האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

Israel Society for Physiology and Pharmacology

THE ISRAEL SOCIETY FOR
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Annual Meeting

Ma'ale Hachamisha

4.1.2012

Annual Meeting

Ma'ale Hachamisha, January 4, 2012

Program Outline

08:30 - 09:20	***Registration and coffee***
9:20-11:20	**Morning Sessions**
11:20 - 11:45	***Coffee break***
11:45-12:45	**Students presentations**
12:45 - 14:00	***Lunch and poster session***
14:00 - 15:00	**Magnes Plenary Lecture**
15:00 - 15:15	***Coffee break and business meeting***
15:15-17:15	**Afternoon Sessions**
17:15	***Concluding Remarks and awarding the winners of poster and student lecture competitions***

Program

08:30 - 09:20 ***Registration and coffee***

9:20-11:20 Morning Sessions

Session A:

A window into the working cells and the door to change cell function: an application of novel tools to monitor cellular functions (Hall A)

Chair: **Ilya Fleidervish** (*Ben-Gurion University*)

09:20-09:50 **Ofer Yizhar** (*Weizmann Institute*)

Light-based modulation of cortical excitation and inhibition.

09:50-10:20 **Michal Hershfinkel** (*Ben-Gurion University*)

Live cell imaging elucidates the physiological role of a Zn-sensing receptor.

10:20-10:50 **Itamar Kahn** (*Technion*)

Regulation of neocortical excitation-inhibition balance revealed by optogenetic fMRI.

10:50-11:20 **Leah Gheber** (*Ben-Gurion University*)

Directionality and intracellular functions of mitotic Kinesin-5 nanomachines.

Session B:

Drug design and delivery to optimize drug targeting (Hall B)

Chairs: **Amnon Hoffman** (*Hebrew University*);

David Stepensky (*Ben-Gurion University*)

09:20-09:50 **Amnon Hoffman** (*Hebrew University*)

Nano-liposphere formulation for improved oral bioavailability of lipophilic and peptide drugs

09:50-10:20 **David Stepensky** (*Ben-Gurion University*)

Quantitative analysis of nano-drug delivery systems intended for intracellular targeting

10:20-10:50 **Ayelet David** (*Ben-Gurion University*)

Novel Polymer-Drug Conjugates for Cancer Therapy

10:50-11:20 **Gershon Golomb** (*Hebrew University*)

Immuno-modulation by Nanoparticles for Anti- inflammatory Therapy

Session C:

Energy sources in health and disease: Oxygen, ATP and mitochondria (Hall C)

Chairs: **Israel Sekler** (*Ben-Gurion university*)

Dov Lichtenberg (*Tel Aviv university*)

09:20-09:50 **Varda Shoshan-Barmatz** (*Ben-Gurion university*)

Mitochondria, VDAC, cell death and cancer

09:50-10:20 **Yael Pewzner-Jung** (*Weizmann Institute*)

Ceramide synthase 2 deficiency leads to oxidative stress in liver

10:20-10:50 **Oren Tirosh** (*Hebrew university*)

Involvement of redox changes in the metabolic status of liver steatosis and mitochondrial biogenesis

10:50-11:20 **Dov Lichtenberg** (*Tel Aviv university*)

Oxidative Stress (OS) is not our enemy; Antioxidants are not always good

11:20 - 11:45 ***Coffee break***

Session D:

11:45-12:45 **Students presentations**

11:45-11:57 **Limor Cohen** *(Ben Gurion University)*

Activation of the novel zinc sensing receptor-GPR39 is highly regulated by extracellular pH

11:57-12:09 **Lior Shaltiel** *(Ludwig-Maximilians-Universität München)*

The visual phenotype of $Ca_v1.4$-deficient mice

12:09-12:21 **Keren Ettinger** *(Hebrew University)*

Nerve growth factor (NGF) stimulation of ERKs phosphorylation by a cross talk between $p75^{NTR}$ and $\alpha9\beta1$ integrin in C2C12 muscle cell model

12:21-12:33 **Anat Marom** *(Technion)*

Optical probing of three-dimensional engineered neural-networks

12:33-12:45 **Moran Dvela** *(Hebrew University)*

Involvement of endogenous ouabain in the regulation of cell viability

12:45 - 14:00 ***Lunch and poster session***

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14:00 - 15:00 **Magnes Plenary Lecture**

Prof. Bruce P. Bean (Harvard University)

Pharmacology of voltage-dependent ion channels

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15:00 - 15:15 ***Coffee break and business meeting***

15:15-17:15 **Afternoon Sessions**

Session E:

Detection of sensory stimuli: from the pleasure of light touch to suffering from devastating pain

Chairs: **Alex Binshtock** (*Hebrew University*)

Avraham Yaron (*Weizmann Institute*)

15:15 - 15:45 **Yehuda Shavit** (*Hebrew University*)

Interleukin-1 (IL-1) Antagonizes Morphine Analgesia and Underlies Morphine Tolerance

15:45 - 16:15 **Avi Priel** (*Hebrew University*)

Probing the pain pathway using natural toxins

16:15 - 16:45 **Avraham Yaron** (*Weizmann Institute*)

Illuminating diabetic neuropathy - novel systems facilitating *in-vivo* and *in vitro* investigations.

16:45 - 17:15 **Ehud Ahissar** (*Weizmann Institute*)

Mechanisms and coding of object localization by whisking rats

Session F:

Modeling human diseases in the lab

Chairs: **Ofer Binah** (*Technion*)

Ronen Beeri (*Hebrew University*)

15:15 - 15:45 **Ronen Beeri** (*Hebrew University*)

Experimental models of volume overload

15:45 - 16:15 **Neville Berkman** *(Hadassah Medical center)*

Using a murine model of "Asthma" to investigate the link between airway hypperresponsiveness and airway remodeling.

16:15 - 16:45 **Jacob Bortman** (*Ben-Gurion university*)

Finite elements model for cardiac function.

16:45 - 17:15 **Ofer Binah** *(Technion)*

Modeling Catecholaminergic Polymorphic Ventricular Tachycardia – a congenital cardiac arrhythmia, using induced pluripotent stem cells-derived cardiomyocytes

17:15 ***Concluding Remarks and awarding the winners of poster and student lecture competitions***

Invited Lectures

Session A

A window into the working cells and the door to change cell function:

an application of novel tools to monitor cellular functions

Light-based modulation of cortical excitation and inhibition

Ofer Yizhar
Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Excitation and inhibition in neural circuits are finely tuned and highly regulated. Severe behavioural deficits in psychiatric diseases have been hypothesized to arise from elevations in the cellular balance of excitation and inhibition (E/I balance) within neural microcircuitry. To evaluate this hypothesis experimentally, we developed optogenetic methods to probe the effects of altered E/I balance in behaving animals. Optogenetic techniques enable cell-type specific, multimodal control over cellular physiology. The optogenetic method utilizes naturally occurring and engineered microbial opsin genes, which encode light-sensitive ion channels, pumps and receptors. Transgenic or viral gene delivery sensitizes the genetically-modified neurons to light and allows manipulation of their activity in vitro and in vivo. Through protein engineering, we developed several novel optogenetic tools that enable real-time bidirectional modulation of cellular excitation and inhibition. We then used these tools to investigate effects of modulating excitation and inhibition in the prefrontal cortex of awake mice. We found that elevation, but not reduction, of the cellular E/I balance within the medial prefrontal cortex leads to profound impairment in cellular information processing, associated with specific behavioral deficits and increased high-frequency power in the 30–80 Hz range. Our results provide direct support for the cellular E/I balance hypothesis in neuropsychiatric disease.

Live cell imaging elucidates the physiological role of a Zn-sensing receptor

Michal Hershfinkel

Department of Morpholgy, Ben-Gurion University, Beersheva, Israel

Zinc is an essential micronutrient with pleiotropic effects on human health. Zinc dyshomeostasis has been linked to neurological disorders such as epilepsy and stroke, impaired wound healing and digestive system disorders such as colitis and diarrhea. We have identified a Zn-Sensing Receptor (ZnR) that mediates intracellular signaling following changes in extracellular zinc concentration. This receptor is found on many cell types where zinc homeostasis plays a physiological role such as neurons, keratinocytes and colonocytes. Using live cell imaging we showed that ZnR triggers calcium release from thapsigargin-sensitive stores via the IP3 pathway. We have further shown that endogenous ZnR activity is mediated by GPR39, and silencing the expression of this protein abolishes Zn-dependent signaling. We then looked for the physiological roles of the ZnR. Measuring ion transport mediated by the major chloride cotransporter in neurons, KCC2, we show that ZnR activity induces pronounced upregulation of KCC2 activity. The ZnR-dependent upregulation of KCC2 leads to a hyperpolarizing shift in the GABA reversal potential and enhances the neuronal inhibitory drive. In colonocytes and keratinocytes, where changes in pH have an important physiological role, we monitored changes in intracellular pH mediated by the Na^+/H^+ exchanger, NHE. We show the ZnR upregulates NHE activity and increases the recovery rate following intracellular acidification. Indeed, ZnR induced an increase in the growth of keratinocytes in a scratch model, this process was decreased by inhibition of NHE activity. Thus, our results support an important role for the ZnR in mediating Zn-dependent enhanced wound healing. In colonocytes, we observed ZnR-dependent survival following exposure to the short-chain fatty acid, butyrate. Surprisingly, this was not mediated by enhanced NHE activity but via increased clusterin expression. Based on our studies we suggest that ZnR is the missing link between zinc and the well-known physiological roles of this ion.

Regulation of neocortical excitation-inhibition balance revealed by optogenetic fMRI

Itamar Kahn

Department of Physiology and Biophysics, The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel

A fundamental challenge in understanding neocortical computation is analysis of the balance of inhibition and excitation. Magnetic resonance imaging (MRI) methods allow us to simultaneously measure the function of multiple brain systems. Fluctuations in the blood oxygenation level-dependent (BOLD) signal serve as the basis of functional MRI (fMRI). In humans we can characterize the functional organization and specialization, and compare the system between health and disease. In animal models we can dissect the circuits underlying these dynamics. In this talk I will discuss recent work aimed at revealing variations in excitation-inhibition balance using BOLD fMRI in animal models. Precise analysis of the balance of excitation and inhibition requires the ability to systematically manipulate neural activity. We achieved such control by using optogenetic drive of neocortical neurons. By employing light trains that were matched in frequency and had either periodically or Poisson distributed onset times we were able to manipulate the likelihood of interneuron inhibition recruitment. Electrophysiological recordings revealed that these stimulus patterns generated dissociable patterns of single-unit, multi-unit and local field potentials (LFP) activity in the neocortex. Specifically, both single-unit and multi-unit activity was greater for Poisson distributed trains, while LFP demonstrated similar levels of activity between the two regimes. Critically, BOLD fMRI activity tracked single-unit and multi-unit responses and diverged from LFP suggesting that it is more likely under these protocols to reflect pyramidal cell activity. Further, intricate temporal dynamics of spike activity were reflected in the BOLD fMRI temporal response. These results suggest that BOLD fMRI while in and of itself reflects pyramidal neurons spike activity, its response dynamics may serve as a surrogate measure for variations in excitation-inhibition balance. I will discuss implications for studying neocortical computation in health and disease and translation of these results to human imaging.

Directionality and intracellular functions of mitotic Kinesin-5 nanomachines

Leah Gheber

Departments of Clinical Biochemistry, Chemistry and the Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University, Beersheva, Israel

Molecular motors from the Kinesin superfamily are nanometric machines that move objects along microtubule filaments. While it has been demonstrated that their function is essential for intracellular vesicle trafficking, cell locomotion and cell division, the mechanism and regulation of their activity have not as yet been established. Our research focuses on the study of Kinesin-5 motor proteins, whose function is essential for chromosome segregation during mitotic cell division. Until recently, these motors were believed to perform their essential mitotic functions as slow, processive microtubule plus-end directed motors. The Saccharomyces cerevisiae Kinesin-5 Cin8 was found, surprisingly, to switch directionality. We have examined Cin8 directionality control using single-molecule fluorescence motility assays and high-resolution live-cell microscopy. On spindles, Cin8 motors mostly moved slowly towards the midzone, in the plus-end direction of the interpolar MTs. Occasionally, Cin8 also moved faster towards the spindle poles, in the minus-end direction of the MTs. *In vitro*, individual Cin8 motors could be switched by ionic conditions from rapid and processive minus-end to slow plus-end motion on single MTs. At high ionic strength, Cin8 motors rapidly alternated directionalities between antiparallel microtubules, while driving steady plus-end relative sliding. Deletion of the uniquely large insert in loop 8 of Cin8 induced bias towards minus-end motility and affected the ionic-strength dependent directional switching of Cin8 *in vitro*. *In vivo*, the deletion mutant exhibited reduced midzone-directed motility and efficiency to support spindle elongation, indicating the importance of directionality control for the function of Cin8.

Session B

Drug design and delivery to optimize drug targeting

Nano-liposphere formulation for improved oral bioavailability of lipophilic and peptide drugs

Amnon Hoffman and Abraham J. Domb
Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem amnonh@ekmd.huji.ac.il

Many of the newly proposed active pharmaceutical ingredients are lipophilic which makes them poorly soluble in the intestinal media and susceptible to first pass metabolism, and thereby poorly oral bioavailable. We developed pharmaceutical solution of nano-lipospheres pro-dispersion that enables to overcome this obstacle via several mechanisms, including: 1) improved solubility in the intestinal media; 2) improved permeation through the unstirred aqueous layer; 3) reduced efflux activity at the intestinal wall; 4) reduced metabolic degradation in the enterocytes. This approach leads to reduced variability in oral bioavailability, and is suitable for a wide range of drugs and drug candidates.

QUANTITATIVE ANALYSIS OF NANO-DRUG DELIVERY SYSTEMS INTENDED FOR INTRACELLULAR TARGETING

David Stepensky

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Many biopharmaceuticals (proteins, peptides, siRNA, etc.) act on intracellular targets and should reach the site of action in specific organelle in order to exert pharmacological effect. Due to the limited permeability and stability of biopharmaceuticals, efficient delivery requires encapsulation of these agents into specialized drug delivery systems (DDSs) targeted to the site of action. DDS targeting on the organ/tissue level has been a topic of intensive research by many investigators. We are extending this approach and investigating DDSs intended for intracellularly-targeted delivery of biopharmaceuticals. Feasibility and potential efficiency of this approach is not yet clear and requires development of novel DDSs decorated with specific targeting residues, and analytical tools for their detailed *in vitro* and *in vivo* characterization.

To this end, we have developed PLGA-based DDSs decorated with peptidic targeting moieties using a novel 3-stage conjugation approach. We applied/developed a panel of analytical tools to assess the amount and stability of the surface-conjugated targeting residues, drug encapsulation efficiency and kinetics of drug release. These tools include HPLC-based analysis of samples obtained during the conjugation process, and assessment of the DDSs at the individual stages of conjugation by spectroscopy and imaging-based tools.

The applied analytical tools allow quantitative analysis of the developed DDSs and their optimization (to enhance the drug encapsulation capacity and efficiency of decoration with the targeting moieties). The optimized DDSs and the developed analytical tools will be used to assess the feasibility of intracellularly-targeted delivery and to identify the primary parameters that limit the targeting efficiency. These outcomes will lead to identification of the DDSs that can efficiently deliver their cargo to the target organelles in the controlled fashion and will assist in preclinical and clinical development of the intracellularly-targeted DDSs.

Novel Polymer-Drug Conjugates for Cancer Therapy

Ayelet David
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The concept of targeted polymer-drug conjugates was developed to address the lack of specificity of low molecular weight drugs for malignant cells. Features needed to design an effective conjugate include: a polymer-drug linker that is stable during transport and able to release the drug in the intracellular compartment of the target cell at a predetermined rate, adequate physicochemical properties of the conjugate, and the capability to target the diseased cell or tissue by an active or a passive mechanism. Manipulation of the intracellular penetration and subcellular fate of macromolecular therapeutics may result in more effective conjugates.
The lecture will demonstrate the design, synthesis and biological activity of targeted polymeric anticancer drugs through the example of the water-soluble N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–drug conjugates. The polymeric drug delivery systems have proved to be effective for chemotherapy and imaging.

Immuno-modulation by Nanoparticles for Anti- inflammatory Therapy

Gershon Golomb

Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem gershong@ekmd.huji.ac.il

Intimal hyperplasia is a universal response of the arterial wall to mechanical injury and it is a major cause of restenosis following angioplasty. Experimental and clinical data indicate that the innate immunity and inflammation are of major importance in the pathophysiology of restenosis. Macrophage recruitment is associated in other clinically important disorders such as myocardial infraction (MI) and endometriosis. We validated the hypothesis that systemic and transient depletion of circulating monocytes inhibits the inflammatory cascade. Monocytes/macrophage depletion was achieved with a systemic injection of nanoparticulated dosage forms (PLGA-based NP and liposomes) containing bisphosphonates (BP), which were formulated for effective phagocytosis. Following phagocytosis the vesicles discharge their encapsulated drug like a Trojan Horses, inactivating the cell with no effect on non-phagocytic cells. We investigated the effect of different BP, NP type (polymeric or liposomal), and size on the formulation properties, biodistribution, and monocytes sub-populations. Bioactivity and mechanism was examined in tissue cultures, and in animal models of restenosis, MI and endometriosis. Partial and transient depletion of blood monocytes following NP systemic injection correlated with the therapeutic effect. Phase I and IIa clinical studies, in stented patients (one IV injection at the time of angioplasty), confirmed the safety and efficacy of the liposomal delivery system in preventing restenosis.

Session C

Energy sources in health and disease: Oxygen, ATP and mitochondria

Mitochondria, VDAC, cell death and cancer

Varda Shoshan-Barmatz, Nir Arbel, Tal Prezma, Laetitia Arzoine, Alexandra Zooravlov and Ilan Sela

Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva, Israel

Cancer cells exhibit altered metabolism characterized by the generation most of their energy by glycolysis. This altered metabolism in cancer cells is reflected in the higher expression levels of the mitochondria-bound hexokinase isoforms, HK-I and HK-II, in tumor cells and their binding to mitochondrial protein the voltage-dependent anion channel (VDAC1). This provides both a metabolic benefit and apoptosis-suppressive capacity that offers the cell a growth advantage and increases its resistance to chemotherapy. VDAC assumes a crucial position in the cell, serving as the main interface between mitochondrial and cellular metabolisms, thus controlling the cross-talk between mitochondria and the rest of the cell. VDAC is also an anchor point for mitochondria-interacting proteins such as HK and Bcl2 and Bcl-xL. Along with its importance in regulating cellular energy metabolism, VDAC is also recognized as a key protein in mitochondria-mediated apoptosis, participating in the release of apoptotic proteins and interacting with anti-apoptotic proteins. Bcl2 and Bcl-xL that are highly expressed in many types of cancer, bind to VDAC1 to promote tumor cell survival through inhibiting cytochrome *c* release and apoptotic cell death.

In this study, through exploitation of the abnormal energy metabolism of cancer cells and considering VDAC functions in cellular metabolism and cell death, we developed VDAC1-based cancer therapies targeting both tumor metabolism and inducing apoptosis. VDAC1 domains interacting with HK, Bcl2 and Bcl-xL were defined using site-directed mutagenesis and VDAC1-based peptides that bind specifically to these proteins were designed. We demonstrate that the expression of these peptides in cells over-expressing HK, Bcl2 or Bcl-xL abolished the cells abilities to avoid apoptosis. Furthermore, cell-penetrating VDAC1-based peptides induced cell death in several cancer cell lines but not in non-cancer cells. When tested on lymphocytes derived from B-cell chronic lymphocytic leukemia (CLL) patients, the peptides induced dramatic and selective cell death of cancer but not non-cancer cells. Moreover, the peptides promote cell death in a panel of genetically characterized cell lines derived from different types of human cancer. These selected cell lines harbor alternations that considerably increase resistance to apoptosis and chemotherapy such as mutations in P53 and/ or MDR proteins and the absence of the pro-apoptotic protein Bax.

These findings suggest that using VDAC1-based peptides to interfere with the anti-apoptotic effect of Bcl2, Bcl-xL and HK may offer a novel strategy for potentiating the efficacy of conventional chemotherapeutic agents.

Ceramide synthase 2 deficiency leads to oxidative stress in liver

Yael Pewzner-Jung
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Ceramide is a key intermediate in the pathway of sphingolipid biosynthesis as well as a second messenger in various intracellular signaling pathways. There are six known ceramide synthases (CerS) in mammals. Each CerS uses a defined subset of fatty acyl CoAs as substrate for N-acylation of the sphingoid backbone in order to generate a particular type of ceramide species. A major challenge in SL research is to determine the role of each ceramides containing different length acyl chains. CerS2, which synthesizes C22-C24-ceramide, is the most ubiquitously expressed CerS and has the broadest tissue distribution. Recently, our laboratory generated a CerS2 *null* mouse. This mouse is devoid of SLs containing very long (C22-C24) acyl chains, while long chain-SLs (C16-C18) are elevated. In addition, sphinganine, the substrate for all CerS, is also increased. The mice develop severe and chronic liver disease, displaying increased rates of hepatocyte apoptosis and proliferation. Considering the major role of oxidative stress in liver diseases, we examined whether reactive oxygen species (ROS) are elevated, which might lead to oxidative stress. ROS levels were elevated, as were 4-HNE, nitrotyrosine, and the antioxidant enzymes glutathione peroxidase (GPX) and glutathione-s transferase (GST). Isolated hepatocytes from CerS2 null mice also have elevated ROS. Examination of the mitochondrial electron transport chain in whole liver as well as in isolated CerS2 null hepatocytes revealed that succinate dehydrogenase and cytochrome c oxidase activities were significantly reduced and this appears to be due to inhibition by C16-ceramide but not C24-ceramide. We are currently investigating the molecular mechanisms that lead to these findings.

Involvement of redox changes in the metabolic status of liver steatosis and mitochondrial biogenesis

Oren Tirosh

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The Robert H Smith Faculty of Agriculture, Food and Environment
The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Accumulating evidence indicates that mitochondria play a key role in non-alcoholic fatty liver disease (NAFLD). The aim of the study was to elucidate the contribution of mitochondrial dysfunction and the activity of peroxisome-proliferator-activated receptor-gamma co-activator 1α (PGC1α) to the development of fatty liver disease. C57BL/6J mice were fed a choline-deficient, ethionine-supplemented (CDE) diet to induce fatty liver. Histological studies demonstrated accumulation of fat vacuoles in up to 90% of hepatocytes in mice fed the CDE diet for 14 days. In addition, a decrease in mitochondrial levels, together with an increase in superoxide radicals' levels were observed, indicating elevation of oxidative stress in hepatocytes. ATP levels were decreased in livers from CDE-fed mice after overnight fasting. This was accompanied by a compensative and significant increase in PGC1α mRNA levels in comparison to control livers. However, there was a reduction in PGC1α protein levels in CDE-treated mice. Moreover, the expression of mitochondrial biogenesis genes nuclear-respiratory-factor-1 (NRF-1), mitochondrial transcription factor A (TFAM), mitochondrial transcription factor B1 (TFB1M) and mitochondrial transcription factor B2 (TFB2M), which are all regulated by PGC1α activity, remained unchanged in fasted CDE-treated mice. These results indicate impaired activity of PGC1α. The impaired activity was further confirmed by chromatin immunoprecipitation analysis, which demonstrated decreased interaction of PGC1α with promoters containing NRF-1 and NRF-2 response elements in mice fed with CDE diet. A decrease in PGC1α ability to activate the expression of the gluconeogenic gene phosphoenol-pyruvate carboxykinase (PEPCK) was also observed. In conclusion**,** Dysfunction of mitochondrial biogenesis process in steatotic livers (CDE-fed mice) involves impaired-activity of PGC1α and oxidative stress, which may contribute to the progression of the disease.

Aharoni-Simon M. et al. *Lab Invest*. 2011 Jul;**91**(7):1018-28.

Oxidative Stress (OS) is not our enemy; Antioxidants are not always good

Dov Lichtenberg, Ilya Pinchuk and Didi Dotan
Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University

The term OS is intuitively defined, having no units, no universal criterion and no correlation between the results of different methods used to assay its level (Dotan et al, 2004). In spite of being ill-defined, OS is commonly regarded a measure of a person's probability to suffer from oxidative damages, i.e. to reflect the individual" Oxidative Status". OS is commonly to blamed of being involved in the pathogenesis of many diseases and antioxidants predicted to be good to us. In fact, meta-analyses of epidemiologic studies indicated that vitamin E supplementation increases mortality, in agreement with our decision analysis (Dotan et al., 2009). This inconsistency between the results and the prediction has been explained on the basis of the possibility that under certain conditions, reducing agents, including vitamin E ,may be pro-oxidative.

The latter explanation has recently been challenged. Given the physiological role of free radicals (signaling for skeletal muscle adaptation) Ristow and his collaborators (2011) raised the possibility that in some cases the common antioxidants may hurt us by quenching essential ROS. Furthermore, their recent publication was entitled "Extending life span by increasing oxidative stress." Our recent analysis shows that intense workout results in increased OS, as evaluated by different criteria, but after one hour of rest OS decreased back to normal supporting the view that the homeostatic level of OS is tidally controlled.

The challenge is to define a criterion to predict who is likely to benefit from antioxidant supplementation.

Session D

Students' presentations

Activation of the novel zinc sensing receptor-GPR39 is highly regulated by extracellular pH

Limor Cohen[1], Israel Sekler [2] and Michal Hershfinkel [1]

Department of Morpholgy [1] and Physiology [2], Ben Gurion University, Israel

Zinc regulates cell survival and proliferation in numerous tissues; however a link between extracellular zinc and cellular signaling events is not well established. Our lab identified a specific zinc sensing G-protein coupled receptor, ZnR, that triggers Ca^{2+} signaling in colonocytes. We further showed that ZnR activity is mediated by GPR39 in colonocytes.

Colonocytes are exposed to frequent pH changes which are of physiological significance for their function. A slightly alkaline pH microclimate, tightly regulated by secreted bicarbonate, was monitored on their apical side. However, absorption of short chain fatty acids and activation of ion transport following intracellular acidification may change the local pH. In addition, during inflammatory processes of intestinal mucosa acidification is encountered.

Since a pair of Histidine residues ($Histidine^{17+19}$) coordinate the binding of Zn^{2+} to ZnR/GPR39, we hypothesize that ZnR activity is regulated by pH. Here we show that the ZnR-dependent Ca^{2+} response is highly regulated by extracellular (and not intracellular) pH, at the physiological pH range, in HEK 293 cells and HT29 coloncytes. We further show that ZnR activation of MAP and PI3 kinase is dramatically reduced when zinc is applied at acidic pH. Similarly, zinc-dependent upregulation of NHE1 activity is eliminated at acidic pH. Thus, ZnR-dependent signaling which lead to enhanced colonocytes survival and ion homeostasis is largely impaired at low pH. Interestingly, acidic pH inhibition of the zinc-dependent Ca^{2+} response shows a non competitive mechanism of inhibition, suggesting that it does not originate from the ligand binding site. Using site directed mutagenesis we identified $Aspartate^{313}$, and not $Histidine^{17+19}$, as the specific pH sensor on ZnR/GPR39. Overall we suggest an allosteric regulatory mechanism for the ZnR/GPR39, by extracellular pH, which is accompanied by the regulation of major signaling events triggered by ZnR in colonocytes.

The visual phenotype of $Ca_v1.4$-deficient mice

Lior Shaltiel[1], Stylianos Michalakis[1], Vithiyanjali Sothilingam[2], Marina Garcia Garrido[2], Susanne Koch[1], Naoyuki Tanimoto[2], Mathias W. Seeliger[2], Martin Biel[1], Christian A. Wahl-Schott[1].

[1]Center for Integrated Protein Science Munich (CIPSM), Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Muenchen, Germany, [2]Division of Ocular Neurodegeneration, Institute for Ophthalmic Research, Centre for Ophthalmology, Eberhard Karls-Universität, Tuebingen, Germany.

CACNA1F encodes the alpha1 subunit of retina-specific $Ca_v1.4$ voltage-gated L-type calcium channels. Retinal network activity crucially depends on Ca^{2+} influx through presynaptic $Ca_v1.4$ L-type Ca^{2+} channels. In ribbon synapses of retinal photoreceptors and bipolar cells sustained Ca^{2+} influx through $Ca_v1.4$ channels is required to couple slow graded changes of the membrane potential with a tonic glutamate release. Mutations in this gene cause congenital stationary night blindness type 2A (CSNB2), Aland Island eye disease (AIED) and cone-rod dystrophy type 3 (CORDX3). The clinical phenotypes of these eye diseases vary substantially regarding the ratio of rod to cone functional impairment. The reasons for this variability are not known.

To gain more insights into the pathophysiology caused by loss of $Ca_v1.4$ function we analyzed the visual phenotype of $Ca_v1.4$-deficient mice. To this end, we combined immunohistochemistry, electroretinography (ERG) and vision-dependent behavioral testing.

Immunohistochemical analysis using synaptic and postsynaptic markers revealed severe synaptic defects in $Ca_v1.4$-deficient mice. Heterozygous $Ca_v1.4$ mice showed mosaic synaptic defects most probably caused by random X-chromosomal inactivation of the healthy allele. Electroretinography revealed a loss of scotopic and photopic photoreceptor function. This loss of retinal network function resulted in impaired perfomance of $Ca_v1.4$ knockout mice in a water maze-based behavioral test of rod and cone function.

In conclusion, loss of $Ca_v1.4$ channels strongly impairs rod and cone retinal function and vision in mice.

Nerve growth factor (NGF) stimulation of ERKs phosphorylation by a cross talk between p75^{NTR} and α9β1 integrin in C2C12 muscle cell model

Keren Ettinger, Yoram Nevo, Shimon Lecht, Hadar Arien-Zakay, Shlomit Mizrachi, Nurit Yanay, Uri Saragovi, Cezary Marcinkiewicz, and Philip Lazarovici

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The mechanism of pleiotropic functions of nerve growth factor (NGF) in skeletal muscles physiology and pathology are not clear. However, anti-NGF transgenic mice are characterized by skeletal muscle myopathy and gene therapy with NGF improved muscle recovery in mice model of dystrophy. Therefore, in the present study, we sought to investigate NGF-induced ERK phosphorylation, as a paradigm to clarify the contribution of its receptors to this signaling cascade using the C2C12 skeletal muscle cellular model. Measuring by RT-PCR and western blotting mRNA and protein expression, we characterized NGF and it receptors p75^{NTR}, α9β1 integrin, and its regulator ADAM12 as the NGF receptors system in this model. Both proNGF and βNGF induced ERK1,2 phosphorylation, a process blocked by MEK inhibitor, PD98059. βNGF and proNGF-induced ERK1,2 phosphorylation was completely blocked by VLO5, a MLD-disintegrin with relative selectivity towards α9. Furthermore, small, non-peptide p75^{NTR} antagonists, Thx-B and LM-24, but not the non-active compound Thx, inhibited by 70-90% βNGF and proNGF-induced phosphorylation, respectively. We propose that NGF-induced ERK1,2 phosphorylation is mediated by a heterologous cross-talk between p75^{NTR} and α9β1integrin whereas, p75^{NTR} and 9 couples to activate β1, which in turn stimulates MEK and ERK phosphorylation. Therefore, the present study establishes an important role to α9β1 in NGF-induced signaling in skeletal muscle model, mimicking the role of trkA in neurons. Further understanding of the interactions between NGF receptors in the skeletal muscle will contribute towards novel therapeutic approaches for skeletal muscle dystrophies.

Optical probing of three-dimensional engineered neural-networks

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Physiological and pharmacological studies of the mammalian brain *in situ* are inherently limited, due to light scattering as well as many other experimental difficulties, motivating the development of many *in vitro* preparations. These *in vitro* models are typically two-dimensional and thus provide only a limited similarity to natural physiology. Conversely, creating and working with three dimensional (3D), biologically relevant central nervous system (CNS) models also present major challenges, such as selecting a suitable 3D scaffold and developing fast 3D imaging systems. Laboratory engineered hydrogel scaffolds have many advantages over alternatives, since hydrogels are typically transparent and can also mimic the properties of the natural extra cellular matrix around cells, thus providing the cells with the appropriate mechanical and biological cues. Hydrogels could thus enable both the growth of brain tissue and of dissociated cells and imaging of the artificial neuronal networks created.

In this study we introduce and present the fundamental characteristics of a dense 3D neuronal network composed of rat primary cortical cells embedded in a hydrogel scaffold, and the 3D optical probing of the network using optogenetic probes. The network was transfected with viral agents for the calcium indicator GCaMP3 and for the light-gated channel Channelrhodopsin 2, facilitating the probing of a large population of neurons. We monitored the activity of large neuronal populations in our 3D network, using a fast custom-developed, temporal-focusing based imaging system with frame rates of up to 200 Hz, and will present the basic characteristics of network activity patterns. Furthermore, we will present viability and immunohistological analysis of the forming networks.

Involvement of endogenous ouabain in the regulation of cell viability

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The endogenous cardiac steroid-like compounds, endogenous ouabain (EO) in particular, are present in the human circulation and are considered putative ligands of the inhibitory binding site of the plasma membrane Na^+, K^+-ATPase. A vast amount of data shows that, when added to cell cultures, these steroids promote the growth of cardiac, vascular and epithelial cells. However, the involvement of the endogenous compounds in the regulation of cell viability and proliferation has never been addressed experimentally. We show that EO is present in mammalian sera and CSF, as well as in commercial bovine and horse sera. The lowering of serum EO concentration by the addition of specific anti-ouabain antibodies caused a decrease in the viability of several cultured cell lines. Among these, neuronal NT2 cells were mostly affected, whereas no reduction in viability was seen in rat neuroendocrine PC12 and monkey kidney COS-7 cells. The anti-ouabain antibody-induced reduction in NT2 cell viability was significantly attenuated by the addition of ouabain and was not observed in cells growing in serum-free media. Furthermore, the addition to the medium of low concentrations (nM) of the cardenolide ouabain, but not of the bufadienolide bufalin, increased NT2 and PC12 cell viability and proliferation. In addition, at these concentrations both ouabain and bufalin caused the activation of ERK1/2 in the NT2 cells. The specific ERK1/2 inhibitor U0126 inhibited both the ouabain-induced activation of the enzyme and the increase in cell viability. Furthermore, anti-ouabain antibodies attenuated serum-stimulated ERK1/2 activity in NT2 but not in PC12 cells. Hence, our findings support the notion that activation of the ERK1/2 signaling pathway is obligatory but not sufficient for the induction of cell viability by EO. Cumulatively, our results suggest that EO plays a significant role in the regulation of cell viability.

Magnes Plenary Lecture

Bruce P. Bean

Pharmacology of voltage-dependent ion channels

Bruce P. Bean

Harvard Medical School

Voltage-gated ion channels are targets of many drugs, including local anesthetics, anticonvulsants, antiarrhythmics, and antihypertensives. In almost all cases, clinically useful drugs are found to bind to ion channels in a manner that is strongly regulated by the voltage-dependent gating of the channels. For example, drugs targeted to voltage-gated sodium and calcium channels typically bind with low affinity to resting closed states and bind with much higher affinity to open states and inactivated states that are reached when the membrane is depolarized. This state-dependent binding, originally characterized by Hille as the "modulated receptor hypothesis" for local anesthetic binding to sodium channels, can explain the ability of many drugs to allow normal activity of nerve and muscle cells while disrupting pathological activity. The lecture will review state-dependent interactions of drugs with voltage-dependent ion channels, focusing on interactions of local anesthetics and anticonvulsants with sodium channels and asking how natural patterns of action potential firing in different types of neurons influence drug block.

Session E

Detection of sensory stimuli:

from the pleasure of light touch to suffering from devastating pain

Interleukin-1 (IL-1) Antagonizes Morphine Analgesia and Underlies Morphine Tolerance

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Pain sensitivity reflects a balance between pain facilitatory and inhibitory systems. To characterize the relationships between these systems we examined the interactions between the analgesic effect of morphine and the anti-analgesic effect of the pro-inflammatory cytokine interleukin-1 (IL-1). Administration of a neutral dose of IL-1 abolished morphine analgesia in mice, whereas acute or chronic blockade of IL-1 signaling significantly prolonged and potentiated morphine analgesia. Morphine-induced analgesia was also extended in strains of mice genetically impaired in IL-1 signaling. The finding that IL–1 produces a marked anti-analgesic effect, suggests that it may also be involved in the development of opiate tolerance. Indeed, genetic or pharmacological blockade of IL-1 signaling prevented the development of tolerance following repeated morphine administration. Moreover, acute administration of iterleukin-1 receptor antagonist (IL-1ra) in mice, either immediately following the cessation of acute morphine analgesia, or following the development of chronic morphine tolerance, re-instated the analgesia, suggesting that blockade of the IL-1 system unmasks the analgesic effect of morphine. These findings suggest that morphine produces an IL-1-mediated homeostatic response, which serves to limit the duration and extent of morphine analgesia and which underlies the development of tolerance.

Probing the pain pathway using natural toxins

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The ability of an organism to sense and react to noxious stimuli through the pain pathway is essential for its survival. Pain sensation can be divided into three principal categories: acute nociception, inflammatory and neuropathic pain. Our overall objective is to obtain novel insights into the molecular mechanisms that underlie acute and inflammatory pains. Venoms from various species contain an evolutionarily honed pharmacopeia of natural toxins that target membrane receptors and ion channels to produce shock, paralysis, pain, or death. These toxins have evolved to interact with functionally important protein domains making them invaluable reagents with which to probe mechanisms underlying receptor and channel activation. I will describe the use of a toxin from the Earth Tiger tarantula that serves as a specific and irreversible agonist for the heat and capsaicin receptor, TRPV1. This toxin contains two independently folded inhibitor cysteine knot (ICK) domains, endowing it with an antibody-like bivalency that results in extremely high avidity for its multimeric channel target, making it a powerful new biochemical tool for probing channel function. I will further describe the use of this toxin to study the mechanisms underlie neurogenic inflammation.

Illuminating diabetic neuropathy - novel systems facilitating *in-vivo* and *in vitro* investigations

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Peripheral neuropathy is a devastating diabetes-related complication imposing vast morbidity and mortality, however its complicated pathophysiology hampers major advances in its research and treatment. We have set to establish novel *in-vivo* and *ex-vivo* models to monitor changes that are taking place in the peripheral nervous system during the course of diabetes.

Our non-invasive *in-vivo* model applies two-photon microscopy to evaluate nerves ends in footpads of mice expressing a fluorescent neuronal genetically encodedd tracer. Using this technology we documented qualitative and quantitative diabetes-specific alterations, the detection of which has previously required employing cumbersome invasive methods. The *ex-vivo* system simulates the native microenvironment of the nerve ending via a unique co-culture of primary neurons and skin. In the experiments conducted we interchanged tissues originating from healthy or disease afflicted animals and could therefore differentially investigate their virtues. The assays demonstrated that axonal degeneration is correlated with a decrease in the supportive competence of diabetic skin, yet not with the basal growth capacity of the neurons or their tentative ability to respond to skin-derived cues. Thus target-organ malfunction rather than intrinsic neuronal factors is the major culprit of early diabetic neuropathy. Overall, the illustrated models offer novel experimental platforms that may facilitate holistic investigation of diabetic neuropathy.

Mechanisms and coding of object localization by whisking rats

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Rats use their large facial hairs (whiskers) to detect, localize and identify objects in their proximal three-dimensional (3D) space. Our data indicate that object location in 3D is encoded in an orthogonal scheme: each primary-afferent can signal object location by a spatial (labeled-line) code for the vertical axis (along whisker arcs), a temporal code for the horizontal axis (along whisker rows), and an intensity code for the radial axis (from the face out). The recoding of these signals as reliable representations in the thalamocortical network appears to be based on an iterative convergence process of several (~4) whisking cycles, involving fast bottom-up and slow top-down waves via the thalamus and somatosensory cortices (S1 and S2) and multiple neural codes. An important factor in this convergence process is the movement of the sensory organ, whiskers in this case. Our rat and human data suggest that perception of object location does not result from sensory processing per se, but rather emerges from a closed-loop motor-sensory convergence process.

Session F

Modeling human diseases in the lab

Experimental models of cardiac volume overload

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Volume overload of the left ventricle of the heart is a pathophysiological state with many common etiologies, including remodeling after myocardial infarction, mitral and aortic regurgitation, and arterio-venous shunts. These states increase diastolic and often systolic wall stress- thus increasing cell death and enhancing further myocardial remodeling, creating a vicious cycle.

Modeling such disease states is problematic, as many of the etiologies for these states cannot be reliably recreated in animal models. This talk will describe the published models for these diseases, and discuss the conundrum of combined etiologies (such as mitral regurgitation induced by remodeling after myocardial infarction). We will discuss in depth one such a model, defining it at the anatomical, physiological and molecular level. We will subsequently describe interventions in this model to simulated treatment in the clinical disease state.

Using a murine model of "Asthma" to investigate the link between airway hypperresponsiveness and airway remodeling

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Airway hyperresponsiveness (AHR) is the cardinal physiological hallmark of asthma. Although chronic airway inflammation is considered to be the primary abnormality in asthma, anti-inflammatory therapy has little impact on AHR. "Airway remodeling" refers to the structural changes present in the airways of patients with chronic asthma such as increased airway fibrosis and smooth muscle and is thought to account for refractoriness to therapy and to contribute to AHR. Extra domain A (EDA)-containing fibronectin [EDA-FN], an alternatively-spliced form of the extracellular matrix protein fibronectin, has been implicated in fibroblast differentiation during wound healing and tissue fibrosis. We have investigated the link between airway fibrosis and hyperresponsiveness using a murine model of chronic allergen-induced experimental asthma. EDA -/- and wild type (WT) mice were sensitized and exposed to inhaled ovalbumin (OVA) or saline for 5 weeks and markers of airway remodeling (peribronchial fibrosis, smooth muscle area, mucus-producing cell number, bronchoalveolar cell counts and airway hyperresponsiveness were evaluated. Fibroblast activation and differentiation were evaluated ex vivo using OVA-treated WT and EDA -/- lung fibroblasts. Exposure to OVA increased EDA-FN expression in lung tissue and primary lung fibroblasts. OVA-treated EDA -/- mice showed reduced airway fibrosis, AHR and impaired expression of TGF-β1 and interleukin (IL)-13 without changes in airway inflammation or other aspects of remodeling. Lung fibroblasts from OVA-treated EDA -/- mice exhibited reduced proliferation, migration, α-smooth muscle actin expression, collagen deposition and impaired TGF-β1 and IL-13 release as compared to WT mice. This model enables us to dissect out the contribution of different aspects of airway remodeling and to better understand the factors contributing to airway hyperresponsiveness.

Finite element models for cardiac function

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Computational approaches that simulate healthy and diseased hearts have the potential to improve our understanding on the functioning of the myocardium and hence to assist in developing better treatments for cardiac diseases, which account for significant morbidity and mortality. The heart is an extremely durable pump with many correction circles that at times damage the heart, rather than supporting it, and may ultimately result in cardiac arrest and death.

For understanding the mechanical behaviour of the heart, including the basic properties of cardiac muscles, a novel modelling approach is developed along the following steps: a) a constitutive mathematical model is constructed based on mechanical experiments on myocardial tissue, b) patient-specific geometry and boundary conditions are acquired, including the micro-structure of the orientation of the heart muscle fibers, from MRI data, c) combining all the above into an organ-scale finite element (FE) model. The ultimate goal of our research is to develop a validated FE model, which, in conjunction with cardiac expertise, will facilitate the quantitative prediction of cardiac diseases.

Heart modelling concepts and preliminary results from an uni-axial stretch experiment used to obtain the constants of the constitutive model of the rat cardiac muscle will be presented. At the end, future research plans will be presented.

Modeling Catecholaminergic Polymorphic Ventricular Tachycardia – a congenital cardiac arrhythmia, using induced pluripotent stem cells-derived cardiomyocytes.

Ofer Binah

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Sudden cardiac death caused by ventricular arrhythmias is a disastrous event, especially when it occurs in young individuals. Among the 5 major arrhythmogenic disorders occurring in the absence of a structural heart disease is Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), which is a highly lethal form of inherited arrhythmias. Our study focuses on the autosomal recessive form of the disease caused by the missense mutation D307H in the cardiac calsequestrin gene, CASQ2. Since CASQ2 is a key player in excitation contraction coupling, the derangements in intracellular Ca^{2+} handling may cause delayed afterdepolarizations (DADs) which constitute the mechanism underlying CPVT. To investigate catecholamine-induced arrhythmias in the CASQ2 mutated cells, we generated for the first time CPVT-derived iPSCs by reprogramming fibroblasts from skin biopsies of two patients, and demonstrated that the iPSCs carry the CASQ2 mutation. Next, iPSCs were differentiated to cardiomyocytes (iPSCs-CMs) which expressed the mutant CASQ2 protein. The major findings were that the β-adrenergic agonist isoproterenol caused in CPVT iPSCs-CMs (but not in the control cardiomyocytes) DADs, oscillatory arrhythmic prepotentials, after-contractions and diastolic $[Ca^{2+}]_i$ rise. Electron microscopy analysis revealed that compared with control iPSCs-CMs, CPVT iPSCs-CMs displayed a more immature phenotype with less organized myofibrils, enlarged sarcoplasmic reticulum cisternae and reduced number of caveolae. In summary, our results demonstrate that the patient-specific mutated cardiomyocytes can be used to study the electrophysiological mechanisms underlying CPVT.

Posters

Poster # 1

The prostaglandin EP_1 receptor downregulates the expression of the enzyme cyclooxygenase-2 by facilitating its proteasomal degradation

Ariz Haddad

The enzyme cyclooxygenase-2 (COX-2) is rapidly and transiently upregulated by a large variety of signals, and as such is implicated in many pathologies including inflammation and tumorogenesis. While there is a plethora of information regarding signals that cause its upregulation, much less is known about mechanisms that actively downregulate COX-2. Here we show that one of the receptors that are stimulated by the COX-2 catalysis products, acts as a regulator of its expression. The G protein-coupled receptor prostaglandin E1 (EP_1) reduces the expression of COX-2 in a concentration-dependent manner through a mechanism that does not require receptor activation. At the posttranslational level, we find that EP_1 decreases the levels of COX-2 by facilitating its proteolysis through the substrate-independent degradation pathway, and does not interfere with the entry of COX-2 into the ER-associated degradation (ERAD) cascade. These findings propose a new role for the EP_1 receptor in resolving inflammation through downregulation of COX-2.

Poster # 2

A novel probe for tracking Syntaxin 1A conformational changes during exocytosis: implications for the Kv2.1-induced facilitation of exocytosis.

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We have constructed a probe that reports, through fluorescence resonance energy transfer (FRET), conformational changes of syntaxin 1A (Syx) during high K^+ stimulation that elevates intracellular Ca^{2+} concentration and induces exocytosis. This probe was utilized in our study of the non-channel function of the voltage-gated potassium channel, Kv2.1, to facilitate exocytosis. Classically, voltage-gated potassium channels are viewed as indirectly exerting an inhibitory function on exocytosis by influencing the membrane potential via K+ fluxes. Recently, we identified a new role for Kv2.1 in facilitating vesicle release from neuroendocrine cells, as well as from soma of neurons. Importantly, the Kv2.1-induced facilitation was shown to be K^+ flux-independent and to be mediated by direct association of Kv2.1 C1a domain with Syx. Further, the facilitation of exocytosis was shown to be mediated by an increase in total number of exocytotic events due to enhanced rate of vesicle recruitment during prolonged cytosolic Ca^{2+} elevation. Hence, it was suggested that Kv2.1 belongs to a group of proteins that regulate vesicles recruitment by virtue of their direct interaction with Syx. Since Kv2.1 was shown by us and others to interact with both the open conformation of Syx and the binary t-SNARE complex, but not with the ternary SNARE complex, we hypothesized that Kv2.1 stabilization of open Syx and/or the t-SNARE complex underlies the Kv2.1-induced recruitment of vesicles. We used the probe to confirm our hypothesis.

* Equal contributors

Poster # 3

Mitochondrial Na^+/Ca^{2+} exchanger modulates glucose dependent Ca signaling and temporal pattern of insulin secretion

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The mitochondrial Na^+/Ca^{2+} exchanger (NCLX) is considered the main extrusion pathway of calcium from mitochondria and thereby, in global intracellular Ca^{2+} signals. The link NCLX - insulin secretion is controversial, due to the lack of the specific pharmacological tools. Taking the advantage on the novel molecular tools that arise from the cloning and functional characterization of this exchanger in our laboratory, we elucidated the physiological role of the NCLX in insulin secretion. To investigate the physiological role of NCLX in pancreatic β cells, the expression of NCLX was knocked down and the activity of NCLX was reduced by a mutation to the catalytic site (NCLX S468T). The effect of knockdown expression of the NCLX as well as the effect of dominant negative NCLX S468T on cytosolic and mitochondrial Ca^{2+} showed a 40% reduction in calcium influx rate via the L-type calcium channels (LTCC) and almost a completely suppression of the mitochondrial calcium efflux, respectively. We therefore determined the effect of NCLX siRNA as well as the effect of NCLX S468T on glucose dependent Ca^{2+} signaling on primary β cells. The glucose dependent changes in rate of cytoplasmic Ca^{2+} was reduced in cell expressing either NCLX siRNA or NCLX S468T indicating that knockdown of NCLX expression or activity is down regulating Ca^{2+} influx by the LTCC. We also determined if silencing NCLX affects mitochondrial metabolism and found that while NCLX siRNA didn't change the glucose dependent metabolic rate determine by monitoring the ratio of $NADPH/NAD^+$, but rather increased the basal metabolic activity. Finally, glucose dependent insulin secreted profile was modulated following the knock down of NCLX leading to a delay in the hormone secretion. In conclusion, mitochondria via NCLX regulate Ca^{2+} signaling through LTCC thereby the temporal profile of insulin secretion.

Keywords: mitochondria, calcium homeostasis, insulin secretion

Poster # 4

Lithium regulates the G protein-activated K^+ channels: possible linkage with bipolar disorder

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Bipolar disorder (BPD), also known as manic-depressive illness, is a brain disorder that causes unusual shifts in a person's mood, energy, and ability to function. Lithium (Li^+) is a widely used treatment for bipolar disorder. Many theories were proposed over the years as to its molecular mechanism, however to this day it still remains unclear. One theory of BPD postulates the involvement of the activity of G proteins as a possible mechanism of the disease. Studies have shown that Li^+ can modulate the function of G proteins. We hypothesized that Li^+ modulation of G proteins will also affect G protein effectors including ion channels. G-protein activated K^+ channels (GIRK) are directly modulated by G-proteins derived from activation of Gαi/o coupled receptors. Our preliminary studies revealed that therapeutic doses of lithium (0.5-2 mM) activated neuronal GIRK1/2 channels expressed in *Xenopus* oocytes by increasing their basal activity. This increase of basal activity strongly depended on the coexpression of G protein alpha subunit ($G\alpha_{i3}$). The specific role of the G protein βγ subunit remains unclear, since at the whole cell level, the effect of lithium was not blocked by coexpression of Gβγ scavenger proteins. Moreover, the effect of lithium seems to depend on channel subunit composition, since it activated homotetrameric GIRK2 channels but not GIRK1/2 heterotetramers. In addition, we found that lithium decreased the GIRK response to $GABA_B$ receptor agonist Baclofen in cultured neurons of mouse hippocampus. We propose that the complex effect of lithium on GIRK channel activity may be related to the overall "mood stabilizing" effect of lithium in bipolar patients. These findings may bring us closer to understanding the complex mechanism of lithium action in the brain and perhaps it will help us understand the pathophysiology of the disorder itself.

Poster # 5

Regulation of GIRK by Gβγ is influenced by channel subunit compositions

Boaz Styr, Moran Rubinstein, Uri Kahanovich, Nathan Dascal

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G protein-activated inwardly rectifying potassium channels (GIRKs) are important mediators of inhibitory neurotransmission. The four mammalian GIRK subunits (GIRK1, 2, 3, 4) form heterotetramers of GIRK1/4 found in the heart and GIRK1/2, GIRK1/3, GIRK2/3 and homotetramers of GIRK2 in the brain, showing region-specific localization. Gβγ is the major activator of GIRK and upon G protein coupled receptor (GPCR) activation, binds and opens the channel. In addition to the GPCR-dependent response (evoked response), GIRKs can also show some basal activity. This basal and evoked activity is regulated by both Gα and Gβγ in a complex fashion that is not yet fully understood.

Our electrophysiological and imaging work in oocytes and HEK cells has shown that heterologously expressed neuronal GIRK1/2 channels have high basal activity, which is Gβγ dependent and can be reversed by added Gα and restoration of the Gαβγ trimer. This is most probably caused by preferential pre-association and/or co-trafficking to the plasma membrane of GIRK1/2 with Gβγ over Gα. We now show that the cardiac GIRK1/4 channel expressed in *Xenopus* oocytes shows lower basal and larger evoked currents at expression levels similar to GIRK1/2. In comparison to GIRK1/2 the channel is also very sensitive to added Gβγ, which reduces the basal at the expense of the evoked current. Thus, it appears that small differences between the homologous GIRK2 and GIRK4 subunits substantially affect Gα and/or Gβγ regulation of GIRK1/2 and GIRK1/4. In contrast to the relatively similar GIRK1/2 and GIRK1/4 channels, GIRK1/3 shows very different properties. GIRK1/3 has a very small basal current even at relatively high levels of expression and has a relatively strong response to GPCR stimulated activation. Surprisingly, added (coexpressed) Gβγ has a biphasic effect on GIRK1/3. While GIRK1/2 and 1/4 show a Michaelis-Menten like dose response to added Gβγ, GIRK1/3 shows a bell shaped response curve indicating that paradoxically, high levels of Gβγ decrease both basal and evoked activity of the channel. The reason behind this effect is being investigated. The pattern of GIRK1/3 regulation by coexpressed Gβγ is similar to that of the homotetramer created by the GIRK1 mutant GIRK1(F137S), thus GIRK1/3 may offer a physiologically relevant approach to study the properties of GIRK1. In summary, we find that GIRK1/2, GIRK1/4 and GIRK1/3 have distinctly different basal and GPCR-evoked activities that are differentially regulated by Gβγ and Gα. These differences are crucial to our understanding of the function of GIRK channels in diverse parts of the brain and in the heart.

Poster # 6

Rapamycin (Sirolimus) protects against hypoxic/reoxygenation damage in primary heart cultures via opening of the ryanodine receptor and PKC involvement

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We have shown previously (El-Ani et al 2011) that rapamycin induced cardioprotection against hypoxia/reoxygenation (H/R) damage in primary heart cultures, via stimulation of Na^+/Ca^{2+} exchanger. Ryanodine receptors play an essential role in excitation-contraction coupling by regulating the delivery of Ca^{2+} from the sarcoplasmic reticulum (SR) to the contractile apparatus. Our goal was to study the interactions between rapamycin and ryanodine receptor in H/R heart cultures and to elucidate the protective mechanism induced by rapamycin. Rat heart cultures were exposed to H/R. Rapamycin (10 μM) and Ryanodine (2 μM) attenuated by 40-50% lactate dehydrogenase (LDH) leakage from heart cultures that were subjected to H/R and increased cell viability as measured by MTT assay. Ryanodine at concentrations known as closing the ryanodine channels (100 μM), inhibited the cardioprotective effect of rapamycin, as detected by LDH release. Moreover, when 100 μM ryanodine was added to hypoxic heart culture, rapamycin did not reduce cytosolic Ca^{2+} ($[Ca^{2+}]_i$) following hypoxia. To study the importance of Ca^{2+} entry to the cell on cardioprotection against H/R by rapamycin, verapamil (a calcium channel blocker) was included during H/R insult. It was found that both rapamycin and verapamil (10-30 μM) each one alone or together gave protection to the cells as detected by LDH release to the medium following H/R. No significant difference was obtained between cultures treated with rapamycin or verapamil or both drugs during H/R.

Chelerythrine (a PKC inhibitor, 2 μM) inhibited the protective effect of rapamycin against H/R. Immunostaining for PKCε of heart cultures treated with rapamycin following H/R showed translocation of PKCε to the nucleus. Immunostaining for PKCε of heart cultures treated with rapamycin and chelerythrine before H/R revealed inhibition of PKCε translocation to the nucleus. Western blotting reveals also increase of 40% for PKCε in H/R cultures, and similar protein expression to control in H/R cultures treated with rapamycin. Based on the current and previous results, we suggest that rapamycin induced cardioprotection against H/R damage in primary heart cultures, via opening of the ryanodine channel to a constant flow that stimulate the Na^+/Ca^{2+} exchanger, and that PKC is involved in this cardioprotection.

Poster # 7

Selective Rerulation of KCNQ2, but not KCNQ3, by Syntaxin 1A and Ca2+-Calmodulin via N-C termini Interactions

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M-channels are slowly activated, non-inactivating, voltage-dependent potassium channels. Heteromeric assembly of subunits, encoded by two members of the KCNQ gene family KCNQ2 (Q2) and KCNQ3 (Q3), recapitulate the functional properties of the M-current. Q2 and Q3 are co-expressed on the cell body and dendrites of hippocampal and cortical neurons. Importantly, Q2 but not Q3, is expressed on neuronal axons, where it might regulate action potential propagation or neurotransmitter release. Previously, we showed that Syntaxin 1A **(Syx)** physically interacts with homomeric Q2 in brain synaptosomes and in *Xenopus* oocytes. In oocytes, this interaction results in a reduction of the current's amplitude and reduction of the channel's activation rate. In vitro pull down revealed that Syx specifically binds the helix A domain located in the C-terminus **(CT)** of both Q2 and Q3. However, binding of Syx to Helix A does not mediate Syx's effect on the channel. Importantly, similar characteristics are shared by calmodulin **(CaM)**, another, well accepted, regulator of KCNQ2 currents. In the present study, we further characterize the effects of Syx and CaM on KCNQ2 versus KCNQ3 currents, using electrophysiological analyses at the level of whole cell and single channel, combined with Fluorescence Energy Transfer **(FRET)** and biochemical analyses. We show direct interactions between the amino- and carboxy- termini of KCNQ2 and KCNQ3, basal and induced, in which the N-terminus **(NT)** plays a functional role in the effect of Syx and the CT in the effect of CaM on channel gating. We suggest a novel mechanism of modulation of KCNQ2 channel gating via rearrangements in the relative orientation of the cytoplasmic domains, induced by both a membrane-anchored (Syx) and a cytosolic (CaM) regulatory proteins.

Poster # 8

Near Infrared Optical Imaging of Epidermal Growth Factor Receptor in Colorectal Tumors.

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The increase in the aging population has led to a growing interest in achieving a better understanding of the aging process and of diseases that are predominantly expressed during advancing age. Since the structural and, in turn, the functional integrity of the mucosa of the gastrointestinal tract (GI) are maintained by constant renewal of cells, a detailed knowledge of the events that initiate and regulate mucosal proliferative processes is essential for a better understanding of the normal aging process as well as age-associated dysfunctions, including malignancy such as colorectal carcinoma. EGF and TGF-α appear to be involved in regulating mucosal proliferation during aging. Aging is associated with increased level and activation of EGF-receptor (EGFR), the common receptor for EGF and TGF-α. Also, colorectal cancer which arises from adenomatous polyps and develops over a relative long period of time is related to overexpression of epidermal growth factor receptor (EGFR) and its oncogenic forms. Therefore EGFR may represent a useful biomarker for GI aging and cancer.

In this study we conjugated EGF with a NIR dye (EGF-NIR, IRDye800CW) and demonstrated its specific and selective pharmacological properties by measuring its receptor binding activities *in vitro*, with different CRC cell lines, using NIR imaging with two lasers Odyssey® InfraredImager. A very significant effect of mucin type III (produced by Brunner's glands to protect the mucosa) on reducing the nonspecific binding of EGR-NIR was measured. *In vivo* imaging of mice bearing rectal orthotopic tumors indicated accumulation of the imaging probe at 24 and 48 hours in the tumor and liver. *In situ* experiments using EGFR positive and negative human CRC biopsies further supported the specificity of binding of EGF-NIR to the receptor positive tumors, as was validated using Western blotting.

EGF-NIR is a promising molecular imaging agent in studies to evaluate EGFR level of expression in GI aging, cancer and different physiological conditions.

Poster # 9

Structural and functional basis for Zn^{2+} selectivity by ZnT zinc transporters

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As opposed to Zn^{2+}, an essential macro-nutrient necessary for a vast variety of cellular functions, Cd^{2+} is a non-physiological ion that permeates cells by metal mimicry, and is exceptionally toxic, leading to kidney, respiratory and skeletal disorders.

Zn^{2+} homeostasis is a tightly regulated process, maintained by a surprisingly great variety of cytoplasmic proteins, such as metallothioneins and membrane embedded transporters, mainly ZIPs and ZnTs. Zips mediate Zn^{2+} influx but as most mammalian cation transporters, do not show selectivity for Zn^{2+} over Cd^{2+} and are therefore a major pathway for cadmium influx; ZnTs mediate Zn^{2+} efflux and sequestration into intracellular compartments and are thereby involved in lowering cytoplasmic Zn^{2+} concentrations. By comparing cytoplasmic and vesicular Zn^{2+} and Cd^{2+} transport rates, we found that ZnTs, in contrast to ZIPs, are highly selective for Zn^{2+} over Cd^{2+} transport.

Modeling of ZnT5 based on the crystal structure of the bacterial Zn^{2+} transporter, YiiP indicated that 3 of the 4 residues which coordinate the Zn^{2+} binding site are evolutionally conserved. However, the fourth, is substituted from an Aspartate (Asp45) found in Yiip to a Histidine residue in mammalian ZnT5 (His451) and ZnT8 (His106). Replacing this Histidine to an Aspartate was sufficient to enhance ZnT-dependent cytoplasmic removal and vesicular uptake of Cd^{2+}. Reciprocally, replacing this Aspartate to a Histidine residue, in the bacterial ortholog, Yiip, abolished Cd^{2+} transport mediated by Yiip. Finally, we show that metal selectivity evolves through a strong reduction in binding affinity but not translocation of Cd^{2+}

Thus, our results identify the first class of mammalian transporters and the structural motif required to discriminate between Zn^{2+} and Cd^{2+} and show that metal selectivity is tuned by a coordination-based mechanism that lowers the thermodynamic barrier to Cd^{2+} binding, making ZnT expressing cells an "end point" to Cd^{2+} transport, by trapping Cd^{2+} ions and preventing their dissemination via both the vectorial and trans-epithelial pathways.

Poster # 10

A new class of 'Salan' Titanium(IV) compounds as anti-cancer drugs

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Cisplatin is a widely used platinum-based metallo-chemotherapeutic drug that is considered an efficient treatment mainly for testicular and ovarian cancers. However, its narrow activity range and severe side effects trigger studies of other potent transition metal complexes.

Titanium compounds have previously been investigated, as safer alternatives to cisplatin, for impairing tumor growth. Two classes of Ti(IV) complexes: derivatives of titanocene dichloride and of budotitane, have been found to be active in this respect and led to reduced side-effects relative to platinum compounds. However, a crucial drawback of these compounds is their rapid hydrolysis in biological media, leading to unidentified aggregates, hampering the elucidation of mechanistic aspects, and resulting in these complexes ultimately failing clinical trials.

To address the hydrolysis issue, we have recently introduced a new class of C_2-symmetrical Ti(IV) complexes of "salan" type diamino bis(phenolato) ligands. These compounds demonstrate exceptionally high hydrolytic stability (up to weeks in mixed water/organic solvent, compared to minutes of the known compounds), as well as enhanced cytotoxic activity towards a variety of cancer cell lines in vitro. The IC_{50} of different derivatives of these compounds towards murine mammary carcinoma, lymphoma, multi-drug resistant lymphoma, as well as human leukemia, melanoma and pancreatic cancer cell lines, is in the range of 0.6 – 5.7μM, as compared to 70-100μM of the known compounds and 50-60μM of cisplatin. Preliminary results indicate that the new compounds impair cell growth in the G1 phase of the cell cycle throw p53 pathway. In vivo experiments are now under way to determine both the safety, as well as the anti-cancer activity of these potential drugs.

Poster # 11

Spatio-temporal characteristics of Na^+ influx in axon initial segment of Layer 5 pyramidal neurons

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In neocortical pyramidal cells, the axon initial segment (AIS) represents a highly specialized structure that plays a pivotal role in synaptic integration and action potential (AP) generation. We previously showed that the average Na^+ channel mediated charge transfer in the AIS is 1.5 - 3 times larger than in the soma. The details of Na^+ channel distribution over the AIS length, however, remain unknown. Here we explored the spatial and temporal pattern of Na^+ influx into the AIS elicited by a single AP using a combination of patch-in-slice recording and high-speed fluorescence imaging of the Na^+-sensitive indicator SBFI. We found that Na^+ influx was maximal in a 18 ± 2 μm (n=5) long region, located at a distance of 8 ± 2 μm from the soma and 27 ± 4 μm from the edge of the myelin. In a simplified compartmental model that encompassed the fundamental morphological features of a layer 5 pyramidal neuron as well as the experimentally determined pattern of Na^+ channel distribution. AP initiation occurred at a distance of ~40 μm from the soma, distal to the point of the maximal Na^+ entry. Our model further revealed that AP initiation requires a "spacer"-resistive element of optimal length to be present between the Na^+ influx "hotspot" and the soma. Thus, placing the hotspot closer to the soma by shortening the spacer element beyond the optimal length caused a significant reduction in neuronal excitability while a more distal location of the hotspot had similar effect. The strength of the synaptic input required to produce an AP was minimal when the spacer length was in the range of 25-40 μm. In this optimal location, the rapidly rising AP was sufficiently isolated from the somatodendritic capacitive load while attenuation of the relatively slowly rising synaptic potentials was insignificant. The optimal spacer length was critically dependent on the resistivity of axoplasm, on axonal and somatic diameters and on the activation rate and density of Na^+ channels.

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Poster # 12

ACTIVATION OF PHOSPHORYLATION PATHWAYS IN EPITHELIAL CELL LINES THROUGH THE ZINC SENSING RECEPTOR

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Zinc is an essential microelement required for cell proliferation. This ion has been recognized as an important signaling molecule in epithelial, neuronal and immune system cells. Extracellular Zn^{2+} activates a specific Zn-sensing receptor, ZnR, which is a Gq-coupled receptor activating the IP_3 pathway in epithelial cells among them the salivary ductal cell line HSY. We demonstrate here that ZnR activity in HSY cells is mediated by GPR39, which was until recently an orphan GPCR. ZnR was shown to induce the activation of two major pathways leading to cell survival and proliferation, the MAP and PI3 kinases but the downstream signaling of this pathway is unknown. Our data indicates that the ZnR-dependent Ca^{2+} release triggers phosphorylation of p70S6k, mediated by physiological concentrations of extracellular Zn^{2+}. Inhibition of the IP_3 and PI 3-kinase pathways, both activated via the ZnR, attenuates the Zn^{2+}-dependent activation of p70S6K. Using a colorimetric assay we demonstrate that activation of the ZnR enhances HSY cell proliferation. Thus, our data indicates that Zn^{2+}, through the ZnR, activates the mTOR pathway to enhance cell proliferation. This may provide an important link between extracellular zinc and epithelial cell proliferation.

Keywords: ZnR, mTOR, proliferation, HSY, epithelial cells

Poster # 13

Dexamethasone influences CD300a inhibitory receptor function on human and murine mast cells

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Background: The gold standard for allergy treatment are glucocorticosteroids (GC). While potent, these drugs induce many side effects. Therefore the possibility to decrease their use is warranted. The key effector cells of allergy, mast cells (MC), which are mainly activated by an IgE-dependent mechanism, are downregulated by different factors including membrane receptors containing Immunoreceptor tyrosine-based inhibitory motifs (IR). We have previously reported the expression and downregulatory function of CD300a, an IR which belongs to the Ig-like superfamily, on human and mouse MC IgE-dependent activation.

Objectives: The present study aimed to evaluate the effect of dexamethasone combined with αCD300a activating antibodies on the function of murine and human mast cells**.**

Materials and methods: Cord blood derived (CBMC) and murine bone marrow derived (BMMC) MC were sensitized o.n. with IgE +/- dexamethasone (10^{-9}M-10^{-8}M). CD300a expression was evaluated by flow cytometry. For cell activation, MC were then incubated +\- αCD300a antibodies (30 mins) followed by a cross-linking antibody (30 min). Activation was measured by beta-hexoseaminidase release.

Results: When incubated with dexamethasone, CD300a expression did not change on CBMC but slightly decreased on BMMC. Dexamethasone alone or with αCD300a antibodies, dose-dependently decreased BMMC activation/ degranulation. Moreover, in CBMC dexamethasone was found to upregulate DOK-1 expression, a protein known to recruit the SHP-1 phosphatase required for IR.

Conclusions: Our study shows a possible co-operation between dexamethasone and CD300a in downregulating MC IgE-dependent activation, possibly due to a synergistic activity on Dok-1. In allergic inflammation it might be therefore possible to spare GC when CD300a is activated.

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Poster # 14

Extracellular Nucleotide Derivatives Protect Cardiomyocytes against Hypoxic Stress

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Rationale: Extracellular nucleotides have widespread effects and various cell responses. Whereas the effect of a purine nucleotide (ATP) and a pyrimidine nucleotide (UTP) on myocardial infarction has been examined, the role of different purine and pyrimidine nucleotides and nucleosides in cardioprotection against hypoxic stress has not been reported.

Objective: To investigate the role of purine and pyrimidine nucleotides and nucleosides in protective effects in cardiomyocytes subjected to hypoxia.

Methods and results: Rat cultured cardiomyocytes were treated with various extracellular nucleotides and nucleosides, before or during hypoxic stress. The results revealed that GTP or CTP exhibit cardioprotective ability, as revealed by lactate dehydrogenase (LDH) release, by propidium iodide (PI) staining, by cell morphology, and by preserved mitochondrial activity. Pretreatment with various P2 antagonists (suramin, RB-2, or PPADS) did not abolish the cardioprotective effect of the nucleotides. Moreover, $P2Y_2^{-/-}$, $P2Y_4^{-/-}$, and $P2Y_2^{-/-}/P2Y_4^{-/-}$ receptor knockouts mouse cardiomyocytes were significantly protected against hypoxic stress when treated with UTP. These results indicate that the protective effect is not mediated via those receptors. We found that a wide variety of triphosphate and diphosphate nucleotides (TTP, ITP, deoxyGTP, and GDP), provided significant cardioprotective effect. GMP, guanosine, and ribose phosphate provided no cardioprotective effect. Moreover, we observed that tri/di-phosphate alone assures cardioprotection. Treatment with extracellular nucleotides, or with tri/di-phosphate, administered under normoxic conditions or during hypoxic conditions, led to a decrease in reactive oxygen species production.

Conclusions: Extracellular tri/di-phosphates are apparently the molecule responsible for cardioprotection against hypoxic damage, probably by preventing free radicals formation.

Poster # 15

Potassium leak channels (K_{2P}) activity is regulated by membrane cholesterol

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Background: Potassium leak channels (K_{2P}) play a central role in setting the membrane resting potential and their activity is regulated by physical and chemical effectors such as temperature, pH, mechanical stretch and phosphorylation. Cholesterol is an essential structural component of mammalian cell membranes and is a main component of lipid rafts, which are cholesterol/sphingomyeline enriched microdomains in the membrane. It was found that membrane cholesterol regulates the activity of several membrane proteins. In this study, we characterized the effect of membrane cholesterol levels on K_{2P} channels activity.

Results: We studied the influence of membrane cholesterol levels on several members of the K_{2P} family: human $K_{2P}2.1$, $K_{2P}3.1$, $K_{2P}5.1$ and $K_{2P}9.1$ channels as well as $K_{2P}0$ channels from *Drosophila melanogaster*, all expressed in *Xenopus laevis* oocytes. Functionality and biophysical properties of the channels were assayed using the two electrode voltage clamp (TEVC) technique. Depletion of membrane cholesterol using MβCD altered the activity of most tested channels. Application of 1mM MβCD reduced $K_{2P}2.1$ currents by 80% and increased $K_{2P}0$ currents 7-fold. Other channels displayed milder responses. In accordance with these results, lipid raft disruption by sphingomyelinase had effects similar to those of MβCD on $K_{2P}2.1$ channels. Conversely, mutant $K_{2P}2.1$ and $K_{2P}0$ channels that are insensitive to phosphorylation were not affected by cholesterol depletion.

Conclusion: The activity of members of the K_{2P} potassium channels is regulated by membrane cholesterol. We speculate that $K_{2P}2.1$ channels reside in lipid rafts and that cholesterol affects K_{2P} channel current through an indirect mechanism.

Poster # 16

VP12 - pharmacological tool for development of novel drugs selective for α2β1 integrin*

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The integrins are family of receptors on the cell surface, heterodimers of α and β subunits, which adhere to multiple ligands and mediate cell-cell and cell-extracellular matrix interaction. Besides their structural function integrins have been identified as signaling molecules, resulting in cytoskeleton reorganization (shape change, adhesion, migration), regulation of cell proliferation and cell survival and apoptosis. The collagen receptor - α2β1 integrin is expressed on many tumor cells such as melanoma, rhabdomyosarcoma, ovarian epithelial carcinoma and mammary carcinoma. A modern approach for cancer treatment is based on inhibition of the cells adhesion, migration, growth and generation of a network of blood capillaries, i.e., angiogenesis. The integrin α2β1 seems to be promising target for inhibition of angiogenesis and tumor progression.

C-type lectin-related protein (CRPL), called VP12 was isolated from Israeli snake *Vipera palestinae* venom and purified by HPLC and ion exchange FPLC. In adhesion assay, VP12 showed a potent inhibitory effect on the adhesion of integrin α2β1 overexpressing K562 cells to collagen I (IC_{50} 0.5 nM), but not on K562 cells transfected with α1 integrin subunit to collagen IV. Also, the direct interaction of VP12 with α2β1 integrin was confirmed in adhesion assay. Cells transfected with the α2 integrin subunit showed a potent, dose-dependent adhesion to immobilized VP12, whereas non-transfected control cells did not interact with this CLRP.

VP12 selectively and potently inhibits α2β1integrin and hence will provide a lead structure to design synthetic α2β1integrin inhibitor drugs. Such collagen receptor inhibitors would enable the design of a variety of drugs towards therapy of different cardiovascular and cancer diseases.

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Poster # 17

C-terminus of $Ca_V1.2$ is the important mediator of CaBP1 regulation

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$Ca_V1.2$ is a voltage dependent calcium channel found is cardiac and brain cells plasma membranes. An intrinsic regulation of this channel is its inactivation through a conformational change of the cytoplasmic domains, by a mechanism which is extensively studied but poorly understood. The mechanism regulating the inactivation differs between tissues and depends on auxiliary proteins that contribute to this process. Two proteins from the same family are involved in calcium dependent inactivation (CDI): the ubiquitous protein calmodulin (CaM) that enhances CDI, and the tissue-specific CaBP1, that does not support CDI. Previous study on HEK cells showed that CaBP1 activity is abolished when the first half of the N terminal (NT) of the calcium channel is absent. In our study, currents via $Ca_V1.2$ channels were measured in *Xenopus* oocytes using two electrode voltage clamp technique. We expressed NT chimeras of the channel, as well as CaBP1, and identified a residual effect of CaBP1 even in the absence of the whole NT. Biochemical assays showed that CaBP1 and CaM interact with the channel at the same site on the CT, while binding two different sites on the NT. Fluorescently tagged CaM and CaBP1 showed that CaM removed the effect of CaBP1 at a molar ratio of ~a 1:1 in the cell, which indicates a competitive nature of action of the two proteins. Alanine mutagenesis of the CaM binding site on the NT did not remove the inactivating effect of CaBP1. Thus, CT but not NT appear to be the important functional and/or anchoring site for the action CaBP1, and the role of the interaction of CaBP1 with the $Ca_V1.2$ NT remains to be determined.

Poster # 18

New Fluorescent Reagents Specific for Ca^{2+}-Binding Proteins

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Ca^{2+} carries information pivotal to cell life and death via its interactions with specific sites in proteins. Although numerous Ca^{2+}-dependent activities are known and fluorescent indicators for Ca^{2+} are commercially available, many proteins responsible for these cellular activities remain unidentified. We previously developed a novel photoreactive reagent, azido ruthenium (AzRu), which strongly inhibits Ca^{2+}-dependent activities. Here, we synthesized new fluorescent ruthenium-containing reagents (AzRu derivatives) containing FITC or EITC, termed FITC-Ru and EITC-Ru, respectively. These reagents were purified and characterized with respect to their excitation and emission spectra, molar absorption coefficients and structural formula. We found that FITC-Ru and EITC-Ru specifically and markedly inhibited the activity of Ca^{2+}-dependent proteins, such as those mediating Ca^{2+} accumulation in the sarcoplasmic reticulum (SR) or in mitochondria. On the other hand, the reagents did not inhibit the activity of Ca^{2+}-independent proteins, such as glucose-6-phosphate dehydrogenase or lactate dehydrogenase. Thus both FITC-Ru and EITC-Ru specifically interacted with Ca^{2+}-dependent proteins and inhibited their activity. The fluorescence intensity of FITC-Ru is enhanced upon binding to the Ca^{2+}-binding proteins, calmodulin, calbindin-D_{28K} or myosin but not to non-Ca^{2+}-binding proteins, such as hexokinase. FITC-Ru fluorescence changes that occur upon binding to calmodulin have been used to determine apparent affinity of the compound. Ca^{2+} decreased protein-bound FITC-Ru levels, suggesting its displacement by Ca^{2+} to occur at calmodulin-Ca^{2+}- binding sites. Using confocal microscopy, we demonstrate that FITC-Ru and EITC-Ru cross the cell membrane and stain cells. In addition, the reagents protect against apoptosis and prevented VDAC oligomerization. In contrast to many reagents that serve as probes for Ca^{2+}, (i.e.Fura-2), FITC-Ru and EITC-Ru are the first fluorescent divalent cation analogs to be synthesized and characterized which specifically bind to Ca^{2+}-binding proteins and inhibit their activity. Such reagents will assist in characterizing Ca^{2+}-binding proteins, thereby facilitating better understanding of the function of Ca^{2+} as a key bio-regulator.

Poster # 19

The VDAC1 and MAVS expression in human cancer and their potential usage as novel tumor biomarkers

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Cancer cells are characterized as highly metabolic cells with increased glycolysis. The voltage-dependent anion channel, VDAC1, an outer mitochondrial membrane protein, mediates ATP, ADP and other metabolite exchanges between mitochondria and cytosol. However, VDAC1 not only controls energy production in high energy-demanding cancer cells but also plays a role in mitochondria-mediated apoptosis.

The mitochondrial anti-viral signaling (MAVS), plays a pivotal role in the induction of antiviral and inflammatory pathways but is also involved in the coordination of apoptotic and metabolic functions. MAVS proposed to regulate cell death by different mechanisms including via modulation of VDAC1 expression levels. Therefore, we have examined the protein expression levels of the two mitochondrial, apoptosis regulating proteins, VDAC1 and MAVS in several tumors. VDAC and MAVS expression level was analyzed using immunohistochemistry in breast, prostate, colon, lung, cervix and melanoma cancers, and by western blot in chronic lymphocytic leukemia (CLL), correlating results with tumor stage and histological grade. In CLL PBMCs VDAC1 expression was increased by 5-fold in comparison to its level in healthy PBMCs donors. Comparing protein expression levels in cancer cells with those in normal cells within the same tissue section showed up-regulation of both VDAC1 and MAVS expression in certain cancer types. These findings point to MAVS and VDAC as novel biomarkers of this disease and their potential use in early tumor detection.

Poster # 20

Role of distal C-terminus of GIRK1 in the organization of GIRK-G protein beta gamma signaling complex

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The G-coupled inward rectifier potassium channel (GIRK) opens after Gβγ associates with the channel. The Gβγ is released after G protein coupled receptor (GPCR) activation, thus controlling excitability in the brain and in the heart. The GIRK channel is assembled from 4 different subunits, labeled GIRK1-4. GIRK2 and GIRK4 can form a homotetramer, while GIRK1 and GIRK3 needs another type of GIRK subunit to assemble a heterotetramer. The GIRK2 homotetramer behaves like a "classic" effector of GPCR, i.e. it has very low activity without GPCR activation (basal activity), and high activity after GPCR activation by agonist (evoked response). In contrast, the GIRK1/2 channel has high basal activity, with a comparably smaller evoked response. We set up to investigate the unique role of the GIRK1 subunit in the GIRK1/2 channel, using electrophysiological and biochemical assays in *Xenopus laevis* oocytes.

In order to isolate GIRK1 function in the channel, we use a GIRK1 mutant, $GIRK1_{F137S}$ (GIRK1*), which can be expressed as a homotetramer. The GIRK1* shows similar characteristics as GIRK1/2, i.e. high basal activity and relatively small activation by GPCR. The GIRK1 subunit has a 121-long distal C terminus, which is not present in the other subunits. We constructed a truncated channel, GIRK1* that lacks the 121 a.a. of the distal C-terminus (Δ121). The Δ121 channel resembles the GIRK2 channel, i.e. it shows low basal activity and high evoked response. Moreover, biochemical assays reveal that the truncated cytosolic domain of the channel binds Gβγ and Gα worse than the cytosolic domain of the full-length channel.

We have previously found that GIRK1/2 increase Gβγ concentrations in the plasma membrane. A recently developed mathematical model that describes GIRK1/2 basal activity, predicts that a single GIRK1/2 channel drags 3-4 Gβγ molecules and 1-2 Gα molecules to the plasma membrane. This preferential expression of Gβγ over Gα can explain the high basal activity of GIRK1/2. Our hypothesis is that the deletion of the distal C terminus hinders the channel from dragging the extra Gβγ's. We tested the constructs using two separate assays, one using fluorescent protein-tagged Gβγ, the other using antibodies in giant membrane patches of the oocyte and labeling Gβγ with fluorescent antibodies. Both assays show that Gβγ expression in the plasma membrane is enhanced with either GIRK1/2 or GIRK1*. GIRK2 and Δ121 show no such enhancement. We conclude that the distal C terminus of GIRK1 is essential for enhanced Gβγ expression in the plasma membrane.