystal nanobalance (EQCN) technique as highly sensitive methods of monitoring the assembly process and the detection of ATP. The RELS technique has provided a simple means for very sensitive detection of metal cation-linked ATP-caped AuNP assembly. Several divalent cation linkers (Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}) have been tested. The basic processes of the formation of functionalized charge-controlled interfaces have been investigated using EQCN and high frequency piezometry [5].

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P10.2

Paracellular and transcellular trasport of ions in human bronchial epithelium

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Defect of water and ion transport caused by mutation in single *cftr* gene leads to the cystic fibrosis - CF. *cftr* gene code anion channel known as Cystic Fibrosis Transport Regulator (CFTR). Defective CFTR protein leads to very viscous secretion. There are three contradictory hypotheses of how viscous mucus is produced in CF patients. First hypothesis claims that there is decreased water transport to apical side of epithelial cell monolayer, the second that there is increased water absorption from the apical side and the third that there is decreased bicarbonate transport in CF patients. There are multiple ions which are transported across the epithelial monolayer i.e. sodium, potassium, chloride, hydrogen and bicarbonate. Until this time there was no method to simultaneously monitor all ions transported through epithelium. In our laboratory we built and successfully tested the multielectrode biosensor system which can measure transport of all the ions.
The 16HBE-14σ cell line was used in experiments. It forms tight junctions and is characterized by high transepithelial electrical resistance (TER). Cells were seeded onto Costar Snapwell inserts (0.45 μm pore size, 1 cm^2 surface area) and grown submerged in culture medium until the TER of cell monolayer was about 1000 Ω. To polarize the cells, the medium from the upper side of the insert was taken off to maintain the air contact at the apical side of monolayer and started the experiments, when TER decreased to about 400 Ω.
To better understand the mechanism of electrolyte transport in epithelial lung cells, the sodium and chloride ions transport was measured at first. Our potentiometric electrode system was equipped with two chloride, two sodium and the reference electrode on each side of cell monolayer. In the first experiment, the sodium gradient across the cell layer was provided (the low sodium fluid on apical side and high on the basolateral side). When the solution flow was stopped, sodium ions were transported across the monolayer from basolateral to apical side. Sodium transport was not changed after introduction of 10μM amiloride (sodium channel blocker).
In the second experiment the chloride ions gradient was provided (low chloride on apical side, high chloride concentration on basolateral side). The electroneutral transport of chloride ions from basolateral to apical side was observed. Then the solution was supplemented with 50 μM of glybenclamide (CFTR channel blocker) and 50 μM of DIDS (other apical chloride channels blocker). No chloride transport was observed but the transepithelial potential reached -25 mV. Thus in polarized epithelium of 16HBE-4σ cell line, sodium is transported via paracellular route while chloride by transcellular one – which is defective in CF.

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