Session 12: Ion transports across biological membranes

Lectures

L.12.1

Short-range interaction of dendritic excitatory and inhibitory synapses

Massimo Ruben, Tiziana Ravasenga, Vincenzi Regio, Andrea Barberis

Fondazione Istituto Italiano di Tecnologia, Italy
Andrea Barberis <andrea.barberis@iit.it>

Activity of individual dendritic excitatory synapses affects the amplitude of synaptic signals at neighboring excitatory synapses, a phenomenon referred to as heterosynaptic plasticity. However, whether such forms of dendritic interplay also involve inhibitory synapses remains elusive. In the first of the present talk I will focus the interaction of two GABAergic inhibitory synapses. We observed that desensitized GABAA receptors at a given GABAergic synapse "A" could laterally diffuse to a contiguous synapse "B" spaced on average by 2-4 microns thus interfering with the amplitude of synaptic signals at synapse B, s that receptor lateral diffusion can mediate a transfer of information between neighboring dendritic inhibitory synapses. In a second form of dendritic synaptic crosstalk I investigated how synaptic plasticity induced at individual glutamatergic spines affects the strength of neighboring GABAergic synapses. To this end we induced "single spine LTP" by pairing the postsynaptic depolarization with glutamate uncaging at individual spines while simultaneously measuring the strength of adjacent dendritic GABAergic synapses by GABA uncaging. We found that GABAergic synapses located within 3 micrometers from a potentiated spine showed depression (iLTD). Overall, we show that local diffusion can modulate both excitatory and inhibitory signaling and that inhibitory synapses participate to heterosynaptic plasticity with profound effects in dendritic integration.

L.12.2

The role of mechanosensitive ion channel Piezo2 in pain sensation

Marcin Szczot

Linköping University, Linkoping, Sweden;
Marcin Szczot <marcin.szczot@liu.se>

Perception of mechanical pain and touch, a fundamental sensory experiences shared across all animal species, requires converting physical pressure into neuronal excitation through ion channels. However, the molecular mechanisms underlying mammalian mechanotransduction remained unclear for decades. In 2010, Piezo2 was identified as a transduction complex component necessary for touch sensation. Our research aims to uncover physiological processes in which Piezo2 becomes also a transducer of painful stimuli. In instances of injury, gentle touch can trigger pain, a phenomenon known as allodynia. Our observation of Piezo2-deficient mice revealed a lack of allodynia following inflammation, suggesting Piezo2's role in detecting light touch even when it turns painful. Clinical tests on humans with PIEZO2 inactivating mutations confirmed this hypothesis; they did not develop mechanical allodynia in response to capsaicin injury. Recently we've also explored acute pain and found Piezo2's conserved role in conducting specific fast pain related to hair follicle innervation. This sensation involves specialized sensory neurons, functionally and molecularly conserved between mice and humans. We show that Piezo2 is required to set sensitivity of this form of pain. These findings highlight acute pain system diversity and specialization in mammals, positioning Piezo2 as a potential pharmacological intervention target.

L.12.3

Ion channels' cooperativity – model, complexity, and experimental insights

Agata Wawrzkiewicz-Jałowiecka[1], Paulina Trybek[2], Beata Dworakowska[3], Piotr Bednarczyk[3]

[1]Silesian University of Technology, Department of Physical Chemistry and Technology of Polymers, Gliwice, Poland; [2]University of Silesia, Institute of Physics, Chorzów, Poland; [3]Warsaw University of Life Sciences, Department of Physics and Biophysics, Institute of Biology, Warsaw, Poland

Agata Wawrzkiewicz-Jałowiecka <agata.wawrzkiewicz-jalowiecka@polsl.pl>

Ion channels are integral proteins that effectively contribute to the shaping of membrane potential in virtually all living cells. Among the features that are relatively poorly understood in ion channel-oriented research, one can point out the possibility of the interchannel cooperativity and its consequences for the net magnitude of ionic current flowing through a channel cluster. In literature, one can encounter examples of the collective behavior of ion channels (like some ATP-sensitive potassium channels or CaV1.3 channels), where they gate non-independently (i.e., the open probability of one channel depends on the open probability of its neighbors).
In this work, we investigate this phenomenon by appropriate models' simulation that enable both positive and negative channel coupling. For the simulated series, we perform kinetic analysis and also apply more advanced coherence and correlation methods of data processing to extract and better understand the main features of the signals from cooperative and non-cooperative clusters. The analogous analysis is then performed for the experimental multichannel traces of ionic currents to evaluate whether the investigated empirical system (i.e., BK channels cluster in glioblastoma cells) exhibits any signs of coupled behavior.

L.12.4

Function and plasticity of GABAergic transmission

Jerzy W. Mozrzymas, Grzegorz Wiera, Katarzyna Lebida, Patrycja Brzdąk, Jadwiga Jabłońska, Anna Lech, Marcin Wyroślak, Przemysław Gmerek

Department of Biophysics and Neuroscience, Wroclaw Medical University, Wroclaw, Poland

Jerzy W Mozrzymas <jerzy.mozrzymas@umw.edu.pl>

Synaptic plasticity is a key substrate of learning and memory. Much is known about the glutamatergic synapses but plasticity of GABAergic inhibition is poorly understood. Our aim was to determine the molecular mechanisms GABAergic plasticity in the hippocampal CA1 field. We found that the inhibition of extracellular protease MMP-3 blocked the GABAergic plasticity induced by transient NMDA application. We used also peptides to block the interaction between adhesion proteins and extracellular matrix. We found that the impaired transsynaptic adhesion between neuroligin-2 and neurexins or interference in integrin-dependent adhesion abolished the induction of NMDA-iLTP. Moreover, administration of a peptide interacting with β1 integrins led to induction of iLTP, while application of fibrinogen activating β3 integrins resulted in GABAergic iLTD. We found that plasticity of inhibitory synapses at GABAergic cells shows interneuron-specificity and dependence on integrins types. Application of RGD-containing peptide evoked iLTP in fast-spiking parvalbumin (FS PV+) or somatostatin (SST+) expressing interneurons but treatment with peptide affecting α5β1 integrins led to iLTP in SST+ and iLTD in FS PV+ GABAergic cells. Additionally, we observed opposite sings of NMDAR-mediated inhibitory plasticity in PV+ and SST+ interneurons and that iLTP depends on the α5-GABAARs incorporation into inhibitory synapses.

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Posters

P.12.1

Effect of Quercetin on mitoBK$_{Ca}$ channel and mitochondrial function in human bronchial epithelial cells exposed to particulate matter

Agnieszka Lukasiak, Adrianna Dabrowska, Miroslaw Zajac, Bednarczyk Piotr

Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences-SGGW, Warsaw, Poland
Agnieszka Lukasiak <agnieszka_lukasiak@sggw.edu.pl>

Exposure to air pollution and airborne particulate matter (PM) has been linked to many health risks involving diseases of respiratory and cardiovascular system. The deleterious effect is linked to reactive oxygen species (ROS) and can lead to subsequent inflammatory response and cell damage. It is well known that mitochondrial potassium channels play important role in cytoprotective response. It has been recently reported that mitochondrial large conductance potassium channel (mitoBK$_{Ca}$) is present in the inner mitochondrial membrane of human bronchial epithelial cell line (HBE) and is activated by quercetin. Here we tried to verify the cytoprotective role of mitoBK$_{Ca}$ in particulate matter (PM<4μM) induced HBE cell damage.
It was observed that PM decreased TEER of HBE cells and the effect was partially abolished by quercetin. In mitochondria quercetin increased oxygen consumption rate and decreased mitochondrial membrane potential. The ROS measurements revealed decrease in PM-induced ROS level after quercetin administration. Additionally, it was observed that PM decreases cell viability, which can be partially restored by quercetin. The toxic effect of PM is also manifested in reduced mitochondrial function.
In summary, PM has toxic effect in HBE cells on cellular and mitochondrial level, which can be partially abolished by quercetin, involving mitoBK$_{Ca}$ activation.

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P.12.2

The role of the potassium channel in damage caused by urban particulate matters

Piotr Bednarczyk[1], Kamila Maliszewska-Olejniczak[1], Karolina Pytlak[2], Adrianna Dąbrowska[1], Agnieszka Łukasiak[1], Mirosław Zając[1], Bogusz Kulawiak[2]

[1]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Warsaw, Poland; [2]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
Piotr Bednarczyk <piotr_bednarczyk@sggw.edu.pl>

Proper functioning of epithelium is essential for maintaining lung health. Recently, potassium channels (BK$_{Ca}$) has been identified in the inner mitochondrial membrane of the epithelium. These proteins control potassium fluxes between mitochondrial intermembrane space and mitochondrial matrix which directly regulates mitochondrial and cell functions. It has been found that activation of mitoBK$_{Ca}$ channels preserves against damage induced by various factors including ischemia/reperfusion. Despite intensive research, the exact mechanism of proper cell function involving the influx of potassium still remains under investigation.
To verify the role of potassium channel in cytoprotection in response to stress induced by PM, we used wild-type epithelium cells (HBE wt) and cells with the deletion of the alpha subunit of the BK$_{Ca}$ channel (HBE Δα BK$_{Ca}$). Using the patch-clamp, it has been shown that in HBE Δα BK$_{Ca}$ cells model, BK$_{Ca}$ channel activity is not observed. Additionally, HBE Δα BK cells displayed mitochondrial dysfunction, lower transepithelial electrical resistance and reduced clone formation capabilities. To determine whether reduced clone formation capabilities are associated with cell cycle phase distribution changes, we conducted a cell cycle analysis. In summary, obtained results indicate that the BK$_{Ca}$ channel is important for bronchial epithelium barrier function.

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P.12.3

Investigating the impact of mutations on ROMK channel activity: a novel bacterial assay and *in silico* model

Mariusz Możajew[1,2], Przemysław Miszta[2], Sławomir Filipek[2], Piotr Koprowski[1]

[1]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2]Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

Piotr Koprowski <p.koprowski@nencki.edu.pl>

The renal outer medullary potassium channel (ROMK) is vital for K^+ transport in the nephron. Mutations in ROMK are linked to antenatal Bartter syndrome type II (aBSII) and contribute to blood pressure variations. Certain aBSII-causing mutations exhibit insensitivity to phosphatidylinositol 4,5-bisphosphate (PIP2), a ROMK activity stimulator. Thus, understanding the functional impact of these mutations is crucial. We developed an *in silico* model to explore ROMK-PIP2 interaction and introduced the Potassium Uptake PIP2 Activation assay (PUPA assay) using a simple bacterial growth system. The PUPA assay combined a growth complementation screen in K^+-transport deficient *Escherichia coli* expressing the K^+ channel with PIP2 synthesis pathway introduction. Screening ROMK mutants with aBSII mutations *via* the PUPA assay revealed bacterial growth phenotypes reflecting the mutations' impact on channel function. Additionally, the PUPA assay experimentally validated the ROMK-PIP2 complex model. Mutants affecting crucial amino acids for PIP2 interaction confirmed the PIP2 binding site structure and emphasized the significance of a unique lysine residue. Interestingly, our findings suggest that PIP2 is not essential for primary ROMK activity and other anionic lipids partially substitute its function. Overall, our study comprehensively investigates ROMK mutations enhancing understanding of their functional consequences, PIP2's role, and alternative anionic lipids in ROMK activity regulation.

P.12.4

The role of ion transport in the prevention of epithelial damage

Jakub Hoser[1], Kamila Maliszewska-Olejniczak[1], Bogusz Kulawiak[2], Mirosław Zając[1], Piotr Bednarczyk[1]

[1]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Warsaw, Poland; [2]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Jakub Hoser <d003227@sggw.edu.pl>

It has been reported that active chloride transport across cell membranes is very important and regulates many cellular functions. It plays a key role in the process of epithelial water secretion which impairment leads to many diseases such as asthma, COPD or cystic fibrosis. Since the apical membrane potential is close to the reversal potential for chloride, the activation of other channels could provide the driving force for chloride secretion improving the airway surface liquid hydration. One of the possible targets is calcium-activated potassium channel (BK_{Ca}) present on apical surface of bronchial epithelium.

To assess the influence of BK_{Ca} channel activation on chloride transport we performed series of Ussing chamber experiments on WT human bronchial epithelial cells line (16HBE14σ) and generated variant of this cell line with CRISPR/Cas9 BK_{Ca} channel deletion. Also, we used a naturally derived activator (quercetin) and synthetic blocker (penitrem A) of BK_{Ca} channel to assess its role in chloride secretion in both cell lines. Interestingly and unexpectedly, our results show that quercetin decreases the chloride currents in both cell lines. This effect could be used for further experiments regarding modifying-activating or blocking protein channels and is expanding our knowledge about cellular and epithelial electrophysiology.

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P.12.5

The role of infrared light in modulation of mitochondrial large-conductance calcium-activated potassium channel

Joanna Jasińska[1], Piotr Bednarczyk[2], Barbara Kalenik[1], Bogusz Kulawiak[1], Antoni Wrzosek[1], Adam Szewczyk[1]

[1]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology PAS, Warsaw; [2]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences -SGGW, Warsaw

JOANNA JASIŃSKA <j.jasinska@nencki.edu.pl>

Mitochondria are not only a cellular source of ATP. They play a significant regulatory role in apoptosis and necrosis pathways, taking part in Ca^{2+} buffering and ROS production. Opening of mitochondrial potassium channels present in the inner mitochondrial membrane has a cytoprotective effect, therefore, they are seen as potential drug targets. Several pharmacological modulators have been proposed. Unfortunately, many of them affect alternative targets, what exposes the need for exploring other approaches to regulate activity of the channels, such as photobiomodulation (PBM). Mitochondria are able to absorb infrared (IR) light, making them potential targets for PBM. Particularly intriguing is the absorption of specific wavelengths in IR band: 820 nm and 760 nm by metal centres of cytochrome c oxidase (COX): oxidised Cu_A and reduced Cu_B, respectively. Taking into consideration possible functional connection of mitochondrial large-conductance calcium-activated potassium (mitoBK$_{Ca}$) channel with COX in the astrocytoma U87 cell line, we conducted patch-clamp experiments with illumination system. In oxidizing conditions induced by ferricyanide, we observed an inhibition of mitoBK$_{Ca}$ channel. However, when the channel was illuminated with 820 nm wavelength, its activity was restored. Importantly, this restorative effect was immediate and durable.

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P.12.6

Lack of BK$_{Ca}$ channel affects mitochondrial function in human bronchial epithelial cells

Karolina Pytlak[1], Kamila Maliszewska–Olejniczak[2], Aleksandra Sęk[1,3], Adam Szewczyk[1], Piotr Bednarczyk[2], Bogusz Kulawiak[1]

[1]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Poland; [2]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Poland; [3]Faculty of Chemistry, University of Warsaw, Poland.

Karolina Pytlak <k.pytlak@nencki.edu.pl>

Airways are exposed on various factors including urban dust, which contain particulate matters (PMs). Recent studies described that PM cytotoxicity might be related to the mitochondrial dysfunction. Pharmacological activation of mitochondrial potassium (mitoK) channels induces cytoprotective mechanisms. This phenomenon was observed during ischemia/reperfusion of brain and heart. Transport of K^+ across the inner mitochondrial membrane is important for regulation of oxidative phosphorylation, reactive oxygen species synthesis and membrane potential. Here, we describe the large conductance calcium-activated potassium (mitoBK$_{Ca}$) channel in the IMM of human bronchial epithelial (16HBE14o-, HBE) cells. We focused on the potential role of the mitoBK$_{Ca}$ channels in cytoprotection against damage induced by PMs. We used the CRISPR/Cas9 technology to develop 16HBE14o- cell line with BK$_{Ca}$ a pore-forming subunit knockout. Loss of mitoBK$_{Ca}$/BK$_{Ca}$ channel changes expression of selected mitochondrial genes and mitochondrial respiration. We also observed that lack of channel leads to loss of monolayer integrity of HBE cells. We also checked effect of BK$_{Ca}$ channel loss on PMs induced cytotoxicity. In conclusion, in our project we want to test whether the mitoBK$_{Ca}$/BK$_{Ca}$ channel is a suitable pharmacological target for cytoprotection against PMs induced damage.

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