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Plenary lectures

1. Is full cis-trans chromophore isomerization required for biological activity of rhodopsin-like systems?

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Rhodopsins (complexes of retinal and opsin) belong to two distinct protein families. Visual rhodopsins (RH) are photosensory pigments (retinal in 11-cis configuration), which start visual cascade. Archaeal rhodopsins, bacteriorhodopsin (bR) and halorhodopsin (hR) function as light-driven ion pumps (retinal in all-trans configuration): bR pumps protons, hR pumps chloride ions. Proton gradient is utilized for ATP production in the cell. Light absorption by rhodopsins triggers their characteristic photoconversion in which cis-trans (in RH) or trans-cis (in bR and hR) isomerization of the chromophore is believed to be a primary step in this reaction. The problem whether full cis-trans isomerization is a prerequisite for full biological activity of rhodopsins is still open. To clarify this problem the experiments with rhodopsin analogues were performed. RH from octopus Paroctopus defleini, bR and hR from Halobacterium salinarum S9 and L-33, respectively, were regenerated with retinal analogues: eight-, seven-, six-, five-membered ring retinal, displaying gradually limited rotation around characteristic double bonds: C_{11}=C_{12} (RH) and C_{13}=C_{14} (bR and hR). Flash photolysis and photoelectric data indicate that gradual confinement of the chromophore double bond rotation limits rhodopsins photoreaction. Experiments with pH sensitive and potentiometric dyes show that even full blocking of double bonds rotation (with five-membered ring) does not lead to termination the rhodopsins activity: bR displays limited proton pumping, hR still pumps chloride ions and RH generates small membrane potential. However, experiments performed in model systems (bR and ATPase in liposomes and RH with transducin) demonstrate that limited activity of rhodopsins is too low to activate ATPase or visual cascade. These results demonstrate that: an appearance of early intermediates is dependent on the flexibility of the chromophore, limited rhodopsins activity can be achieved with blocked trans-cis isomerization of the chromophore, and full chromophore cis-trans isomerization is required for full biological activity of rhodopsins.

2. Spectral properties of dyes-potential sensitizers in photodynamic therapy and diagnosis

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The dye which can be applied as sensitizer in photodynamic therapy (PDT) or photodynamic diagnosis (PDD) of cancer has to fulfill the following conditions: has to be more efficiently incorporated into cancer than into healthy cells, has to be expelled possibly quickly from healthy cells and as a result of illumination the stained cancerous cells must be destroyed (in PDT treatment) or be strongly fluorescent in the cells (for PDD applications). Even chemically very similar dyes could exhibit in cells very different interactions with the surroundings and therefore can be differently suitable for medical applications. The photodynamic reactions occur frequently with the participation of very photochemically active triplet states. Therefore the knowledge of the efficiency of triplet state generation is useful for the selection of sensitizers for PDT. This efficiency can be established by Laser Induced Optoacoustic Spectroscopy (LIOAS) (Braslavsky & Heibel, 1992, *Chem. Rev.,* **92**, 1381). The yield of dye fluorescence helps us select proper candidates for PDD. Because of an important role of singlet oxygen (1O2) in photodynamic reactions the sample must be studied in oxygen atmosphere and bubbled by nitrogen. The absorption of dye-sensitizer has to be located in a region of low absorption of the cell material. For these reasons the knowledge of absorption, fluorescence and photothermal properties of dye-sensitizer is necessary. The blood cells obtained from patient have to be predominantly used for medical purposes therefore, the initial selection of dyes can be made using artificial model system which mimics to the some extend the cell properties. In interpretation of the spectral results obtained on the basis of such simple models one has to remember that the efficiency of the triplet generation as well as the yield of fluorescence in models and in cells could be different, but for the set of dyes the sequence of the values of these parameters in models and in cells are usually the same. As model systems the solutions of dyes, the dye-polymer solutions or dye in polymer films were used. The dye strongly interacting with polymer chains usually interact strongly with lipids and proteins, therefore it is located in the cell membrane rather than in the inner material of the cells. The dyes with low values of these parameters observed in models can be excluded. After such preliminary

selection the promising candidates have to be investigated in cells. The resting lymphocytes were obtained from blood of a healthy donor, the stimulated lymphocytes in such cells were changed by chemical reaction in a such a way that they are similar to pathologically changed cells. The cancerous cells from continuous culture line of cells were also investigated. The location of sensitizers in the cells was established by means of fluorescence microscopy.

The dyes recently intensively investigated by us in models and in the cells are stilbazolium merocyanines (Gruda *et al.*, 1987, *Anticancer Res.* **7**, 1125; Gruda & Bolduc, 1984, *J. Org. Chem.* **49**, 3300; Staśkowiak, *et al.*, 2004; *J. Photochem. Photobiol. A: Chem*. **163**, 127; Staśkowiak *et al.*, 2004, *J. Photochem. Photobiol. A: Chem*., in press). Some of them (for example Mero B (1-(1,1'-hydroxyhexyl)-4-[(4-oxocyclohexa-2,5-dienylidene)etylidene]-1,4-dihydropyridyne)) exhibit strong fluorescence therefore can be used in PDD. This compound has efficient triplet generation, but it is easily photo-destroyed in the cell in such a short time that the dose of light delivered before the dye degradation is not high enough for the photodynamic action. Therefore, it is not suitable for PDT even having efficient generation of triplet. Lower yield of fluorescence, but also high efficiency of the triplet generation is shown by Mero T* (1-(11'-hydroxyundecyl)-4-[(3-dimetoxy-4-oxocyklohexa-2,5-dienylidene)ethylidene]-1,4-dihydropyridyne salt HCl). The introduction of this dye the into cells and their illumination causes efficient destruction of cancerous cell material. From the investigation of cancerous cells obtained from various cell lines (Wiktorowicz *et al.*, 2004, *Acta Biochim. Pol.*, in press) it follows that the same dye introduced into cells from various donors causes different photodynamic effects. It seems that the optimal sensitizer for a given patient has to be chosen before the PDT treatment.

From our results the following conclusions can be drawn: (i) different dyes, even chemically very similar, in human cells exhibit different spectral properties, such as the yield of triplet state generation and the ability to trigger the PDT reactions; (ii) properties of dyes in simple model systems and in human cells are usually different, but some conclusions concerning possibility of a given dye application in PDT or PDD can be drawn; (iii) to be able to compare of various sensitizers they have to be studied in the cells obtained from the same donor; (iv)before PDT treatment the „behavior" of a sensitizer proposed for a given patient should be established, in such a way the optimal sensitizer for the person can be selected; (v) the dyes exhibiting in the cells, not only efficient triplet state generation, but also high photostability are promising sensitizers for PDT, therefore such dyes can be selected on the basis of LIOAS, absorption and fluorescence spectra.

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3. Ion Cyclotron Resonance (ICR) and Ion Parametric Resonance (IPR) in magnetotherapy — hopes or illusions

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There has been considerable concern and controversy over the last forty years concerning the health risks associated with growing exposure of humans to extremely low frequency electromagnetic fields (ELF-EF).

Positive and negative effects of electromagnetic fields on living organisms relate to field characteristics and exposure conditions. Effective magnetotherapy (magnetostimulation) is contingent upon finding such field parameters and exposure times that offer positive effects in a wide range of pathologic conditions at the same time limiting any negative action. The achievement of this goal depends on the understanding of interactions of EF with living organisms. This work discusses the efforts of researchers which culminated with the hypothesis (useful but controversial) of ion cyclotron resonance (ICR) developed further as ion parametric resonance (IPR). This history dates back to the 70's and 80's when several reports (chiefly by Blackmann and coworkers) demonstrated that exposure to radio-frequency electromagnetic radiation (low frequency amplitude modulated) of chick brain resulted in the release of calcium ions (Ca^{++}). Seemingly of little importance at first, this finding led to intense research, discussions and continuing controversies. Finally, a new mechanism (ICR) of interaction of weak magnetic fields with living organisms was described (Liboff). Ion release takes place when two parallel magnetic fields: constant B and low frequency alternating ω, such that $\omega = qB/m$ (q – ion charge, m – ion mass) are applied. This phenomenon is known in physics as the cyclotron magnetic resonance. Authors of the hypothesis and most of their followers have advanced the ICR mechanism to explain the release of calcium ions from brain tissue (Blackmann) and diatoms (Smith), the healing of fractured bones, and changes in calcium ion flux through membranes of human lymph cells (Liboff). The ICR hypothesis was criticized by Stewart and other researchers who view cyclotron resonance as a misnomer possible exclusively in vacuum and not in tissues. Moreover, the calculated orbital radius of calcium ions under the experimental conditions of Blackmann would be equal to 1.1 m which is unrealistic. Some authors (Durney, Hendee, Wang, Prasad) could not confirm any resonance effect in different experimental settings. Of particular concern is the failure by Prasad to reproduce the resonance effects under conditions exactly the same as described by Liboff for the transport of $^{45}Ca^{++}$ ions through lymphocyte membranes. To explain some special requirements for the resonance effect to take place (dependence on resonance frequency harmonics and amplitude of the alternating field), Lednev proposed another hypothesis called the ion parametric resonance (IPR). An ion inside a Ca^{++}- binding proteins (such as calmodulin) approximated by a charged oscillator. The breaking and forma-

tion of coordination bonds between the calcium ion and chelating groups on the calcium-binding protein corresponds to transitions between specific energy levels of the ion-protein complex. An applied constant magnetic field causes the energy difference between the bound and anbound states to split into two sublevels (vibrational). A shift in the probability of ion transition between differential energy levels (first excited states to the ground state) occurs when a combination of static and alternating magnetic fields is equal to the cyclotron frequency of this ion or to some its harmonics. This implies that the applied magnetic fields might alter the equilibrium dissociation constant between calcium and corresponding calcium-binding protein. Observatin of Lednev-like resonance has been reported in mitogen-activated limphocytes (Yost), neurite outgrowth experiment (Blackmann) and recently for the transport Ca^{++} through purified plasma membrane vesicles (Koch). Numerous experiments have failed to find resonance responses and Lednev mechanism has been criticized (Adair, Hendee, Prasad).

The selection of current pulses (magnetic field generators) used in instruments for magnetotherapy or magnetostimulation is usually done with the trial and error approach without knowing the mechanisms involved. The Viofor JPS instrument represents a novel approach to this problem based on a (correctly) defined pulse pattern used to generate the magnetic field aimed at achieving the maximum number of harmonic components. Depending on the ICR or IPR option, the instrument offers resonances for various ions. Nevertheless, dispute continues whether ICR or IPR has any relevance to the effects of magnetotherapy. Debate has recently heated up after one of the fathers of the ICR hypothesis and author of numerous publications in this field (Liburdy) was accused of falsifying some experimental results. Much of the controversy, as well as discrepancies and conflicting results may be attributed to the interdisciplinary nature of magnetotherapy (physics, biology, medicine), equivocal results of experiments, and methodological errors. The number of publications in magnetobiology today, their quality, and growing commitment of researchers give hope that the problems discussed here will soon lose their controversial nature, preventive measures will be developed, and clinical use of magnetic fields will extend.

4. Antibiotic amphotericin B in lipid membranes

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Amphotericin B (AmB) is a polyene antibiotic applied in treatment of deep seated mycotic infections. Amphiphilic nature of AmB is responsible for both binding of the drug to lipid membranes and formation of specific molecular structures, including pores. The analysis of electronic absorption spectra and fluorescence excitation and emission spectra of AmB incorporated to liposomes reveals formation of various molecular structures of the drug. The results of the experiments carried out with the application of polarized light indicate both horizontal and vertical orientation of AmB with respect to the plane of lipid membranes. The possible effects of these two pools of AmB on structural properties of lipid membranes will be discussed based on the results of monomolecular layer technique experiments and FTIR analysis of lipid multibilayers modified with AmB.

5. The role of lipid phase state in the interaction of isoflavones and phenothiazines with model membranes

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The existence of liquid-ordered microdomains in liquid-disordered biological membranes is now well established. The basic molecular mechanism of raft formation is a spontaneous segregation of certain kinds of membrane constituents (lipids and proteins) caused by their preferential interactions and following it spatial separation of less- and more-ordered membrane components. These microdomains are known as lipid rafts and are supposed to play an essential role in many cellular processes e.g. intracellular transport, exocytosis and signalling. Due to the presence of multidrug transporter proteins like P-glycoprotein in lipid rafts these microdomains can be involved also in multidrug resistance (MDR) processes.

Reversal or modulation of multidrug resistance involves direct interactions of chemosensitizers with transporter proteins as well as alteration of protein activity induced by changes induced in surrounding lipid matrix. Several lines of evidence confirm that many of multidrug resistance modulators interact with lipid bilayers and alter their biophysical properties. Phenothiazine derivatives and flavonoids studied previously in our lab belong to the group of membrane–active compounds that are able to interfere with the drug resistance processes. Since MDR and its reversal may involve lipid rafts it seemed interesting to investigate the role of the phase state of lipid bilayer in the interaction between potential MDR modulators and model membranes.

The interaction of phenothiazine derivatives with lipid bilayers was studied using commercially available thioridazine (TDZ) and newly synthesised 2–trifluoromethyl–10–(4–[methylsulfonylamid]buthyl)–phenothiazine (4FPhMS). Alteration of bilayer fluidity upon the influence of phenothiazines was assessed by means of DPH fluorescence polarisation measurements. We found 4FPhMS to increase the fluidity of DPPC bilayer in gel state and to decrease it in liquid-crystalline state. Both effects were dose–dependent – their magnitude increased when 4FPhMS concentration was in-

creased. Thioridazine was tested against four different lipids: EYPC, DPPC, DMPC and DMPG. TDZ like 4FPhMS altered the properties of the gel phase of lipid bilayers —we recorded the rigidifying effect exerted by this drug.

As a representing group of isofolavones four compounds were studied: daidzein, formononetin, genistein and biochanin A. These flavones are metabolitically related since due to the naturally occurring process of O-demethylation formononetin is turned to daidzein and biochanin A to genistein. We found, however, that demethylation process does not alter the overall character of the influence of these isoflavones on the lipid bilayer properties. Formononetin and daidzein increased the fluidity of the lipid bilayers in gel state, while biochanin A and genistein decreased fluidity of liquid–crystalline bilayers. The DPPC main phase transition parameters were weaker altered by formononetin and daidzein then by biochanin A and genistein.

Different effects exerted by studied compounds on lipid bilayer in gel and liquid–crystalline state may result from the different location of these molecules in membranes. Generally flavones are supposed to penetrate lipid bilayers deeper than phenothiazine derivatives. In case of isoflavones number and positions of hydroxyl groups attached to the isoflavone core may also play an important role. In phenothiazines the character of the side group located at the end of short hydrocarbon chain may be as important as the type of group substituting drug molecule in the position 2 of the ring system.

6. On the applicability of the Kedem-Katchalsky and mechanistic transport equations

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The transport of non-electrolytic substances across biological membranes (as well as across artificial membranes) may be conveniently described by means of the Kedem-Katchalsky (KK) practical equations, i.e. Eqs.:

$$J_v = L_p \Delta \mathrm{P} - L_p \sigma \Delta \Pi \,, \tag{1}$$

$$j_s = L_p (1-\sigma) \overline{c}_s J_v + \omega \Delta \Pi \,, \tag{2}$$

where J_v and j_s are the volume flow and solute flow; L_p, σ and ω are the coefficients of filtration, reflection and diffusive permeability; ΔP and $\Delta\Pi$ are the mechanical and osmotic pressure differences; and $\overline{c}$ is the mean concentration. The convenience results, primarily, from the formulation of the flows J_v and j_s as a function of the two most basic stimuli, i.e. ΔP and $\Delta\Pi$.

These equations were derived on the basis of linear thermodynamics of irreversible processes. Their applicability is, as assumed, limited to membranes which are homogeneous in terms of transport properties. Moreover, in the KK formalism, one does not differentiate between the microscopic structures of the membranes, while in research practice, one deals mostly with porous membranes. As regards transport properties, it is rational to divide membranes into homogenous and heterogeneous. At this juncture, it must be explained that a porous membrane may be considered homogeneous if its pores do not vary in their linear dimensions (cross-section radiuses). A membrane, in turn, whose pores vary in their dimensions ought to be treated as heterogeneous in terms of transport properties. Considering this division of membranes, it becomes easily noticeable that the KK equations apply to homogeneous porous membranes. With respect to these membranes, their individual terms describe filtration ($L_p \Delta P$), osmosis ($\sigma\Delta\Pi$), solute diffusion ($\omega\Delta\Pi$) and convection ($(1-\sigma)\overline{c}J_v$), respectively. It must be stressed that the parameter ω, defined by the formula

$$\omega = \left(\frac{j_s}{\Delta \Pi}\right)_{J_v = 0} \tag{3}$$

is the coefficient of diffusive solute permeability. This follows from the fact that, at J_v=0 (which is the case when $\Delta P = \left| -\sigma \Delta \Pi \right|$), there are no volume flows in individual pores of a given membrane.

This situation is understandable (with the exception of Staverman's definition $(\sigma = -L_{pD} / L_p)$, wherein the parameter ($-L_{pD}$), as a negative value, does not carry any physical sense).

Unfortunately, the KK equation for the flow j_s j_s does not apply to heterogeneous porous membranes. If this equation is made to refer to these membranes, we note that, at $\Delta \mathrm{P} = \left| -\sigma \Delta \Pi \right|$, the volume flow also assumes a negative value (J_v = 0). Yet, locally (in individual pores), there will exist volume flows, varying in value and direction. Hence, it follows that the parameter ω given by the formula (3) is not a coefficient of diffusive transport of the solute only. What is more, the term $\omega\Delta\Pi$ will not formulate only the solute diffusive transport, and the term $(1-\sigma)\overline{c}J_v$ - only the convective solute transport.

In an attempt to face this problem, we have recently developed a mechanistic formalism of membrane substance transport, as an alternative to the KK formalism (Kargol and Kargol (2000, *J.Biol.Phys.* **26**, 307; 2001, *J.Membrane Sci.* **191**, 61; 2003, *Gen. Physiol. Biophys.* **22**, 51; 2003, *Biophys. Chem.* **103**, 117).

The construction of this formalism has been based on a heterogeneous porous membrane with the statistical number N pores permeable to the solvent. In order to facilitate our considerations, we have assumed – as a model — that the pores of the given membrane are arranged in one direction, starting with the smallest pores (of cross-section radiuses $r_1^{\min}$), and ending with the largest pores $r_N^{\max}$.

With regard to such a membrane, it is possible to find such a solute (s) of the molecule radius r_s that the following relation will be satisfied:

$$r_1^{\min} = r_w < r_2 ... < r_s < ... < r_N^{\max} ,$$

where r_w is the radius of the solvent (water) molecules.

In this case, the membrane may be divided into Part (a) which contains a certain number $n_a$of semipermeable pores (its reflection coefficient $\sigma_a = 1$) and Part (b) which contains $n_b = N\ n_a$ permeable pores (reflection coefficient $\sigma_b = 0$).

If, on the membrane, there appears a mechanical pressure difference ΔP, as well as an osmotic pressure difference $\Delta\Pi_S$, then across its Parts (a) and (b) volume flows J_{va} and J_{vb} given by the following equations, shall permeate:

$$J_{va} = J_{vwa} = L_{pa}\Delta\mathrm{P} - L_{pa}\Delta\Pi , \quad (4)$$

$$J_{vb} = L_{pb}\Delta\mathrm{P}, \quad (5)$$

where P_{pa} and P_{pb} are filtration coefficients of the pores n_a and n_b.

Starting from these equations, we arrive at the following practical transport equations of mechanistic formalism for the volume flow J_{vM} and the solute flow j_{sM}:

$$J_{vM} = L_p\Delta\mathrm{P} - L_p\sigma\Delta\Pi_s , \quad (6)$$

$$j_{sM} = \omega_d\Delta\Pi + (1-\sigma)\bar{c}L_p\Delta\mathrm{P} = (1-\sigma)\bar{c}L_p(\Delta\Pi_s + \Delta\mathrm{P}) , \quad (7)$$

where L_p is the filtration coefficient; σ is the reflection coefficient; ω_d is the coefficient of solute diffusive permeability.

Respective parameters of these equations are given by the formulas:

$$L_p = L_{pa} + L_{pb} , \quad (8)$$

$$\sigma = \frac{L_{pa}}{L_p} , \quad (9)$$

$$\omega_d = L_{pbs}\bar{c} \approx (1-\sigma)\bar{c}L_p , \quad (9)$$

where L_{pbs} is the diffusive solute conductance of the pores n_b (Part (b) of the membrane).

Eqs. (6) and (7) apply both to the homogeneous and heterogeneous porous membranes. Moreover, they offer a wholly unambiguous interpretation.

7. A new antioxidant? — defence of red blood cells

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After exposition to NIR membrane fluidity decreases, polarity decreases in the vicinity of polar heads, rate of hemolysis is dropped compare to the control value, zeta potential, measured electrophoretically, was changed. Lower values of electrophoretic mobility for irradiated erythrocytes suggest changes in volume charge density.

Agglutination process was studied by observation of antibody — antigen reaction for blood group tests in media with different antibody concentration. Observation of agglutination process indicates that NIR radiation affects antibody — antigen reaction. NIR changes surface structure of the erythrocytes. After irradiation the number of sites bonding antibodies decreases.

For NIR exposed erythrocytes osmotic fragility does not change significantly, but the shape of static hemolysis curve does. This demonstrates the different distribution of osmotic fragility for erythrocyte population than that for control samples what illustrates unification of osmotic properties of irradiated erythrocyte population. Lower rate of hemolysis and higher swelling time for NIR modyficated erythrocytes suggest improved elastic properties of RBC membranes.

Further studies show the protective action of NIR radiation before oxidative stresses. The dose of protective radiation depends on the intensity of the oxidative stress however do not exceed to the values when NIR radiation irreversibly changes the membrane structure.

We found also that the concentration of deoxyhemoglobin increased about 30% after exposition red cells to near infrared radiation (NIR). Raman and fluorescence spectra show that low oxygen affinity and increased resistance to autoxidation are due to dehydration process. The increasing hydrophobicity after exposition to NIR stabilizes the low affinity T structure for species: deoxy and met — hemoglobins. Additionally in the reduction reactions the heme iron of methemoglobin is more readily reduced to oxyhemoglobin when in the tense conformation (T state) relative to the R relaxed conformation.

From this findings it follows that NIR radiation could play a key role in the stabilisation of the aging processes in erythrocytes.

8. FCS — The Fluorescence Correlation Spectroscopy

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The rapid development of nano-sized structures and their emerging applications require the development of experimental techniques that will enable not only their effective research but also the evaluation of large samples in industrial setups. A number of research techniques are used to study the structure and properties of supramolecular aggregates. Most of them, however, due to complex protocols, are difficult to adopt for the large scale applications. A large scale method should provide data regarding single aggregates in statistical significant numbers. Fluorescence Correlation Spectroscopy (FCS) is one such technique. It is based on the measurement of the time a particle needs to pass the detection volume (confocal volume). A subsequent appropriate statistical analysis enables the particle properties and their statistical distributions to be determined. The capabilities of

the method are illustrated on the example of the conformational state of nucleic acid molecules. The DNA molecule is capable of adopting various conformations, depending on physico-chemical conditions and the presence of cationic, amphiphilic and hydrophobic compounds. DNA conformation is an important parameter when supramolecular aggregates are designed for gene therapy. In this case, the complete characterization of the sample from the point of view of its uniformity as well as selected properties is an important technological issue. In order to develop a reliable methodology for such applications, the FCS technique needs to be calibrated and tested for selected formulations. Since the method requires fluorescent labeling, the effect of dye association on DNA molecule conformation was determined. This experiments shows that the dye should be carefully selected. In our hands, only Pico-Green labels the nucleic acid without altering its conformation. The effect of other compounds on DNA conformation was evaluated. A variety of cationic and amphiphilic compounds commonly used for the construction of transfection formulations were used. We have shown that FCS technique is a sensitive method to monitor the DNA condensation process induced by different agents. We have been able, using very low quantities of chemicals in simple and fast experiments, to determine the minimum concentration of certain compounds needed to complete the condensation process. The information available from FCS experiments is qualitatively different from that of other experiments, hence they provide particle distributions, a parameter crucial for any commercial application in which uniformity of the sample is required.

9. Modulation of GABAergic synaptic transmission in the central nervous system

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The fast signal transduction between neurons in the central nervous system (CNS) is mediated by synaptic transmission. Signal integration is an extremely complex process and a single neuron may receive up to several thousands synaptic inputs. Basically, synapses can be divided into excitatory and inhibitory ones depending on whether the transmission results in an increase or decrease of the membrane voltage. Thus, the signal integration in neurons can be regarded as a balance between excitation and inhibition. When, the excitatory drive temporarily "prevails", the membrane voltage may reach the excitation threshold and the action potentials are generated. The kinetic properties of synaptic currents play thus a crucial role in determining the excitation/inhibition balance in neuronal signal transduction. In the adult CNS, the excitatory fast synaptic transmission is mediated by glutamate while the major role in synaptic inhibition is played by GABA. The properties of synapses can be strongly modulated by several endogenous and exogenous factors. In the present report, an outline of our recent work aiming to describe the mechanisms of modulation of GABAergic synaptic transmission by selected endogenous (pH, zinc ions) and exogenous (chlorpromazine) factors is presented.

The synaptic currents can be routinely recorded in the whole-cell configuration of the patch-clamp technique. In these conditions, the recording electrode is measuring the synaptic currents flowing at any of postsynaptic sites of the neuron. Synaptic current recordings in control conditions and in the presence of selected drug concentration provide important information on drug action on synaptic events. However, it needs to be borne in mind that recordings of synaptic currents are usually insufficient for a thorough investigation of the underlying mechanisms. The reason for this is that the synaptic currents represent a current response of receptors to the agonist application of an *a priori* unknown peak concentration and time duration. Thus, a standard approach based on analysis of dose-response relationships is not available. Dose-response analysis requires thus is recordings of current responses to exogenous agonist application. There is, however, an important prerequisite that needs to be met to make the information extracted from dose-response recordings relevant to the synaptic transmission. Namely, the appearance of the neurotransmitter in the synaptic cleft is very short (at most hundreds of microseconds) and therefore the speed of agonist application must be very fast to mimic the conditions of synaptic application. The ultrafast perfusion system, able to exchange the solutions within less than 100 μs has been introduced in the mid nineties (Jonas et al., 1995, [in:] Sakmann B., Neher E. (eds.), *Single-channel recording*. Plenum Press, New York and London, p.231). The basic strategy, that turned out to be very successful in pharmacological studies, is to monitor in parallel the effect of considered drugs on synaptic currents and to construct the respective dose-response relationships for current responses elicited by ultrafast agonist applications. Implementation of this approach allowed to describe the effect of a number of drugs on $GABA_A$ receptors in terms of the modulation of the receptor gating.

It has been long recognized that changes in pH modulate the $GABA_A$ receptors but the mechanism of this effect was not clear. In a recent study (Mozrzymas *et al.*, 2003, *J. Neurosci.* **23**, 7981), synaptic currents and current responses to rapid GABA applications were recorded at pH varying from 5.0 to 8.0. It was found that weakly acidic pH (around 6.5) caused an enhancement of the synaptic currents (with respect to pH=7.2) while stronger acidification resulted in current inhibition. The underlying mechanism was studied by analyzing the current responses to rapid applications of various GABA concentrations. Quantitative analysis of these data provided evidence that the major effect of pH change is to affect the binding rate of GABA (increase in pH enhances the binding rate) and the desensitization process (increase in pH increases the rate and extent of desensitization).

Zinc ions are abundantly present in the CNS (especially in hippocampus) and it is known that these divalent cations inhibit $GABA_A$Rs. However, the underlying

mechanism of this effect has not been studied in details. Combination of synaptic current recordings and measurements of the current responses to rapid GABA applications allowed to conclude that zinc ions decrease the receptor affinity as well as decrease the rate and increase the extent of desensitization (Barberis *et al*. 2000, *J. Neurosci.* **20**, 8618). In addition the transition from closed bound to open bound state is slowed down by the zinc ions.

Chlorpromazine (CPZ) is a phenothiazine widely used in treatment of psychiatric disorders. However, this drug is prone to give rise to several side effects including epileptic-like symptoms. Since one of the mechanisms of epilepsy is a disfunction of the inhibitory synaptic transmission, it seemed interesting to study the effect of this drug on GABAergic synaptic transmission. It was found that CPZ reduced the GABAergic synaptic currents in a dose-dependent manner. Basing on the analysis of current responses to rapid GABA applications it was concluded that CPZ reduced the binding rate and increased the unbinding rate of the agonist (Mozrzymas et al., 1999, *J. Neurosci.* **19**, 2474).

Studies on the mechanisms underlying $GABA_A$ receptor modulation by pH and CPZ allowed to estimate the time course of the agonist transient during the synaptic transmission. It was found that the clearance of the neurotransmitter in very fast (ca. 100 μs) and the averaged peak current is ca. 1.5 mM GABA that does not suffice to reach saturation.

In conclusion, the extreme non-equilibrium conditions of postsynaptic receptor activation, resulting from a very brief presence of agonist in the synaptic cleft, is a critical factor in shaping the kinetics and susceptibility of synaptic currents to pharmacological modulation. The combination of standard synaptic current recordings and the analysis of current responses to ultrafast agonist applications offer a unique possibility to reliably explore the receptor gating and its modulation at the time scale of synaptic currents.

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10. Digital signal processing in the uterine contractions analysis

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The history of investigating the mechanical activity of the uterus by recording the intrauterine pressure goes back to the end of last century. Analysis of the uterine contractility in the non-pregnant states has provided information about physiological changes during the menstrual cycle (Bulleti *et al.*, 2002, *Fertil Steril.* **77**, 1156). There is need to develop methods of recording uterine activity as well as mathematical interpretation of recorded time series (Challis & Kitney, 1990, *Med. & Biol Eng & Comput.* **28**, 509). The uterine activity signals are often very noisy and there are problems with determining the contractions correctly. The use of digital signal processing method in obstetrics and gynaecology requires strict cooperation between a clinician and a biophysicist.

The aim of this paper is to review of the digital signal processing methods in the uterine contractility studies.

Spontaneous uterine activity was recorded directly by a dual micro-tip catheter (Millar Instruments, Inc. USA). The device consisted of two ultra-miniature pressure sensors. The sensors produced electrical signals, which varied in direct proportion to the magnitude of measured pressure. One sensor was placed in the fundus (sensor X), the other one in the cervix (sensor Y). After amplification, analog signals were passed to a PC computer for conversion to digital form by means of an analog-to-digital converter. Converted signals were recorded on a computer hard disk. Fig. 1 shows the scheme of the digital processing system for investigation of uterine contractions.

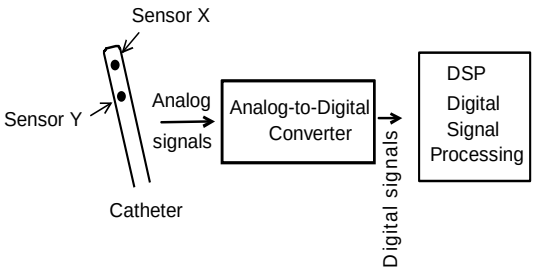

Fig. 1. The scheme of the signal processing system. The distance between sensors is 30 mm.

For illustrations three patients: were selected: a healthy patient A (with normal contractions during the first day of menstruation), a patient B with dysmenorrhea (with contractions during the first day of menstruation) and a patient C with fibromyomas in the follicular phase were selected. The study was approved by the regional ethics committee.

For analysis of the recorded signals we used programs written in MATLAB (MathWorks, Inc., USA) a high-performance language for technical computing. Very helpful appeared toolboxes for use with MATLAB – Signal Processing Toolbox and Wavelet Toolbox.

Intrauterine pressure signals may be analyzed in the time domain or in the frequency domain. Parameters in the time domain such as area under curve recording (AUC), maximal amplitude of contractions and various statistical quantities (mean, standard deviation, median, skewness and so on) are easily computed even for short time windows. In frequency domain signal is decomposed by means of spectral analysis into its sinusoidal components. We have for each frequency f the amplitude A and phase φ. We can plot the power of each component as a function of frequency. Power spectral analysis may be performed by means of fast Fourier transform or autoregressive modeling (Szamatowicz et

al., 1997, *Acta Obstet Gyn Scand*. **76**, 973; Muthuswamy & Thakor, 1998, *J. Neurosci. Methods*. **83**,1).

Contrary to the Fourier decomposition, which is global and provides the information integrated over the whole signal, the continuous and discrete wavelet transforms allow extracting local and global variations of the recorded contractions (Oczeretko et al., 2003, *Annales Academiae Medicae Bialostocensis* **48**, 135; Eswaran et al, 2002, *J. Matern. Fetal Neonatal Med.* **11**, 158).

From the medical diagnostics point of view it is very important to identify time delays between contractions in two signals. Time delays may be positive and negative. If the signal from the uterine fundus is taken as a reference signal and if the signal from the uterine os is taken as the signal for evaluation then various patterns of propagation of contractions can be detected:

- normal propagation – the uterine fundus contracts before the uterine os – positive time delays,
- inverted propagation – the uterine fundus contracts after the uterine os – negative time delays,
- simultaneous propagation - the uterine fundus and the uterine os contract simultaneously – time delay = 0.

Cross-correlation function is sensitive to the direction of lag and may be used to identify time delays between the contractions in two time series (Steinmeier et al., 2002, *Crit Care Med.* **30**, 1969). In the case of patient A, positive time delays can be observed. For the patient with dysmenorrhea and for the patient with fibromyomas the patterns of propagation are very complex. In the case of dysmenorrheic signals there are longer time intervals of synchronization (positive time-shifts). Signals of the patient with fibromyomas have disturbed synchronization.

In recent years the physiological signals obtained from the brain and the heart have been investigated for possible deterministic chaotic behavior. The human uterus is undoubtedly a complex system. We used the techniques of surrogate data analysis to testing for nonlinearity in the uterine contraction signals. The approximate entropy was the test statistics. The results showed that the spontaneous uterine contractions are considered to contain nonlinear features (Oczeretko et al., 2004, *Riv. Biol.-Biol. Forum*, **97**, 157).

Digital signal processing is based on the development of mathematics and computer technology. The modern hospital can offer a wide range of physiological measurements (ECG, EEG, electromyography and many others). Digital signal processing methods play an important role not only in medical diagnostics but also as the basis of clinical research studies. Digital signal processing algorithms help us to a better understand of uterine contractility.

11. Monte Carlo simulation of cooperative phenomena in lipid membranes modified by carotenoids

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The aggregation of a carotenoid in lipid membrane is investigated. The monomeric form of the carotenoid in cell membrane promotes the protection of the lipid phase against oxidative damage. The carotenoid in aggregated form absorbs the short wavelength radiation more, which is the important physiological function of e.g. lutein in retina membranes. In the present work, intermolecular interactions affecting aggregation of carotenoids in lipid membranes are studied. The method applied is a comparison of properties of the computer model lipid bilayer taken to the equilibrium by the Monte Carlo process with the experimental results cited in literature. The model allows the change of orientation of a carotenoid molecule from parallel to the membrane surface to the perpendicular one. Computer simulations show that the fraction of a corotenoid in the aggregated form depends on: (1) possible orientations of carotenoid molecule with respect to the membrane surface; (2) the region of the membrane in which the carotenoid fraction parallel or nearly parallel to the membrane surface is distributed, when different orientations are possible; (3) cluster structure of a lipid membrane especially near the main phase transition temperature; the cluster borders acts as a “sink for impurities” and make aggregation of carotenoid molecules easier.

The method applied allows taking into consideration the ensemble of membrane molecules great enough to observe the main phase transition.

12. Adaptive molecular mechanisms in the bacterial membrane: molecular dynamics simulation studies

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The lipid matrix of the cell membrane consists mainly of phospholipids with two asymmetric hydrocarbon chains, of which one is fully saturated in the γ position and the other is mono- or poly-unsaturated in the β position. The double bonds have predominantly the *cis* conformation. The most abundant phospholipid of the animal cell membrane is phosphatidylcholine (PC), whereas the bacterial membrane contains commonly phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) in the proportion 3:1. It has been observed that in bacteria under physiologically stressful conditions (e.g. elevated temperature, starvation, desiccation, organic solvents), lipid composition of the membrane alters. Adaptation to environmental changes may go via a conversion of the *cis* to *trans* conformation of double bonds in the β-chain

or via a change of the relative proportion between PE and PG, in favour of PG. Molecular dynamics simulation studies were carried out to reveal differences between lipid bilayers composed of *cis*- and *trans*-monounsaturated phospholipids as well as to explore, still unknown, physicochemical properties of the mixed PE-PG bilayer. Analyses of the computer models showed that the packing of chains in the *trans*-monounsaturated bilayer is tighter than in the *cis*-monounsaturated bilayer but is similar to that in the saturated bilayer. Despite that, the order and reorientational motion of hydrocarbon chains are similar in *cis*- and *trans*-monounsaturated bilayers, but translational diffusion of *cis*-monounsaturated phospholipids is slower than *trans*. These results, together with published experimental data, suggest that the *trans* conformation that appears as a result of environmentally induced *cis-trans* isomerisation of the double bond does not directly decrease membrane fluidity, as is still believed, but acts indirectly. In contrast to *cis*-unsaturated chains, *trans*-unsaturated chains interact favourably with rigid molecules like carotenoids or cholesterol. Due to such interactions, the structure of the lipid matrix of the membrane becomes more rigid and hydrophobic, thus less permeable for polar molecules and ions. In this way, the bacterial cell membrane integrity is better preserved and the cell can better tolerate stressful external conditions.

13. Interaction of selected surfactants with model membranes

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For a long time the ionic surfactants, especially amphiphilic quaternary ammonium salts showing biological activity, have been widely used in many fields of industry. Among others, they are used as disinfectants in pharmaceutical and cosmetic industries. That is why many papers are devoted to the interaction of the surfactants with biological and model membranes. The roles of counterions in these interactions are rarely discussed. The significance of counterions in the interactions of dodecyltrimethylammonium halides (DTAX) (Różycka-Roszak & Pruchnik, 2000, *Z. Naturforsch.* **55c**, 240; Różycka-Roszak & Pruchnik, 2000, *Z. Naturforsch.* **55c**, 753) and N-dodecyl-N,N -dimethyl-N-benzylammonium halides (DBeAX) (Różycka-Roszak & Przyczyna A., 2003, *Chem. Phys. Lipids* **123**, 209) with phosphatidylcholine (DPPC) bilayers, without or with addition of cholesterol (DPPC/chol), as well as 1-decyloxy-3-carbamoylpyridinium salts (PSX, where X = Cl^-, Br^-, I^-, NO_3^- , ClO_4^- and BF_4^-) (Różycka-Roszak, Przyczyna & Pernak, 2004, *Biophysical Chem.* **109**, 271) with DPPC, DPPC/Chol and phosphatidylethanolamine (DPPE) liposomes will be discussed.

The bilayer gel to bilayer liquid-crystalline phase transition of DPPC was more affected by DBeAX than DTAX. This was attributed to benzyl group. DBeAX compounds differ from DTAX with regard to replacement of a methyl group by a benzyl group. From ^{1}H NMR studies it follows that the benzyl group of DBeAX is inserted into the phospholipid bilayer (Różycka-Roszak & Przyczyna 2003, *Chem. Phys. Lipids* **123**, 209; Różycka-Roszak & Cierpicki, 1999, *J. Colloid Interface Sci.* **218**, 529) and the depth of the insertion depends on the kind of counterion. Due to the insertion of the benzyl group, DBeAX causes greater perturbation of phospholipid bilayer than DTAX. In the presence of cholesterol, opposite as it was in the case of DPPC bilayer, the benzyl group does not insert into gel phase of phospholipid bilayer. In the liquid -crystalline phase, benzyl group changes its position and locates into lipid bilayer.

The effects of PSX compounds on DPPC and DPPE thermotropic phase behaviour are in some ways quite different. All of them also decreased the transition enthalpy of DPPC bilayers, however they had a dual effect on the transition enthalpy of DPPE. Namely, at low concentrations the PS-X salts studied significantly increased the main transition enthalpy of DPPE (perchlorate and tetrafluoroborate the least among them), and decreased it at high concentrations. These differences may be partly related to significantly lower area per lipid in DPPE than DPPC bilayer, and consequently to a deeper insertion of PSX molecules into DPPC than DPPE bilayer.

The character of the interaction of all the surfactants studied with liposomes and the consequent changes in phospholipid organisation depend on the kind of counterion. Results are discussed in terms of counterion ability to modify water structure, counterion molecular geometry and the ability of PSX amide group to form hydrogen bonds with lipids.

14. Mitochondrialny kanał potasowy regulowany przez ATP

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Kanały jonowe to białka umożliwiające przepływ jonów przez błony biologiczne. Funkcja białek kanałowych błony plazmatycznej została w ostatnich latach stosunkowo dobrze poznana. Są one zaangażowane we wszystkich procesach decydujących o prawidłowym funkcjonowaniu komórek. Wiele substancji chemicznych stosowanych w medycynie specyficznie oddziałuje z kanałami jonowymi błony plazmatycznej. Nadal jednak dysponujemy ograniczoną ilością informacji o strukturze i funkcji kanałów jonowych obecnych w błonach wewnątrzkomórkowych. Wewnątrzkomórkowe błony biologiczne stanowią ponad 90% błon komórki i we wszystkich rodzajach błon wewnątrzkomórkowych wykryto kanały jonowe (Szewczyk, 1998). Dobrze poznane są jedynie dwa wewnątrzkomórkowe białka

kanałowe: poryna wystepująca w zewnętrznej błonie mitochondrialnej oraz kanał wapniowy obecny w endo/sarkoplazmatycznym retikulum (Szewczyk, 1998). Aktywność wielu innych kanałów jonowych obserwowano w błonach wewnątrzkomórkowych m.in. w pęcherzykach synaptycznych, błonie jądrowej oraz w błonach mitochondrialnych. Doniesienia opublikowane ostatnio wskazują na kluczową role mitochondrialnych kanałów jonowych w tak ważnych zjawiskach jak kardioprotekcja, egzocytoza czy transmisja synaptyczna.

Głównym obiektem naszych badań jest mitochondrialny kanał potasowy regulowany przez ATP (kanał mitoK$_{ATP}$). Stanowi on interesujący przykład udziału wewnątrzkomórkowego kanału jonowego w tak złożonym zjawisku jak kardioprotekcja (Szewczyk & Marban, 1999, *Molec. Membr. Biol.* **15**, 49). Kanał mitoK$_{ATP}$ został zidentyfikowany w 1991 roku w wewnętrznej błonie mitochondriów wątrobowych. Następnie kanał mitoK$_{ATP}$ opisano w mitochondriach serca i mózgu (Dębska *et al.*, 2001, *Brain Res.* **892**, 42). Badając właściwości mitoK$_{ATP}$ stwierdzono, że podobnie do kanału K$_{ATP}$ z błony plazmatycznej, kanał mitochondrialny jest hamowany przez pochodną sulfonomocznika stosowana w terapii cukrzycy typu II — glibenklamid. Następnie wykazano, że aktywatory kanałów potasowych oddziałują z kanałem mitoK$_{ATP}$.

Funkcja kanału mitoK$_{ATP}$ nie jest w pełni poznana. Kanał ten może katalizować napływ jonów potasowych do wnętrza mitochondriów i wspólnie z antyportem K^+/H^+ regulować objętość matriks mitochondrialnej. Kanał mitoK$_{ATP}$ może także powodować częściową depolaryzację wewnętrznej błony mitochondrialnej ułatwiając tworzenie gradientu jonów H^+. Obie te hipotezy znalazły częściowe potwierdzenie eksperymentalne. Stwierdzono, że niektóre aktywatory kanałów potasowych zwiększają szybkość pęcznienia izolowanych mitochondriów. Mierząc wartość ΔpH w mitochondriach obserwowano, że po podaniu aktywatora kanałów potasowych następowało zwiększenie ΔpH przy jednoczesnej częściowej depolaryzacji potencjału błonowego.

Kanał mitoK$_{ATP}$ ten jest hamowany przez ATP (prawdopodobnie działające od strony cytoplazmatycznej). Kanał jest również hamowany przez pochodne sulfonomocznika np. glibenklamid oraz przez kwas hydroksydekanowy. Należy jednak podkreślić, że w przeciwieństwie do receptora sulfonomoczników z błony plazmatycznej, białko wiążące pochodne sulfonomoczników w mitochondriach (mitoSUR) jest receptorem o niskim powinowactwie.

Próby oczyszczenia mitoK$_{ATP}$ nie doprowadziły do identyfikacji białka odpowiedzialnego za obserwowaną aktywność. Kanał mitoK$_{ATP}$ prawdopodobnie jest podobny do kanału potasowego o właściwościach prostowniczych - Kir6.1 - znanego z błony plazmatycznej.

Ostatnio opublikowano informacje wskazujące, że aktywator kanałów potasowych — diazoksyd ma właściwości kardioprotekcyjne. Zaobserwowano, że uszkodzenie mięśnia sercowego w procesie niedokrwienia/reperfuzji jest mniejsze, gdy obecny jest aktywator kanałów (diazoksyd) oddziałujący z kanałem mitoK$_{ATP}$. W latach 1999-2001 została opublikowana cała seria prac dotycząca udziału kanału mitoK$_{ATP}$ w osłonie mięśnia sercowego przed skutkami niedokrwienia. I tak, opisano oddziaływanie mitoK$_{ATP}$ z cytoszkieletem komórek mięśnia sercowego, opisano prawdopodobny udział mitoK$_{ATP}$ w kardioprotekcji indukowanej przez opioidy, wskazano na potencjalny udział kinazy białkowej C i mitoK$_{ATP}$ w zjawisku kardioprotekcji.

Od lat znane jest zjawisko „hartowania" serca (ang. ischemic preconditioning). Zjawisko to oznacza zwiększoną tolerancję krótko niedokrwionego, a następnie reperfundowanego mięśnia sercowego na skutki następnego, nawet długotrwałego niedokrwienia. W wyniku „hartowania" serca następuje znaczna redukcja strefy zawałowej w sercach poddanych temu procesowi w porównaniu z kontrolnymi sercami. I tak stwierdzono, że aktywatory kanałów potasowych np. diazoksyd chroni mięsień sercowy przed uszkodzeniem wynikającym z niedokrwienia w podobnym stopniu, jak proces „hartowania" serca. Ponieważ wcześniej wykazano, że diazoksyd specyficznie aktywuje mitoK$_{ATP}$ stąd wysnuto wniosek, że aktywacja mitoK$_{ATP}$ jest odpowiedzialna za działanie kardioprotekcyjne tej substancji. Nieznany jest natomiast mechanizm tego zjawiska: nie wiemy, dlaczego aktywacja transportu jonów potasowych w mitochondriach „osłania" mięsień sercowy przed skutkami niedokrwienia?

Przez wiele lat wewnątrzkomórkowe kanały jonowe stanowiły trudny i tajemniczy obiekt badawczy. Aktywność wewnątrzkomórkowych kanałów jonowych była obserwowana we wszystkich badanych błonach natomiast ich postulowana funkcja była raczej wynikiem spekulacji niż interpretacji danych doświadczalnych. W ostatnich dwóch latach dokonał się prawdziwy przełom w tych badaniach. Dziś wiemy, że wewnątrzkomórkowe kanały pełnią ważną rolę w komórkowej homeostazie jonowej. Klonowanie kolejnych kanałów wewnątrzkomórkowych umożliwi ich pełną charakterystykę strukturalną i farmakologiczną, co z kolei umożliwi zrozumienie ich funkcji w komórce.

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15. μ-Opioid receptor modulation of the voltage-dependent channel currents in prefrontal cortex neurons

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The neurons of the prefrontal cortex are responsible for operational memory. Their damage leads to memory impairment, causes emotional and personality abnormalities. An altered functional profile of these neurons may be observed in numerous mental disorders. In order to understand the mechanisms, by which μ opioid receptor modulates the function of prefrontal neurons, it is necessary to investigate signal transduction pathway

from the opioid receptor to the voltage-gated ionic channels expressed in these neurons. Opioid receptors have been identified in the membranes of cortical neurons. These receptors are supposedly coupled to a G protein, which induces the production of second messengers, modifying the function of numerous effectors on cytoplasmic or cell membrane level.

Our study was designed to test the effect of μ opioid receptor activation on the function of prefrontal cortical voltage-gated channels. The neurons were obtained from rats. Slices were prepared from the cerebral prefrontal cortex tissue. Currents were recorded in voltage-clamp configuration from enzymatically mechanically dispersed cells. The DAMGO compound ([D-Ala^2, MePhe^4, Gly-OL^5]-enkephalin) was used to activate the μ opioid receptor and naloxone as the μ receptor antagonist. The tested compound (DAMGO or naloxone) were infused into the solution through a large diameter micropipette. It was found that μ opioid receptor activation leads to inhibition or activation of numerous voltage-gated ionic channels expressed in pyramidal neurons and interneurons of the prefrontal cortex.

16. Chemical physics of kinetin

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Kinetin belongs to the group of cytokinins, small molecules which act as plant hormones. At present very intensive study of cytokinins deals mainly with their biological activity. It is thus a proper time to enrich these efforts with the analysis of physical and chemical properties of these molecules. We have chosen for our studies kinetin as a representative molecule of cytokinins.

Chemical aspects of our studies include analysis of the exchange of tautomeric forms of adenine residues under defined physical conditions. A further step of these efforts deals with the processes of protonation of a group of adenine N_6 derivatives. Finally, we analyze the solubility of kinetin in water-ethanol solution. These data are the starting point of our analysis of hydrophobicity of kinetin.

Physical aspects of our studies concern the analysis of molecular structure and dynamics of kinetin. We have prepared crystals of kinetin and its phosphate salt. A thorough analysis of intermolecular interactions determine a basis for the analysis of biological aspects of possible intermolecular interactions of kinetin with a receptor molecule in a living cell. We also analyze the physical properties of the crystals, taking into account the structural phase transitions. The analysis of molecular dynamics refers mainly to the dynamics of protons in the net of hydrogen bonds.

The presentation includes the data obtained in cooperation with students and scientists from Adam Mickiewicz University and Institutes of Polish Academy of Sciences.

17. Ion channels in plant tonoplast exmined by the patch-clamp technique

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The vacuole occupies more than 90% of a mature plant cell. It plays a very important role as a source of ions, nutrients and as a deposit of toxic compounds. The tonoplast, a membrane surrounding the vacuole participates in osmoregulation, signaling and transport. Ion channels in the tonoplast are keys to understand these processes. The patch-clamp seems most suitable technique to examine vacuolar ion channels. There are following types of ion channels characterized by the technique: slow vacuolar channels (SV), fast vacuolar channels (FV), vacuolar (VK) channels, voltage-gated Ca^{2+} channels (VVCa), IP_3-, and cADPR-gated Ca^{2+} channels, malate (VMAL) and chloride (VCl) channels.

The most abundant are SV channels, although they have not been found in Characean algae. They are permeable to K^+ but also to divalent cations including Ca^{2+}. SV channels activate slowly reaching saturation within 1 - 3 s. Their I/V characteristics shows clear rectification. The channels conduct cations from the cytoplasm to the vacuole. SV channels require high, unphysiological $[Ca^{2+}]_c$ to activate. Mg^{2+} lowers that threshold. FV channels are also permeable for cations but they differ from SV channels both in kinetics (instantaneous activation) and Ca^{2+}-dependence (activation at physiological $[Ca^{2+}]_c$). VK channels were found in guard cell vacuoles. They are selective for potassium, do not rectify but depend on cytosolic pH. Calcium permeable VVCa, IP_3-gated and cADPR-gated channels are responsible for Ca^{2+} release and thus play an important role in cell signaling. VMAL channels were characterized in plants exhibiting CAM metabolism. They carry malate to the vacuole. Its accumulation occurs during the night when stomata are open and mobilization during the day when it is a source of a carbon for photosynthesis.

The anion channel characterized recently in our laboratory in the liverwort *Conocephalum conicum* shows similarities to VCl channels of higher plant vacuoles. It is permeable to Cl^-, NO_3^-, SO_4^{2-} and to a les extent to malate. It opens at negative cytoplasm/vacuole potentials and thus conducts anions to the vacuole. The channel is weakly calcium-dependent and is insensitive to ATP, cAMP, protein kinase A. Activation of the channel occurs when Ca^{2+} on the cytoplasmic side is replaced by Mg^{2+}, Sr^{2+} or Ba^{2+}.