האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

Israel Society for Physiology and Pharmacology

Annual Meeting הכנס השנתי

September 25th 2008

Ma'ale Hachamisha

P R O G R A M & A B S T R A C T S

ת כ נ י ת ו ת ק צ י ר י ם

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Israel Society for Physiology and Pharmacology

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The Israel Society for Physiology and Pharmacology is most grateful to the following sponsors whose generous support has made this meeting possible

SIGMA-ALDRICH®

PROGRAM

8:30-9:30 Registration

9:30-11:10 Early Morning Sessions A and B *(In Parallel)*

Session A: "TRP and other Ionic Channels" (Hall A)

Chair: ***Baruch Minke*** *(Hebrew University)*

9:30-9:50 **Baruch Minke** *(Hebrew University):* TRP channels: what are they and why are they important?

9:50-10:10 **Gideon Bach** *(Hadassah Hospital):* TRP-ML: endocytosis and cation channels - implications for lysosomal storage disorder.

10:10-10:30 **Noam Zilberberg** *(Ben-Gurion University):* Regulated gating of K2P channels.

10:30-10:50 **Ofer Yifrach** *(Ben-Gurion University):* Inverse modes of coupling in leak and voltage-activated K^+ channel pore gates underlie their distinct roles in electrical signaling.

10:50-11:10 **Eitan Reuveny** *(Weizmann Institute):* Control of channel function of the G protein coupled potassium channel.

Session B: "Neuroadaptations to Chronic Substances of Abuse: Addiction, Withdrawal, and Reinstatement" (Hall B)

Chairs: ***Gal Yadid*** *(Bar-Ilan University) and* ***Abraham Zangen*** *(Weizmann Institute)*

9:30-9:45 **Einav Sudai** *(Bar-Ilan University):* Structural neuroadaptations after chronic use of cocaine: implications of DHEA in extinction and reinstatement.

9:45-10:00 **Abraham Zangen** *(Weizmann Institute):* Forgetting to be addicted by brain stimulation: From animal models to human application.

10:00-10:15 **Rami Yaka** *(Hebrew University):* Role of the glutamatergic system in cocaine craving.

10:15-10:30 **Aviv Weinstein** *(Hebrew University):* The acute dose response effects of THC on cognitive-motor skills and brain mechanism in regular users of marijuana.

10:30-10:45 **Irit Akirav** *(Haifa University):* The role of cannabinoids CB1 receptor in extinction and plasticity.

10:45-11:00 **Zvi Vogel** *(Weizmann Institute):* Regulation of microglial activity by cannabinoids.

11:10-11:30 **Coffee Break**

11:30-13:10 Late Morning Sessions C and D *(In Parallel)*

Session C: "Neuronal Imaging: From Neuron to Brain" (Hall A)

Chairs: ***Inna Slutsky*** *(Tel Aviv University)* ***and Adi Mizrahi*** *(Hebrew University)*

11:30-11:50 **Noam Ziv** *(Technion):* Maintaining the CNS synapse: Insights from live imaging experiments.

11:50-12:10 **Shy Shoham** *(Technion):* Microscopic optical control over neural populations.

12:10-12:30 **Inna Slutsky** *(Tel Aviv University):* Regulation of temporal code at individual hippocampal synapses.

12:30-12:50 **Edi Korkotian** *(Weizmann Institute):* Spine apparatus: a mysterious organelle.

12:50-13:10 **Adi Mizrahi** *(Hebrew University):* Using two-photon calcium imaging to study neural networks in the mouse.

Session D:"New Insights into Angiogenesis and Cardiovascular Physiology" (Hall B)

Chair: ***Asher Shainberg*** *(Bar-Ilan University)*

11:30-11:50 **Eli Keshet** *(Hebrew University):* VEGF and ischemic heart disease.

11:50-12:10 **Alexander Battler** *(Rabin Medical Center):* Cell therapy for heart disease 2008.

12:10-12:30 **Asher Shainberg** *(Bar-Ilan University):* Protection of the heart from ischemic stress by uracil nucleotides.

12:30-12:50 **Amos Katz** *(Barzilai Medical Center):* ZnT-1 in the heart: molecular basis and potential clinical applications.

12:50-13:10 **Ronen Beeri** *(Hadassah Hospital):* Myocardial remodeling after myocardial infarction and mitral regurgitation - mechanistic insights.

13:10-14:00 **Lunch**

14:00-15:00 **Poster Session**

15:00-15:15 **Business Meeting**

15:15-16:15 Students' Presentation Competition

Chair: ***Yoav Paas*** *(Bar-Ilan University)*

15:15-15:27 **Yoni Haitin** *(Tel-Aviv University):* Gated motions and interactions between the C-termini of the I_{KS} channel subunits.

15:27-15:39 **Ilanit Itzhaki** *(Technion):* Calcium handling in human embryonic stem cell-derived cardiomyocytes.

15:39-15:51 **Guy Katz** *(Bar-Ilan University; Tel-Aviv University):* Verapamil is the drug of choice in treatment of ventricular tachycardia in calsequestrin-mutant mice.

15:51-16:03 **Elhanan Magidovich** *(Ben-Gurion University):* The C-terminal domain of the *Shaker* voltage-activated potassium channel is intrinsically-disordered.

16:03-16:15 **Maoz Nesher** *(Hebrew University):* Physiological roles of endogenous ouabain in sodium homeostasis and vascular tone regulation.

16:15-16:30 Coffee Break

16:30-17:30	**The Magnes Memorial Lecture** **Prof. Michel Lazdunski** **CNRS-Nice Sophia Antipolis University** **"Sensing with Ion Channels"**

17:30-17:45 **Ceremony Awarding the Winners of Posters' and Student Lectures' Competitions**

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Abstracts of Invited Presentations

Session A

“TRP and other Ionic Channels”

(Hall A)

TRP Channels, What Are They and Why Are They Important

Ben Katz, Shaya Lev, Moshe Parnas and Baruch Minke

Department of Physiology and the Kühne Minerva Center for Studies of Visual Transduction, Faculty of Medicine, Hebrew University, Jerusalem 91120, Israel.

TRP channels constitute a large and diverse family of proteins that are expressed in many tissues and cell types. The TRP superfamily is conserved throughout evolution from nematodes to humans. The name TRP is derived from a spontaneously occurring *Drosophila* mutant lacking TRP that responded to a continuous light with a Transient Receptor Potential (therefore, it was designated TRP by Minke). The *Drosophila* TRP and TRP-like (TRPL) channels, which are activated by the inositol lipid signaling cascade, were used later on to isolate the first mammalian TRP homologues. TRP channels mediate responses to light, nerve growth factors, pheromones, olfaction, taste, mechanical, temperature, pH, osmolarity, vasorelaxation of blood vessels, metabolic stress and pain. Furthermore, mutations in members of the TRP family are responsible for several diseases. Although a great deal is known today about members of the mammalian TRP channels, the exact physiological function and gating mechanisms of most channels are still elusive.

Potentially, a phospholipase C (PLC)-mediated signal transducion can activate the TRP channels by at least three putative mechanisms: by second messengers, by removal of inhibition and by change in membrane lipids–channel interactions. We have investigated the mechanism which assumes that conversion of PIP_2 to DAG or PUFA, which cause membrane lipid packing modifications, changes the plasma membrane lipids and activate the channels by modifying channel-lipids interactions. To support this hypothesis, we have used expression system of *Drosophila* cell-line to express the TRPL channel together with constructed enzymes that cause PIP_2 hydrolysis or phosphorylation of phosphoinositides without activation of PLC or production of DAG. In this system upon addition of a dimerizing drug, PIP_2 was selectively depleted by constitutively membrane-targeted yeast Inositol polyphosphatase 5' phosphatase, without the production of DAG, $InsP_3$, or subsequent calcium signals. The induction of TRPL-dependent current by the dimerizing drug suggests that neither DAG excitatory binding as a second messenger nor removal of PIP_2 inhibitory binding activates the channel. The data strongly suggests that the conversion of PIP_2 to DAG is the mechanism that activates the channels.

Mucolipin Proteins and Lysosomal Function; Implications for Mucolipidosis Type IV

Gideon Bach, David Zeevi, Ayala Frumkin, Aviram Kogot-Levin, Vered Offen-Glasner

Department of Human Genetics, Hadassah Hebrew University Hospital, Jerusalem 91120, Israel.

Mucolipidosis type IV (MLIV) is a neurodegenerative lysosomal storage disorder characterized by psychomotor retardation and ophthalmological abnormalities. Severely affected as well as some milder patients have been described. Over 80% of the MLIV patients are Ashkenazi Jews, with a heterozygotes frequency of 1/100. MLIV has been characterized as a lysosomal storage disorder due to the accumulation of lipids together with water-soluble substances in lysosomes of cells in every tissue and organ of MLIV patients. Mutations in the transient receptor potential (TRP) channel protein family member, mucolipin 1 (TRPML1, MLN1), have been implicated in MLIV pathogenesis.TRPML1 is a member of the mucolipin proteins family, containing 2 other proteins, namely, TRPML2 and TRPML3. The physiological function of these proteins is still unclear. TRPML1 was found to be an outward rectifying, non selective cation channel, localized to lysosomes/late endosomes, functioning in the late stages of the endocytosis process. Channel activity and selectivity was studied hitherto in heterologous, over-expression systems, of mutated TRPML1 that was mostly directed to the plasma membrane. In order to study the interaction of these proteins in an endogenous system, we have prepared antibodies against native TRPML1, 2, and 3. We identified the subcellular localization of each endogenous TRPML protein in lysosomal compartments in addition to extralysosomal compartments for TRPML2 and TRPML3. We also observed direct interactions between each of the TRPML proteins as determined by co-immunoprecipitation assays. Furthermore, after treating cell lysates with a chemical crosslinker, we found that all three TRPMLs (including their respective proteolytic isoforms) associate to form a complex of large size. Deficiency of TRPML2 and TRPML3 lead to lysosomal storage in addition to mitochondrial degeneration, which is less prominent for TRPML1.

Thus, the question for the function of TRPML1, as a lysosomal pH regulatory protein, as suggested in some reports, or as a key player in the endocytosis process, as well as the other TRPMLs, should be addressed not only for the single proteins but also in the complex form and in their native cellular location.

Regulated Gating of K_{2P} Channels

Asi Cohen, Yuval Ben Abu and Noam Zilberberg

Life Sciences Department and the Zlotowski Center for Neuroscience, Ben Gurion University, Beer Sheva 84105, Israel.

Leak potassium currents exert control over cell excitability by shaping the duration, frequency and amplitude of action potentials, in part through their influence over the resting membrane potential. It has been established that these currents are carried by dedicated potassium selective leak channels. By now, 15 human (*KCNK* genes) and numerous fly, nematode and plant channels have been reported. Like native leak currents, activity of the 2P domain channels is tightly regulated by agents as disparate as molecular oxygen, neurotransmitters, pH, arachidonic acid, membrane tension and phosphorylation. To study the strategies for K_{2P} channel gaiting we had employed biochemical and electrophysiological techniques. Single *Drosophila* KCNK0 channels switch between two long-lived states (one open and one closed) in a tenaciously regulated fashion. Gating is dictated by the carboxy-terminal tail that integrates simultaneous inputs from multiple regulatory pathways acting via several protein kinases. Similarly to voltage gated potassium channels C-type inactivation, KCNK0 gating responds to outer pore blockers, potassium, homologous mutations, and chemical modifiers. Thus, our results suggest that a similar mechanism may underlie regulated gating of KCNK0 and C-type inactivation of voltage gated potassium channels. Moreover, we identified the same gating mechanism to control K_{2P} channels' response to external signals, that is, inhibition of the human $K_{2P}2.1$ channel by external protons. We have identified two histidine residues, located in the first external loop of the channel, that govern the response of the channel to external pH. We demonstrate that these residues are within physical proximity to glutamate 84, homologous to *Shaker* Glu-418 or KcsA Glu-51 residues, all previously argued to stabilize the outer pore gate in the open conformation by forming hydrogen bonds with pore adjacent residues. We thus propose a novel mechanism for pH sensing in which protonation of extracellular histidine residues generates a local positive charge that serves to draw Glu-84 away from its natural interactions, facilitating the collapse of the selectivity filter region. In accordance with this proposed mechanism, low pH modified $K_{2P}2.1$ selectivity toward potassium. Moreover, the proton-mediated effect was inhibited by external potassium ions and was enhanced by a mutation (S164Y) known to accelerate C-type gating. We thus conclude that both intra and extracellular signals can induce gaiting of K_{2P} channels by promoting C-type-like external pore structural rearrangements.

Inverse Modes of Coupling in Leak and Voltage-Activated K^+ Channel Pore Gates Underlie Their Distinct Roles in Electrical Signaling

Yuval Ben-Abu[1], *Yufeng Zhou*[2], *Noam Zilberberg*[1] *and Ofer Yifrach*[1]

[1]Department of Life Sciences and the Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel.

[2] Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven CT, USA.

Voltage-activated (Kv) and leak (K_{2P}) potassium channels play key, yet distinct roles in electrical signaling in the nervous system. Here, we examined how differences in the operation of the activation and slow inactivation pore gates of Kv and K_{2P} channels underlie their unique roles in electrical signaling. We report that (1) leak potassium channels possess a lower activation gate, (2) the activation gate is an important determinant controlling the conformational stability of the K^+ channel pore, (3) the lower activation and upper slow inactivation gates of leak channels cross-talk and (4) in contrast to Kv channels, where the two pore gates are negatively-coupled these two gates are positively-coupled in K_{2P} channels. Our results thus demonstrate how basic thermodynamic properties of the K^+ channel pore, particularly conformational stability and coupling between the pore gates, underlie the specialized roles of Kv and K_{2P} channel families in electrical signaling.

Control of Channel Function of the G Protein Coupled Potassium Channel

Eitan Reuveny

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, 76100, Israel.

Regulation of cellular excitability is mediated, in part, by the activation of G protein coupled receptors. These receptors, through specific activation of associated G proteins, directly and/or indirectly modulate the activity of ion channels and various transporters. One of the classical examples for such regulation is the activation of the G protein coupled potassium channels (GIRK) by G proteins. This activation involves the intimate association of the G protein with the channels in concert with other intracellular factor to stabilize the channel's open conformation. To understand such interactions it is necessary to develop sensitive means for the detection of intrinsic subtle motions of the channel molecule during activation, and of its associated G protein *in vivo*. This can be accomplished by using fluorescence resonance energy transfer (FRET) as a molecular ruler for such motions. One of the limitations of such an approach is the fact that it is rather difficult to specifically fluorescently label the cytosolic face of membrane proteins in situ. To overcome this limitation, genetically encoded labeling is used in conjunction with membrane restricted FRET measurements. An example will be given using the GIRK channel as a model for a G protein effector, its intrinsic conformational rearrangements and its mode of association with G proteins during resting and activated states will be shown.

Session B

”Neuroadaptations to Chronic Substances of Abuse: Addiction, Withdrawal, and Reinstatement”

(Hall B)

Structural Neuroadaptations After Chronic Use of Cocaine: Implications of DHEA in Extinction and Reinstatement

Sudai E., Gispan I. and Yadid G.

Faculty of Life Sciences and the Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan, Israel.

The neurosteroid dehydroepiandrosterone (DHEA) affects brain-cells morphology and differentiation as well as neurotransmission. Recently, DHEA was suggested as a potential treatment to attenuate cocaine-seeking behavior and relapse, but its precise mechanism is not yet known. We tested the possibility that DHEA protects against the decreased neurogenesis caused by cocaine consumption in the dentate gyrus of the hippocampus.

Rats were trained (FR-1 schedul) to self-administer cocaine (0.13 ml/infusion; 1.5 mg/kg/20s) and number of lever responses was recorded. When stable maintenance was attained, rats underwent cocaine extinction. Each day, 90 min prior to placement in the operant chambers rats were injected with DHEA (2 mg/kg) or saline. Following the extinction phase, one group of rats was injected with 5-bromodeoxyuridine (BrdU 50 mg/kg) and 28 days latter they were euthanized and their brains stained with antibodies to BrdU and NeuN. Another group received a priming injection of cocaine (10 mg/kg, i.p.), 28 after the extinction phase.

DHEA treatment attenuated cocaine-seeking behavior. Furthermore, DHEA-treated rats responded less to cocaine priming. These behavioral changes were associated with a significant increase (normalization) in neurogenesis in the dentate gyrus of the hippocampus, relative to saline-treated rats. In conclusion, our results may indicate that the mechanism of action by which DHEA decrease relapse to cocaine usage is in part involve neurogenesis in the dantae.

Forgetting to be Addicted by Brain Stimulation:
From Animal Models to Human Application

Dino Levy[1], *Revital Amiaz*[2] *and Abraham Zangen*[1]

[1]Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel
[2]Psychiatry Clinic, Sheba Medical Center, Tel-Hashomer, Israel.

Addiction represents the pathological usurpation of neural processes that normally serve reward-related learning resulting in a strong habitual memory. Present treatment strategies for drug addiction and especially the prevention of craving and relapse are limited and their effectiveness is still questionable. We addressed the issue of treating drug addiction as a problem of weakening or erasing the strong addictive memory trace. Previous studies using pharmacological approach demonstrated that upon retrieval, established memories, like addiction, become labile and enter a transient state, in which they can be changed, weakened or erased. However, this pharmacological approach is of limited value in humans because of toxicity. Intracranial electrical stimulation (ICS) and transcranial magnetic stimulation (TMS) have been shown to alter neuronal activity, synaptic transmission, plasticity and behavior. To this end we used acute and repeated ICS of reward related brain areas in rats and TMS in humans, immediately after retrieval of the addictive memory trace, to try and induce localized neuronal adaptations that may change, weaken or erase the addictive memory trace. Presumably, this will result in reduced drug-addictive behavior. We shall present data showing that in rats 10 days of ICS of the medial prefrontal cortex (PFC) reduced cocaine-seeking behavior and the motivation for its consumption. Furthermore, acute ICS of the basolateral amygdala, applied immediately after presentation of drug-associated cues, blocked reinstatement of cocaine self-administration. Finally, in humans, 10 days of repetitive TMS of the dorsolateral PFC immediately after presentation of smoking related cues reduced cigarette consumption and cue induced craving. Hence, stimulating reward related brain regions might be a novel strategy for treating addiction.

Role of the Glutamatergic System in Cocaine Craving

Rami Yaka

Department of Pharmacology, School of Pharmacy, Faculty of Medicine, the Hebrew University of Jerusalem.

Cocaine-induced modifications of glutamatergic synaptic transmission in the brain reward system play a key role in adaptations that promote addictive behaviors. In particular, the activation of ionotrophic glutamate N-Methyl-D-Aspartate receptor (NMDAR) in the ventral tegmental area (VTA) is critical for the initiation of cocaine sensitization. However, the role of NMDARs in the nucleus accumbens (NAc), the brain region that mediates the expression of sensitization, remains to be explored.

Using locomotor-sensitization as a behavioral paradigm to study cocaine effects, we found that repeated cocaine injections resulted in an increase in all three major subunits of the NMDAR, NR1, NR2A and NR2B in the NAc, 21 days after the last cocaine injection. However, no change in these subunits was found one day following the last cocaine injection. These changes were associated with an increase in the GluR1 subunit of the AMPA receptor. Interestingly, we found a time dependent increase in ERK activity (pERK) which correlated with the increase in NMDAR subunits 21 days following cessation of cocaine injections. Further, the increase in both GluR1 and ERK activity was completely abolished after blockade of NMDAR during the development of sensitization. Finally, inhibition of ERK abolished the increase of GluR1 21 days after the last cocaine injection.

Taken together these results suggest that the development of cocaine sensitization triggers an increase in the expression of NMDAR subunits in the Nac, which in turn enhances the activity of ERK. This enhanced ERK activity drives the expression and membranal redistribution of the GluR1 subunits, which results in an increased excitability of NAc neurons following prolong cessation of drug intake. These findings are part of the long-term neuroadaptations that results in the expression of cocaine sensitization and may underlie the basis of cocaine craving.

The Acute Effects of THC on Cognitive-Motor Skills and Brain Mechanism in Regular Users of Marijuana

Dr. Aviv Weinstein[1,4*], Orit Brickner[1], Dr. Hedva Lerman[1], Dr. Mazal Greemland[1], Dr. Miki Bloch[2], Dr. Hava Lester[4], Prof. Roland Chisin[4], Prof. Yosef Sarne[6], Prof. Raphael Mechoulam[3], Dr. Rachel Bar-Hamburger[5], Dr. Nanette Freedman[4], Prof. Einat Even-Sapir[1]

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[4]Department of Nuclear Medicine, Hadassah – Hebrew University Medical Centre, Jerusalem.
[5]Israeli Anti Drug Authority, Givat Shaul, Jerusalem.
[6]Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv.

The aim of this study was to investigate acute effects of 13mg and 17mg Δ9-Tetrahydrocannabinol (THC) on skills important for coordinated movement and driving and on brain metabolism during performance of the virtual reality maze task in regular users of marijuana. Fourteen regular users of marijuana tested on two separate days. On each test day, subjects smoked 2 low nicotine cigarettes, one with, and one without THC. 17mg THC was included in the cigarette on one test day and 13mg on the other day. During smoking, heart rate and blood pressure were monitored, and the subjects performed a virtual reality maze task followed by 3 other cognitive tasks (Wisconsin Card Sorting Test, a "gambling" task, and estimation of time and distance from an approaching car).

Brain-imaging- twelve regular users of marijuana underwent 2 Positron Emission Tomography (PET) scans using [^{18}F] Fluorodeoxyglucose (FDG), one while subject to the effects of 17 mg THC, the other without THC. In both sessions, a virtual reality maze task was performed during the FDG uptake period. After smoking a cigarette with 17 mg THC, regular marijuana users hit the walls more often on the virtual maze task than after smoking cigarettes without THC; this effect was not seen after cigarettes with 13mg THC. Smoking cigarettes with 13 and 17mg THC increased subjective ratings of pleasure and satisfaction, drug "effect" and drug "high". Finally, 17mg THC increased brain metabolism during task performance in areas that are associated with motor coordination and attention in the middle and medial frontal cortices and anterior cingulate, and reduced metabolism in areas that are related to visual integration of motion in the occipital lobes. In regular marijuana users, 17mg THC may impact on cognitive-motor skills and brain mechanisms that modulate coordinated movement and driving.

The Role of Cannabinoids CB1 Receptor in Extinction and Plasticity

Irit Akirav

Department of Psychology, University of Haifa.

Cannabinoids have wide therapeutic application for a number of important medical conditions, including anxiety. However, the interest in their therapeutic applications has been restrained by the fear of potentially harmful consequences on memory.

Given the well-established role of the hippocampus in learning and memory processes, it is likely that the adverse effects of cannabinoids and specifically marijuana on spatial learning tasks, short-term memory, and attention are attributable to its actions within this brain region. However, optimal performance of tasks which assess working memory, attention and cognitive flexibility require effective communication between several interacting brain regions; deficits may, therefore, arise as a consequence of transmissional interference at a variety of loci. Thus, we aimed to examine the role of CB1 receptors in memory and plasticity in the hippocampus-accumbens-amygdala circuit.

The data so far suggest (i) a differential role of the cannabinoid system in memory and plasticity according to the brain structure under examination, and (ii) that cannabinoids might represent a therapeutic target for treatment of diseases characterized by impaired extinction of fear.

These advances in our understanding of the negative or positive consequences of cannabinoids may lead to important new insights into neuronal function which are likely to result in the development of new therapeutic strategies for the treatment of a number of key psychiatric disorders.

Regulation of Microglial Activity by Cannabinoids

Zvi Vogel[1,2], *Ewa Kozela*[1], *Maciej Pietr*[1], *Rivka Levy*[1], *Neta Rimmerman*[1], *Ana Juknat*[2]

[1] Neurobiology Department, Weizmann Institute of Science, 76100 Rehovot, Israel

[2] Dr Miriam and Sheldon Adelson Center for the Biology of Addictive Diseases, Tel Aviv University, 69978 Tel Aviv, Israel.

Cannabinoids have been shown to exert anti-inflammatory activity in various in vivo and in vitro experimental models, including several neurodegenerative diseases. However, the mechanisms of their anti-inflammatory effects are currently unknown. Using BV-2 mouse microglial cell line we investigated the signaling pathways involved in the anti-inflammatory effects of cannabinoids as well as their influence on expression of genes known to be involved in inflammation. Using lipopolysaccharide (LPS, a bacterial endotoxin) to activate microglial cells, we found that LPS induced the production and secretion of proinflammatory cytokines including interleukin-1beta (IL-1beta) and IL-6. The production and secretion of IL-1beta and IL-6 were reduced by the two major cannabinoids present in marihuana, delta9-tetrahydrocannabinol (THC) and cannabidiol (CBD). However, the mechanisms of their anti-inflammatory action did not involve the currently known cannabinoid receptors (CB1 and CB2). Moreover, we found that THC and CBD act through different mechanisms.

NFkB is an important modulator of the inflammatory response. LPS leads to the degradation of IL-1 receptor kinase (IRAK-1) and IkB (NFkB cytoplasmic inhibitor) proteins and via this pathway activates NFkB. We found that CBD, but less so THC, reversed the LPS-induced IRAK-1 and IkB degradation. Gene array profiling showed that CBD reversed many of the LPS induced changes in BV-2 microglial gene expression while THC had a relatively minor effect.

In conclusion, although both THC and CBD exert inhibitory effects on the production of inflammatory cytokines in microglial cells, their activity seem to involve different intracellular pathways.

Session C

“Neuronal Imaging: From Neuron to Brain”

(Hall A)

Maintaining the CNS Synapse: Insights from Live Imaging Experiments

Noam Ziv

Faculty of Medicine and Lorry Lokey Interdisciplinary Center for Life Sciences & Engineering, Technion, Haifa.

The human brain consists of a vast number of neurons interconnected by specialized communication devices known as synapses. It is widely believed that activity-dependent modifications to synaptic connections - synaptic plasticity - represents a fundamental mechanism for altering network function, giving rise to emergent phenomena commonly referred to as learning and memory. This belief also implies, however, that synapses, when not driven to change their properties by physiologically relevant stimuli, should retain these properties over time. Otherwise, physiologically relevant modifications would be gradually lost amidst spurious changes and spontaneous drift. We refer to the expected default tendency of synapses to hold onto their properties as "synaptic tenacity".

Imaging studies indicate that many synapses do maintain their location and overall organization over long durations. However, at the same time, other imaging studies reveal that synapses are sites of intense molecular dynamics and membrane trafficking processes. Given the lack of obvious barriers between synaptic compartments, adjacent axonal or dendritic segments, and neighboring synapses, the tenacity exhibited by synaptic junctions is by no means an obvious outcome. Yet, to date, not much is known on the principles that govern synaptic tenacity and allow these minute structures to maintain their structure and function in face of the intense molecular dynamics and other “erosive forces” associated with synaptic transmission.

We have begun to examine the relative importance of specific molecules and processes in determining synaptic tenacity. Specifically we are currently applying imaging technologies combined with molecular and genetic approaches to study the stability of synaptic structures and relationships between synaptic stability and network activity. These approaches and the insights they have provided will be described.

Microscopic Optical Control Over Neural Populations

Shy Shoham

Faculty of Biomedical Engineering, The Technion - I.I.T., Israel.

Spatiotemporal patterns of activity carried across populations of neurons are the fundamental representation of information within the nervous system, with even the very simplest of neural circuits being composed of thousands to many millions of individual nerve cells. Physical control of complex neural activity patterns can be used experimentally to gain a better understanding of neural information representation and processing, and medically in neuro-prosthetic interfaces.

I will describe the development of new optical and computational tools for controlling two fundamental aspects of neural populations: activity patterns and network connectivity. First, I will review the recent development of optical systems allowing control of increasingly complex spatiotemporal activity patterns in neural populations, focusing on holographic photo-stimulation which has several fundamental advantages in this application. A second part of the talk will introduce a new approach for exact, flexible control of neurite growth in three-dimensional neural cultures, and its possible applications.

Endogenous Amyloid-β Regulates Temporal Code at Single Hippocampal Synapses

Efrat Abramov, Iftach Dolev, Eyal Ruff, and Inna Slutsky

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Accumulation of cerebral Ab plays a critical role in development of synaptic and cognitive deficits in Alzheimer's disease (AD). However, physiological Ab functions as well as primary mechanisms that initiate early Ab-mediated synaptic dysfunctions remain obscure. Using combination of optical imaging, electrophysiology and biochemistry we explore effects of endogenously-released Ab peptides on synaptic transfer function at individual presynaptic terminals and synaptic connections in non-transgenic hippocampal neurons in hippocampal cultures and slices. Inhibition of Ab degradation rapidly enhances synaptic vesicle release probability, leading to increase in excitation-inhibition ratio and, consequently, elevation in ongoing neuronal activity. The Ab-mediated presynaptic effect depends on the pattern of neuronal activity: simple spikes cause increase in the amount of vesicle release, whereas complex spikes bursts trigger temporal redistribution of synaptic weights in excitatory neuronal connections. Notably, either elevation or reduction in Ab levels reduces presynaptic facilitation by bursts. These findings suggest that Ab enables optimal burst transfer at physiological conditions. Reduction in synapse capacity to transfer bursts triggered by a deficit in Ab clearance might be a primary pathological event initiating a compensatory synapse loss and memory decline in AD.

Spine Apparatus: A Mysterious Organelle

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Spine apparatus is an essential component of dendritic spines of cortical and hippocampal neurons, yet its functions are still enigmatic. Synaptopodin (SP), an actin-binding protein, is tightly associated with the spine apparatus, it may play a role in synaptic plasticity, but it has not yet been linked mechanistically to synaptic functions. We studied endogenous and transfected SP in dendritic spines of hippocampal neurons and found that spines containing SP generate larger responses to flash photolysis of caged glutamate than SP-negative ones. A chemical LTP induced by the delivery of GFP-GluR1 into spine heads is highly associated with SP-positive spines. SP is linked to calcium stores, since their pharmacological blockade eliminated SP-related enhancement of glutamate responses, and release of calcium from stores produced an SP-dependent delivery of GluR1 into spines. Thus, SP plays a crucial role in the calcium store-associated ability of neurons to undergo long-term plasticity.

Using Two-Photon Calcium Imaging to Study Neuronal Networks in the Mouse Auditory Cortex

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Physiological studies of the cortical networks have been mostly focused on either single-cell recordings or large-scale imaging techniques, leaving the intermediate level of cortical neural networks largely unstudied. Here, we studied the functional micro-architecture and network dynamics of populations of neurons in the mouse auditory cortex (AC) using *in vivo* 2-photon calcium imaging. We characterized single-neuron and population responses of L2/3/4 neurons in the AC of anesthetized mice. We imaged somatic calcium activity in response to pure tones in 1437 cells (n=11 mice). Specifically, we imaged up to 258 neurons from single animals and up to 35 cells simultaneously at depths of up to 500mm below the pial surface. Using this method, we derived each neuron's frequency response area (FRA) and its location in 3D space, thereby characterizing the functional micro-architecture of the network at single cell resolution. We identified large-scale architectonic features like columnar organization, tonotopic organization, and a decrease of receptive field similarity with distance. We show that while large-scale organizational principles exist in the AC, the micro-architecture is heterogeneous and sparse.

Session D

”New Insights into Angiogenesis and Cardiovascular Physiology”

(Hall B)

Adjustments to Sub-Lethal Chronic Hypoxia in the Heart

Eli Keshet

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A key energy-saving adaptation to chronic hypoxia that enables cardiomyocytes to withstand severe ischemic insults is hibernation, i.e. a reversible arrest of contractile function. While hibernating cardiomyocytes represent the critical reserve of dysfunctional cells that can be potentially rescued, a lack of a suitable animal model has hampered insights on this medically important condition.

We developed a transgenic mouse system for conditional induction of long-term hibernation and the first system to rescue hibernating cardiomyocytes at will. Via myocardium-specific induction (and, in turn, de-induction) of a VEGF-sequestering soluble receptor, we show that VEGF is indispensable for adjusting the coronary vasculature to match increased oxygen consumption, and exploit this finding to generate a hypo-perfused heart. Importantly, ensuing ischemia is tunable to a level where large cohorts of cardiomyocytes are driven to enter a hibernation mode, without cardiac cell death. Relieving the VEGF blockade even months later resulted in rapid re-vascularization and full recovery of contractile function. Further, we show that left ventricular remodeling associated with hibernation is also fully reversible. The unique opportunity to uncouple hibernation from other ischemic heart phenotypes (e.g. infarction) was used to determine the genetic program of hibernation; uncovering HIF target genes associated with metabolic adjustments and induced expression of several cardio-protective genes. Autophagy, specifically self-digestion of mitochondria, was identified as a key pro-survival mechanism in hibernating cardiomyocytes. This novel system may lend itself for examining the potential utility of treatments to rescue dysfunctional cardiomyocytes and reverse maladaptive remodeling.

Protection of the Heart from Ischemic Stress by Uracilnucleotides

Asher Shainberg[1], *Smadar Yitzhaki*[1], *Or Golan*[1] *and Edith Hochhauser*[2]

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Massive amounts of nucleotides are released during ischemia in the cardiovascular system. Whereas the effect of purine nucleotides (ATP) in myocardial infarction was intensively studied, the cardioprotecting role of pyrimidine nucleotides (UTP) under hypoxic condition has not been demonstrated. The principal aim of our study is to elucidate the protective effects of UTP and pyrimidinergic receptor activation against detrimental factors of ischemia/hypoxia as well as to investigate the mechanism by which the relevant pyrimidine receptor is coupled to its respective intermediate effectors, and the downstream cascade, which exerts distinctive cardioprotective responses.

We found that UTP significantly reduced cardiomyocyte death induced by hypoxia. Even incubation (1 hour) with UTP, 24 hours before exposing the cells to hypoxic conditions, protected the cells. The cardioprotective effect of UTP was reduced in the presence of the non-selective P2 receptor antagonist - suramin. In addition, UTP caused a transient increase of $[Ca^{2+}]_i$ level in cardiomyocytes, which might trigger the cardioprotection. PPADS or RB_2, other antagonists of P2 receptors, abolished $[Ca^{2+}]_i$ elevation caused by UTP.

To further define the role of UTP on ischemic heart in vivo, heart function was tested 24 h post left anterior descending (LAD) ligation in UTP (0.44 μg/kg) treated rats. UTP's beneficial effect in LAD ligated hearts was expressed by high ATP levels, improved mitochondrial activity (Complex I, II and IV) and reduced infarct size. A modest reduction (12%) in the mitochondrial membrane potential was demonstrated when the cultured cardiomyocytes were subjected to UTP. This reduction was abolished by the P2Y receptor antagonist, reactive blue 2, but not with 5 hydroxydecanoate, a mitochondrial K_{ATP} channel inhibitor, or by BAPTA-AM, an intracellular calcium chelator. Using Rhod-2 loaded cardiomyocytes, we found that UTP reduced mitochondrial calcium elevation following hypoxia. In conclusion, early or late UTP preconditioning is effective, demonstrating reduced infarct size and superior myocardial function. The reduction in mitochondrial calcium overload can partially explain the beneficial effect of UTP in cardiac protection following ischemic injury.

ZnT-1 Function in the Heart; Molecular Basis and Potential Clinical Applications

Amos Katz

Department of Cardiology, Barzilai Medical Center, Ashkelon
Department of Physiology, Faculty of Health Sciences Ben Gurion University, Beer Sheva, Israel, Cardiac Arrhythmia Research Laboratory, Soroka University Medical Center Beer Sheva and Ben Gurion University, Beer Sheva.

Background:

ZnT-1 is a ubiquitous protein that until recently was studied in the context of zinc metabolism as a protector against zinc toxicity. Recently, we found that ZnT-1, inhibits the entry of divalent ions via the L-type calcium channels (LTCC) channels that play key role in cardiac function. We studied ZnT-1 effects on the LTCC activity in different cell types; *Xenopus* oocytes, cardiomyocytes, HEK 293 cells in culture, rats and mice hearts and isolated human tissue from patients undergoing surgery attempting to elucidate its, yet unknown activity and mechanism of action in the heart.

Methods and Results:

In *Xenopus* oocytes, two electrode voltage clamp measurements showed that ZnT-1 co expressed with the LTCC led to reduction of the LTCC current with no apparent shift in the current -voltage relationship.

In isolated rat cardiomyocytes, ZnT-1 expression was increased by transfection, while siRNA designed to inhibit ZnT-1 expression, decreased the expression of ZnT-1 in cardiomyocytes. The nifedipine sensitive Ba^{2+} influx was reduced in ZnT-1 transfected cells to, while siRNA increased the influx. This was without changes in expression of the LTCC α_1c subunit.

In *in-vivo* experiments rapid pacing in rat atria led to shortening of the Effective Refractory Period (ERP) that coincides with higher expression of ZnT-1 in the atria.
In human subjects ZnT-1 expression was augmented in atrial fibrillation patients compared to patients in sinus rhythm.

In either absent or excess of the β-subunit ZnT-1 did not inhibit the LTCC current in *Xenopus* oocytes. Direct interaction between ZnT-1 and the LTCC β-subunit was demonstrated in HEK293 cells by co-immunoprecipitation of ZnT-1-myc and β-subunit.

Conclusions:

Our findings are consistent with ZnT-1 being a major physiological inhibitor of the cardiac LTCC and it molecular mechanism involves direct interaction with the β-subunit. Consequently, ZnT-1 seems to be crucial factor in cardiac physiology and pathophysiology process.

Myocardial Remodeling After Myocardial Infarction and Mitral Regurgitation - Mechanistic Insights

Ronen Beeri, MD, FACC, Director

Cardiovascular Research Center, Heart Institute Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Mitral regurgitation (MR) is a frequent complication of myocardial infarction (MI) and left ventricular (LV) dysfunction that doubles mortality, but its additive contribution to LV remodeling is debated and has not been addressed in controlled fashion. Specifically, its influences on the molecular processes in remodeling have not been addressed so far. We have created a new sheep model, based on a LV-LA shunt and a septal MI, which does not cause MR by itself. This allows us to separate the influences of MR and MI. MIs were created in 12 sheep, and 6 had the shunt implanted, consistently producing regurgitant fractions of ~30%. LV end-systolic volume progressively increased by 190% with MR versus 90% without MR. Preload-recruitable stroke work declined with MR, with decreased remote-zone sarcoplasmic reticulum Ca2+- ATPase levels, and decreased isolated myocyte contractility. In remote zones, pro-hypertrophic Akt and gp130 were up-regulated in both groups at 1 month, but significantly lower and below baseline in the MR group at 3 months. Pro-apoptotic caspase3 remained high in both groups. Matrix metalloproteinase (MMP)-13 and membrane-type MMP1 were increased in remote zones of MR versus infarct-only animals at 1 month, then fell below baseline. MMP tissue inhibitors rose from baseline to 3 months in all animals, rising higher in the MI+MR-group border zone. Thus we conclude that MR creates an additive drive to remodeling, in all levels assessed.

We proceeded to assess whether early repair of MR can reverse the remodeling process. We sutured the shunt closed in 6 sheep after 1 month. Sheep in the MI+MR (unrepaired) and repaired groups remodeled during the first month, with a rise in EDV and ESV; but after shunt closure reverse remodeling occurred, with a significant reduction of EDV and ESV, compared with continued rises without repair . The decrease in dP/dt relative to baseline at 3 months was smaller in the repaired and MI-only groups than in the unrepaired group. Pro-hypertrophic gp130 showed an increase followed by exhaustion below baseline in the MI+MR group, but remained elevated at 3 months in the repaired animals, as with MI only; a similar pattern was observed for anti-apoptotic pAkt. In the repaired group, matrix metalloproteinase (MMP)-2 significantly decreased in the MI+MR group in remote and border zones at 3 months. At that time, the MMP inhibitor TIMP-4 increased dramatically in the remote and border zones of repaired sheep. We thus concluded that early repair of ischemic type of MR reverses remodeling.

In order to dissect the different aspects of the remodeling process, we used a gene therapy approach. Twelve sheep received a viral vector (Adeno-Associated Virus-AAV6) percutaneously; half received AAV.SERCA2a and half received reporter gene. Results showed that SERCA2a overexpression reduced LV remodeling at 3 months.

Sheep receiving SERCA2a had well-maintained preload-recruitable stroke work at sacrifice vs. a significant decrease with reporter gene, with similar results for LV dP/dt. Although LVEF decreased in both groups, it was better maintained at sacrifice with SERCA2a . This was associated with a lower ESV with SERCA2a. Sheep receiving SERCA2a showed a 63% rise in pAkt vs. 54% reduction with reporter gene. STAT3 was also 39% higher at sacrifice with SERCA2a than reporter gene. Caspase-3 rose over 5-fold and to a comparable extent over 1 month in both SERCA2a and reporter gene animals, and decreased by only 19% from 1 to 3 months, remaining elevated in both groups at sacrifice. These data support the concept that transgenic modulation of myocyte function can affect LV remodeling in the MI+MR model, but may not ablate all aspects of the remodeling process.

Further work is in progress to dissect out different aspects of the remodeling process, thence derivating potential targets for intervention. An effort is also made to create a rodent model.

Abstracts of Student Lecture Competition

Gated Motions and Interactions Between the C-Termini of the I_{KS} Channel Subunits

Yoni Haitin[1], Reuven Weiner[2], Dana Shaham[2], Enbal Ben-Tal[1], Asher Peretz[1], Liora Shamgar[1], Olaf Pongs[3], Joel Hirsch[2] and Bernard Attali[1]

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Kv7.1 α subunit assembles with the KCNE1 auxiliary subunit to form the cardiac voltage-dependent I_{KS} K^+ channel. Mutations in either Kv7.1 or KCNE1 genes produce the long QT syndrome, a life-threatening ventricular arrhythmia. Here we studied the static interactions and the voltage-dependent molecular rearrangements of the C-termini of I_{KS} channel subunits. Kv7.1 and KCNE1 subunits were tagged with ECFP and/or EYFP and expressed in *Xenopus* oocytes, in which simultaneous spectral analysis of the fluorescence resonance energy transfer (FRET) combined with two electrode voltage-clamp recording of K^+currents were performed. Direct interactions between the C-termini of Kv7.1 and KCNE1 were further explored by the use of purified His/GST tagged recombinant peptides in a series of *in-vitro* pull-down experiments. In the channel closed state, a strong constitutive FRET signal between the C-termini of Kv7.1 and KCNE1 was observed. This static FRET was even stronger with a C-terminal truncation mutant of Kv7.1 (Δ622-676). In addition, significant static FRET signals were observed when 1:1 molar ratio of C-terminally tagged Kv7.1-CFP and Kv7.1-YFP were co-expressed. Double labeling of Kv7.1 (N- and C-termini, YFP-Kv7.1-CFP) also resulted in a marked FRET signal. A voltage-dependent change in the FRET signal was recorded upon channel opening at +30 mV concomitantly with I_{KS} K^+ currents, suggesting spatial rearrangement of Kv7.1 and KCNE1 C-termini during the gating process. A significant FRET increase between the N- and C-termini of the double-tagged Kv7.1 was also recorded at +30mV. However, no voltage-dependent FRET changes were detected between the C-termini of Kv7.1. Notably, both K^+ currents and dynamic FRET changes were abolished by coexpressing the KCNE1 LQT5 mutant D76N along with Kv7.1. *In-vitro* pull-down of purified truncation/deletion mutants of the C-termini of Kv7.1 and KCNE1 indicated that the KCNE1 C-terminus physically interacts with the coiled-coil helix C of the tetramerization domain of Kv7.1.

In all, our results suggest that channel gating is accompanied by a spatial rearrangement of the channel complex that propagates to the intracellular C-termini of both subunits. The D76N KCNE1 mutant locks the channel in the closed state and abolishes the voltage-dependent dynamic FRET signal. In addition, the tetramerization domain of Kv7.1, serves as an intracellular docking site for KCNE1.

Calcium Handling in Human Embryonic Stem Cell-Derived Cardiomyocytes

<u>Ilanit Itzhaki</u>[1], Jonathan Satin[2], Sophia Rapoport[1], Elizabeth A. Schroder[2], Leighton Izu[2], Gil Arbel[1], Rafael Beyar[1], C. William Balke[2], Jackie Schiller[1], Lior Gepstein[1]

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The suitability of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) to serve as potential candidates for the emerging field of cardiovascular regenerative medicine depends partly on their ability to present a mature cardiomyocyte phenotype. The objective of the current study was to characterize the calcium handling phenotype in developing hESC-CMs. To this end real-time-PCR, immunocytochemistry, simultaneous patch-clamp/laser-scanning confocal Ca-imaging and surface-membrane labeling with Di-8-ANEPPS were employed. Immunostaining and real-time-PCR studies demonstrated the presence of the sarcoplasmic reticulum (SR) Ca-release channels, RyR2, and Inositol-1,4,5-trisphosphate (IP3) receptors. Store Ca function was manifested as action-potential (AP)-induced Ca transients. Time-to-target plots showed that these AP-Ca transients traverse the width of the cell via a propagated wave of intracellular store Ca release. The hESC-CMs also exhibited local Ca-events ("sparks") that were localized to the surface membrane. The presence of Caffeine-sensitive intracellular Ca stores was manifested following application of focal, temporally-limited, puffs of caffeine in three different age groups: early-(with the initiation of beating), intermediate-(10 days post initiation of beating;dpb) and late-(30-40dpb) stage hESC-CMs. Ca store gradually increased during *in-vitro* maturation. Similarly, Ryanodine application decreased the amplitude of the spontaneous Ca transients. The function of an IP3-releasable Ca-pool was demonstrated in the hESC-CMs in experiments utilizing caged-IP3 photolysis and antagonist application (2-APB,2μM). Finally, these Ca stores are shown to contribute to the hESC-CMs spontaneous activity. In summary, our study establishes the presence of a functional SR Ca-store and shows a unique pattern of Ca handling in early-stage hESC-CMs, a pattern similar to that presented in mature adult CMs that lack a highly-organized transverse-tubules system.

Verapamil is the Drug of Choice in Treatment of Ventricular Tachycardia in Calsequestrin-Mutant Mice

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Background: Abnormal automaticity evoked by delayed depolarization is responsible for catecholaminergic polymorphic ventricular tachycardia (CPVT), a lethal human arrhythmia caused by recessively inherited mutations in cardiac calsequestrin (CASQ2). D307H is the first human mutation in the CASQ2 gene that was described in Beduin Arabs from Galilee. CASQ2 is a sarcoplasmic reticulum (SR) protein, which plays an important role in excitation-contraction coupling in the heart. CASQ2 associates with triadin and junction to form a complex with the SR Ca^{2+} release channel (the cardiac ryanodine receptor). CASQ2 is also considered to be a major SR Ca^{2+} storage protein. The mechanism by which either defects or total absence of calsequestrin results in arrhythmia is not yet clear. The therapy of choice, β-adrenergic blockers, completely controls the arrhythmia in less than 50% of cases. We have generated gene-targeted mice with either homozygous D307H mutation ($CASQ2^{D307H/D307H}$) as well as a CASQ2 Knock Out ($KO^{-/-}$).

The Aim of current experiments was to characterize the effects of different anti-arrhythmic drugs on the prevalence of arrhythmias in the CPVT mouse model.

Methods: A telemetry transducer was implanted under the back skin. Heart rhythm telemetry was obtained in awake animals at rest, during treadmill exercise and after intra-peritoneal (IP) injection of epinephrine [0.5 μg/g]. Pharmacological screen was done by repeating this protocol after IP injection of different anti-arrhythmic drugs.

Results: CASQ2 mutant mice suffered from complex ventricular arrhythmia at rest and developed bidirectional and polymorphic ventricular tachycardia (VT) during stress and IP epinephrine. No significant arrhythmia was found in control mice. Pretreatment by L-type Ca channel blocker, Verapamil, effectively prevented the arrhythmia. Verapamil (1-2.5μg/g) showed better results in preventing arrhythmias in $CASQ2^{D307H/D307H}$ mice than in $KO^{-/-}$. A higher dosage (5μg/g) abolished the arrhythmias in the $KO^{-/-}$ as well. Other anti-arrhythmic agents: β-adrenergic blocker (Propranolol [10μg/g], Sotalol [10μg/g]) and class-I anti-arrhythmic drugs (Lidocaine 50μg/g, Flecainide 20μg/g and Procainamide 300μg/g) had little if any effect. Nifedipine (5μg/g), a specific L-Type Ca-channel blocker, had only partial beneficial effect on both, $CASQ2^{D307H/D307H}$ and $KO^{-/-}$.

Conclusions: Verapamil has the highest effectiveness in preventing CPVT in CASQ2 mutant mice. Other anti-arrhythmic agents including β-adrenergic blockers have little effect. The fact that high dosage of Nifedipine (5μg/g) did not prevent the arrhythmias indicates that the mechanism by which Verapamil abolishes the arrhythmias is not fully via L-Type Ca channel.

The C-Terminal Domain of the *Shaker* Voltage-Activated Potassium Channel is Intrinsically-Disordered

<u>Elhanan Magidovich</u>[1,2], *Irit Orr*[1], *Deborah Fass*[3], *Uri Abdu*[1] *and Ofer Yifrach*[1,2]

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Membrane-embedded voltage-activated potassium channels (Kv) bind intracellular scaffold proteins, such as the Post Synaptic Density 95 (PSD-95) protein, using a conserved PDZ-binding motif located at the channels' C-terminal tip. This interaction underlies Kv-channel clustering and is important for the proper assembly and functioning of the synapse. Recent bioinformatics analysis showed that the C-terminal domain of many voltage-activated K^+ channels belong to the growing class of intrinsically disordered proteins. Protein segments that belong to this group exhibit unusual sequence characteristics that oppose folding and are, therefore, unstructured or intrinsically disordered under native physiological conditions. Here we present various lines of experimental evidence on the archetypical *Shaker* Kv channel demonstrating that indeed its C-terminal tail is intrinsically disordered. We have cloned, expressed and purified the C-terminal domain of the *Shaker* Kv channel. Spectroscopic analyses, including CD spectroscopy and NMR have clearly indicated that the purified C-terminal domain of the *Shaker* channel is unstructured. This observation is further supported by the unusual hydrodynamic properties of the C-terminal domain. Analytical size-exclusion chromatography and light scattering analyses of the isolated domain revealed an unusual Stocks radius - a reflection of its extended rather than globular chain conformation. The isolated domain still retained its functional activity: we have demonstrated, using pull-down assays, that the isolated C-terminal domain, out of the membrane context, is still able to bind discs large (PSD-95 homologue) its scaffold protein partner. Moreover, co-transfection experiments of both membrane-targeted C-terminal domain and its discs large scaffold protein partner into drosophila Schneider cells, demonstrated successful channel clustering *in vivo*. Combined with phylogenetic analysis, we have previously suggested that the C-treminal intrinsic disordered domain of the Kv channel family encodes a fishing rod like mechanism for ion channel binding to scaffold proteins. Experiments designed to examine this hypothesis are currently being conducted.

Physiological Roles of Endogenous Ouabain in Sodium Homeostasis and Vascular Tone Regulation

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Digitalis-like compounds (DLC) is a family of steroids synthesized in and released from the adrenal gland. DLC bind to and inhibit the activity of the ubiquitous cell surface enzyme Na+, K+-ATPase. These steroids have also been shown to induce intracellular signaling and Ca++ oscillations in several cell types. Although ouabain, one of the DLC steroids, has been implicated in several pathologies such as hypertension, cancer and mood disorders, its physiological role has not been elucidated. To address this issue the circulating endogenous ouabain levels were reduced by long-term administration of specific anti-ouabain antibodies to normal rats. Several physiological parameters were monitored during and after this application. Experiments were performed on Wistar rats. Animals were infused continuously for 28 days with anti-ouabain antibodies or rabbit IgG as a control (both 100 μg/day). Infusions were delivered through a jugular vein cannule by osmotic pumps (Alzet, 2ML4) implanted subcutaneously. Animals were housed in metabolic cages for 24 hours for water and food consumption and urine excretion measurements. Once a week, blood pressure (BP) was measured by tail-cuff plethysmography. After 28 days animals were sacrificed and the thoracic aorta was isolated. Aortic rings were used to study the phenylephrine-induced contraction and the vasorelaxing potency of Atrial Natriuretic Peptide (ANP). Body and organs weights gains and water and food consumption during the 4 weeks of treatment was similar in the two groups. No difference in BP and heart rate between the groups was detected. Importantly, a significant reduction in natriuresis and sodium clearance on day 2 was observed in the ouabain-antibodies treated rats as compared to the control group. Natriuresis returned to control levels in the second week of the experiment. The in-vitro studies showed that the sensitivity and maximal response of the aorta to phenylephrine were reduced in aortic rings from ouabain-antibodies treated rats. This reduction was not due to changes in α adrenergic receptor properties as indicate by the same EC50 values. On the contrary, the sensitivity of the aortic rings to ANP-induced vasodilatation was significantly increased in aortic rings from the ouabain-antibodies treated rats as compared to the control group. These results demonstrate for the first time that circulatory ouabain has physiological roles in sodium homeostasis and vascular reactivity in normal rats. Moreover, these findings point to a possible cross-talk between the DLC and the ANP hormonal systems.

*Abstracts of Posters**

**Arranged alphabetically according to the family name of the first author*

Poster #1

The Anti-Apoptotic Protein Bcl2 Regulates Apoptosis Via Interaction with the Mitochondrial Protein, VDAC1

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The anti-apoptotic proteins of the Bcl2 family are expressed at high levels in many types of cancer. The mechanism by which these proteins regulate apoptosis is still not fully understood, yet it is well established that their activity is mediated via interaction with mitochondria. Accumulated findings indicate that the Bcl2 family interacts with the outer mitochondrial membrane protein, VDAC (voltage-dependant anion channel), a β-barrel protein recognized as a key protein in mitochondria-mediated apoptosis.

In this study, the interaction of the Bcl2 with VDAC is studied. We demonstrate that purified Bcl2 interacts with VDAC-reconstituted into a planar lipid bilayer and reduced its channel conductance. In addition, synthetic peptides corresponding to the VDAC1 N-terminal region and selected cytosolic loops bound specifically, in a concentration- and time-dependent manner, to immobilized Bcl2, as revealed by real time surface plasmon resonance (SPR) technology. Moreover, expression of the VDAC1-based peptides in cells over-expressing Bcl2 prevented its protection against staurosporine-induced release of cytochrome *c* and subsequent cell death. These results point to Bcl2 as promoting tumor cell survival through binding to VDAC1, thereby inhibiting cytochrome *c* release and apoptotic cell death. Moreover, these findings suggest that interference with the binding of Bcl2 to mitochondria by VDAC1-based peptides may correspond to a practicable modality by which to potentiate the efficacy of conventional chemotherapeutic agents.

Poster #2

Human Umbilical Cord Blood Collagen-Adherent, Nestin-Positive Progenitors with Neurogenic Potential Induce Neuroprotection in an In Vitro Ischemic Model Involving Soluble Antioxidant(s) and Angioneurins

Hadar Arien-Zakay,[1] *Shimon Lecht,*[1] *Marian M Bercu,*[1] *Rinat Tabakman,*[1] *Ron Kohen,*[2] *Hanan Galski,*[3] *Arnon Nagler,*[3] *and Philip Lazarovici*[1]

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Human umbilical cord blood (HUCB) is a valuable source for cell therapy since it confers neuroprotection in stroke animal models. However, the responsible sub-populations remain to be established and the mechanisms involved are unknown. To explore HUCB neuroprotective properties in a PC12 cell-based ischemic neuronal model, we used an HUCB mononuclear-enriched population of collagen-adherent cells, which can be differentiated in vitro into a neuronal phenotype (HUCBNP). Upon co-culture with insulted-PC12 cells, HUCBNP conferred ~30% neuroprotection, as evaluated by decreased lactate dehydrogenase and caspase-3 activities. HUCBNP decreased by 95% the level of free radicals in the insulted-PC12 cells, in correlation with the appearance of antioxidants, as measured by changes in the oxidation-reduction potential of the medium using cyclic-voltammetry. An increased level of nerve growth factor (NGF), vascular endothelial growth factor and basic fibroblast growth factor in the co-culture medium was temporally correlated with a -medium neuroprotection effect, which was partially abolished by heat denaturation. HUCBNP-induced neuroprotection was correlated with changes in gene expression of these angioneurins, while blocked by K252a, an antagonist of the TrkA/NGF receptor. These findings indicate that HUCBNP-induced neuroprotection involves antioxidants and angioneurins, which, by paracrine and/or autocrine interactions between the insulted-PC12 and the HUCBNP cells, conferred neuroprotection.

Poster #3

Quantitative Assessment of Neuronal Differentiation in Three-dimensional Collagen Gels Using Enhanced Green Fluorescence Protein Expressing PC12 Pheochromocytoma Cells

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There is a paucity of quantitative methods for evaluating the morphological differentiation of neuronal cells in a three-dimensional (3-D) system to assist in quality control of neural tissue engineering constructs for use in reparative medicine. Neuronal cells tend to aggregate in the 3-D scaffolds, hindering the application of two-dimensional (2-D) morphological methods to quantitate neuronal differentiation. To address this problem, we developed a stable transfectant green fluorescence protein (GFP)-PC12 neuronal cell model, in which the differentiation process in 3-D can be monitored with high sensitivity by fluorescence microscopy. Under 2-D conditions, the green cells showed collagen adherence, round morphology, proliferation properties, expression of the nerve growth factor (NGF) receptors TrkA and p75NTR, stimulation of extracellular signal-regulated kinase phosphorylation by NGF and were able to differentiate in a dose-dependent manner upon NGF treatment, like wild-type (wt)-PC12 cells. When grown within 3-D collagen gels, upon NGF treatment, the GFP-PC12 cells differentiated, expressing long neurite outgrowths. We describe here a new validated method to measure NGF-induced differentiation in 3-D. Having properties similar to those of wt-PC12 and an ability to grow and differentiate in 3-D structures, these highly visualized GFP-expressing PC12 cells may serve as an ideal model for investigating various aspects of differentiation to serve in neural engineering.

Poster #4

Short Peptides (Haptides) Homologous to C-termini of Fibrinogen Affect Rat Cardiovascular System by eNOS Inhinbition

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Haptides, a family of 19-21mer synthetic peptides homologous to C-termini sequences in fibrinogen chains β and γ and homologous sequence on the C-terminal of microfibril-associated-protein-4 (Cmfa), Haptides Cβ, preCγ and Cmfa, respectively, were tested for their cardiovascular effect. In isolated perfused rat hearts, Haptides in concentration range of 50 & 83μg/ml significantly depreciated myocardial function. No such effect was recorded after exposure to a control peptide and whole fibrinogen.

Sodium nitroprusside as NO donor reversed the inhibitory effect of Haptides on perfused hearts activity, but L-NAME, an eNOS inhibitor, did not augment the Haptide mediated inhibitory effect. ^{FITC}Haptides perfused into heart circulation were deposited on the internal surface of cardiac blood vessels, but not in the cardiomyocytes in the cardiac muscles. Furthermore, Haptides had no effect on the contraction of isolated cultured cardiomyocytes. Haptides enhanced contraction of isolated intact aortic rings, but had no effect on rings stripped off endothelium. They significantly decreased eNOS activity in heart homogenates. Our accumulated results suggest that the adverse effect of Haptides alone, or possibly also as part of the larger mixture of fibrin degradation products, may occur due to Haptide induced vasoconstriction by inhibition of eNOS activity, causing temporary ischemia and impaired myocardial function.

Poster #5

Inverse Modes of Coupling in Leak and Voltage-Activated K+ Channel Pore Gates Underlie Their Distinct Roles in Electrical Signaling

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Voltage-activated (Kv) and leak (K2P) potassium channels play key, yet distinct roles in electrical signaling in the nervous system. Here, we examined how differences in the operation of the activation and slow inactivation pore gates of Kv and K2P channels underlie their unique roles in electrical signaling. We report (1) that leak potassium channels possess a lower activation gate, (2) that the activation gate is an important determinant controlling the intrinsic conformational stability of the K+ channel pore, (3) that the lower activation and upper slow inactivation gates of leak channels cross-talk and (4) that in contrast to Kv channels, where the two pore gates are negatively-coupled (Panyi, G. & Deutsch, C. *J Gen Physiol* **128**, 547-59 (2006)), such that the opening of the lower activation gate stimulates the closure of the upper slow inactivation gate, these two gates are positively coupled in K2P channels. Our results demonstrate how basic thermodynamic properties of the K+ channel pore, particularly, intrinsic conformational stability and coupling between the pore gates, underlie the specialized roles of Kv and K2P channel families in electrical signaling.

Poster #6

VEGF-Related Protein Isolated from *Vipera palestinae* Venom, Promotes Angiogenesis*

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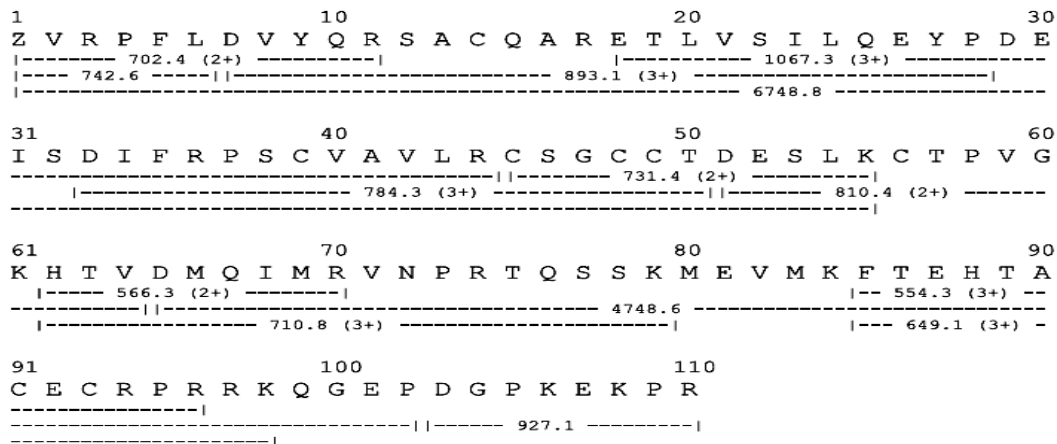
Therapeutic angiogenesis requires the design of novel pro-angiogenic drugs such as VEGF agonists. Since snake venoms represent a rich source of potential, lead compounds for drug development we sought to isolate and characterize VEGF from the Israeli snake viper venom. *Vipera palestinae* VEGF (vpVEGF) was purified from *Vipera palestinae* venom using two steps of reverse-phase HPLC. Structurally, vpVEGF belongs to the VEGF-F1 family of snake venom proteins, which potently stimulated dermal human microcapillary endothelial cells (dHMVEC) proliferation in a VEGFR-2 dependent manner. This growth factor appeared to be a chemoattractant for migration of these cells and stimulated their radial migration in a collagen gel. The stimulatory effect on dHMVEC was correlated with activation of the Erk1/2 signaling pathway. *In vivo* vpVEGF induced angiogenesis in a Japanese quail chorioallantoic membrane angiogenic assay and in a Matrigel plug assay upon subcutaneous injection in mice. Although in the quail assay vpVEGF showed lower activity than human recombinant VEGF-A_{165}, in mammalian-related systems there were no significant differences. The experiments with dHMVEC, as well as angiogenesis in vivo suggest that the pro-angiogenic effect of vpVEGF is related to its interaction with VEGFR-2. vpVEGF provides a novel VEGF analogue to study angiogenesis and towards development of VEGF-mimetic drugs.

Primary structure of vpVEGF:



**Brown et al., Growth Factors 2007, 25: 108-117.*

Acknowledgement

This study was supported in part by Israel Science Foundation (PL).

Poster #7

Astrocytic-Derived Nitric Oxide Modulates Synaptic Function

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Nitric oxide (NO) is produced in the brain by neurons, astroglia and endothelial cells, and is known to participate in diverse signaling pathways. While neurons are capable of rapid release of small amounts of NO serving as neurotransmitter, most studies of NO production by astrocytes have assumed slow activation of an inducible form of NOS (NOS2) under a variety of stress stimulations such as ischemia or inflammation, occurring over a time-scale of hours and days. In the neocortex, the neuronal NOS isoform has been documented only in a small percentage of GABAergic neurons. Hence, one of the most intriguing questions regarding NO biology in the brain is the cellular source of NO acting as a retrograde synaptic messenger.
We used NO imaging with the NO indicator 4,5-diaminofluorescein-2 diacetate (DAF-2DA) and electrophysiological methods in acute neocortical slices from CD1 mice to test the hypothesis that astrocytic-derived NO participates in modulating synaptic strength.

NO imaging experiments demonstrated that robust astrocytic NO production can occur on a time scale of seconds and minutes, and is not dependent on de-novo protein synthesis (buskila et al; 2005). SR-101, a selective marker for astrocytes, was highly co-localized with the diffuse staining pattern of DAF-2DA, confirming that most NO producing cells are astrocytes. Bath application of the selective NOS2 inhibitor 1400W (3 μM) blocked the astrocytic DAF-2DA fluorescence, but not the neuronal fluorescence. We next recorded from pyramidal neurons in layer 2/3 of the neocortex, and examined the effect of inhibiting NOS2 on excitatory synaptic transmission. 1400W reduced the frequency of spontaneous unitary EPSC's without affecting their amplitude. Moreover, when inducing LTP by pairing the pre- and the postsynaptic spikes, 1400W reduced the potentiation of the EPSC's amplitude from 144±18% to 122±18%. Taken together, our results suggest that astrocytic-derived NO is involved in modulating synaptic function.

Poster #8

Effect of Digitalis on Neuronal Cell Viability: Possible Implication in Amyotrophic Lateral Sclerosis

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Digitalis-like compounds (DLC) are steroid hormones synthesized by and released from the adrenal gland. These compounds, the structure of which resembles that of plant and amphibian steroids such as ouabain and bufalin, interact with Na^+, K^+-ATPase. The Na^+, K^+-ATPase is a major plasma membrane transporter for sodium and potassium in all mammalian cells. Numerous studies have shown that Na^+, K^+-ATPase activity is reduced in the brain of Alzheimer's disease (AD) patients and in the spinal cord of a transgenic mouse model for amyotrophic lateral sclerosis (ALS). It has also been found that DLC have a dual effect on epithelial and muscle cell viability: low concentrations exert a protective effect, whereas high concentrations induce apoptosis. We raised the hypothesis that neuronal degeneration is due, in part, to alterations in DLC metabolism in the brain and suggested a role for these compounds in the etiology of neurodegenerative diseases. This hypothesis was addressed by the determination of DLC in the CSF of AD and ALS patients and in normal controls, using a specific and sensitive ELISA. DLC levels were significantly lower in samples from ALS patients as compared to normal subjects. No difference between normal and AD patients was found. The effect of digitalis compounds on cell viability was examined in neuron/endocrine cell lines NT2 and PC12, using the MTT assay. Low concentration of ouabain (10^{-9}-10^{-8}M) protected the cells against serum deprivation-induced cell death, while high concentrations (10^{-7}M-10^{-3}M) induced cell death. Bufalin exert neuronal cell death in lower concentrations than ouabain. Interestingly, the addition of antibodies against ouabain to NT2 cells growing in complete media (10% FBS) resulted in a significant dose-dependent reduction in cell viability as compared to cells treated with normal rabbit IgG. Furthermore, the addition of 10 nM ouabain to the ouabain-antibodies-treated cells attenuated the effect of the antibodies on cell viability. These effects were not seen in PC12 cells. We conclude that low concentrations of ouabain stimulate cell growth and that the endogenous DLC exert constitutive stimulation of neuronal cells survival. Our results are in accord with the suggestions that the reduction in DLC in CSF of ALS patients may be part of mechanisms of cell death involved in this disease.

Poster #9

Rapamycin (Sirolimus) Protects Against Hypoxic Damage in Primary Heart Cultures Via Na^{+}/Ca^{2+} Exchanger Activation

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Rapamycin (sirolimus) is an antibiotic that inhibits protein synthesis through mammalian target of rapamycin signaling and is used as an immunosuppressant. Recently, rapamycin-impregnated stents have also been used to reduce coronary restenosis. We hypothesized that rapamycin induced cardioprotection against hypoxia/reoxygenation damage in primary heart cultures, via stimulation of Na^{+}/Ca^{2+} exchanger in cells that were loaded with indo-1 to record cytosolic $[Ca^{2+}]_i$. Rapamycin inhibited sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2A), as measured by $^{45}Ca^{2+}$ accumulation into the sarcoplasmic reticulum in a dose and time dependent manner. Increase of extracellular calcium or treatment with thapsigargin-SERCA2A inhibitor, increased diastolic $[Ca^{2+}]_i$ of heart cultures that were attenuated by immediate application of rapamycin in buffer containing 140 mM Na^{+} but not in Na^{+}-free buffer that increased $[Ca^{2+}]_{i.}$ Moreover, caffeine not only failed to increased cytosolic $[Ca^{2+}]_{i.}$ following treatment with rapamycin, but even attenuated it. Hypoxic heart cultures treated with KBR_{7943}-specific inhibitor of Na^{+}/Ca^{2+} exchanger, confirmed these results, since KBR_{7943} inhibited the cardioprotective effect of rapamycin in hypoxia/reperfusion as detected in lactate dehydrogenase (LDH) release, desmin immunostaining and stimulating of $[Ca^{2+}]_{i.}$ Thus, rapamycin induced cardioprotection against hypoxia/reperfusion damage by preventing Ca^{2+} overload via activation of sarcolemmal Na^{+}/Ca^{2+} exchanger.

Poster #10

Further Insights into the Mechanism Underlying the Cardiac Steroids-Induced Ca^{++} Oscillations

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Cardiac steroids (CS), such as ouabain, digoxin and bufalin are compounds prepared from the seeds and dried leaves of the genus Digitalis and amphibian skin are used for centuries as cardiac stimulants in the western and eastern medicines. This group of steroids was identified in mammalian tissues and is considered a hormone family involved in numerous physiological roles and pathological states. The only established receptor for CS is the plasma membrane Na^+, K^+-ATPase. The ability of ouabain to induce slow Ca^{++} oscillations in kidney epithelial COS7 cells was discovered by Aperia and coworkers. In series of elegant studies these investigators have demonstrated that this phenomenon is mediated by Na^+, K^+-ATPase localized in specific membrane micro-domain, which interact with IP_3 receptor and Ankyrin B proteins. In the present study we have found that additional CS such as bufalin, digoxin and digitoxin also induce these oscillations which appear 10-20 minutes following the addition of the CS. The effects of extracellular Na^+ and Ca^{++} on the CS-induced oscillations were studied. The results of these experiments show that influx of Ca^{++} into the cell is an essential part of the oscillatory machinery. The removal of extracellular Ca^{++} reversibly inhibited CS-induced Ca^{++} oscillations. Furthermore, under these conditions CS did not induce any rise in cytosolic Ca^{++}. The addition of Ca^{++} to Ca^{++}-free media containing CS generated an immediate Ca^{++} oscillatory response, indicating a previous assembly of the CS-induced oscillatory machinery. The replacement of extracellular Na^+ with N-methyl-D-glucamin completely abolished the CS-induced Ca^{++} oscillations suggesting the involvement of Na^+/Ca^{++} counter-transport in this phenomenon. This study reveals new components in the molecular mechanism governing the CS-induced Ca^{++} oscillations in COS7 cells.

Poster #11

A Bioinformatics Assay of Age, Gender and Blood Parameters

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A massive body of data exists on the variation in the blood parameters for very large populations. The causes of extreme values for blood test results have been extensively studied, as each test was chosen because extreme values are clinically important. However, the causes of variation within the normal interval have not been extensively studied. Here we present an analysis of the complete blood picture from a large cross-sectional study (NHANES3), involving over 29,000 non-institutionalized USA residents. Using bioinformatics approaches, we derive from these data an "age-array" by deriving age and gender signatures for each parameter. Complex patterns of change with age and gender are observed for most parameters. Moreover, multiple parameters change in concert, following several distinct patterns. Applying clustering algorithms to this array, blood parameters are grouped according to the similarity of their change with aging. To further investigate these patterns, clustered parameters were analyzed for the best combination of critical ages that divide life into periods of different change (e.g. the end of growth). Using a special algorithm, based on maximizing the goodness of fit of b-splines and scanning a wide range of possible age ranges, was used to propose critical ages in which in different blood-related systems. Our result suggests that each set of blood parameters follows a different pattern of change, although some ages are common to many sets. We also provide a list of possible critical ages, which may serve as the starting point for the study of aging specific processes.

Poster #12

Protection of Cardiomyocytes Against Stress by Extracellular Nucleotides

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Cardiomyocytes survival after heart disease and heart failure is essential for its function. Many considerable efforts are accomplishing in order to reduce cardiac damage and improve its function. In this study we examined the cardioprotective effect of extracellular nucleotides (purines and pyrimidines), which can activate various cell responses by common purinoceptors, on cardiomyocytes, which exposed to hypoxic stress damage. For this purpose, we treated cardiomyocytes with various extracellular nucleotides prior or following to the hypoxic conditions, and compared biochemical variables with untreated cardiomyocytes. We discovered that pretreatment with extracellular triphosphate nucleotides remarkably reduced LDH leakage from the cells and led to cell viability as demonstrated by propidium iodide (PI) staining. The effect was concentration and time dependent as even 48 hours pretreatment revealed cardioprotection. We also demonstrated that extracellular nucleotides-pretreated cells maintained mitochondrial membrane potential, preserved complex II (succinate dehydrogenase) activity, and high cytosolic ATP level following hypoxia. The cardioprotective effect was also demonstrated by the deoxy forms of the triphosphate nucleotides, but not with the nucleotides in their diphosphate or in their nucleoside forms. To verify the mechanism of the receptor which coupled to its effectors, we treated the cells with different P2 antagonists. We found that suramin, which has a broad-spectrum antagonism to P2 receptors, partially abolished the protective effect. To investigate the protective effect against hypoxia-reoxygenation damage, we treated the cells with the extracellular nucleotides following hypoxia before reoxygenation. LDH measurements revealed that 24 h after the hypoxic conditions, post treated cells presented lesser LDH release. In conclusion, therapeutic activation of purinergic and pyrimidinergic receptors may be effective and promising targeting in patients at risk of myocardial ischemic damage. Further exploration and understanding the downstream cascade may be helpful in manufacturing greater potent agonists.

Poster #13

Association Between Specific SNPs in Na^+, K^+-ATPase Isoforms and Bipolar Disorders

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The sodium- and potassium-activated adenosine triphosphatase (Na^+, K^+-ATPase) is a major plasma membrane transporter for sodium and potassium. The possible involvement of Na^+, K^+-ATPase in bipolar affective disorders (BD) was suggested more than 50 years ago. BD has consistently been associated with abnormalities in the Na^+, K^+-ATPase activity in erythrocytes. Furthermore, the levels of digitalis-like compounds, endogenous inhibitors of the Na^+, K^+-ATPase in the parietal cortex of BD patients were significantly higher than in normal individuals and depressed patients. We conjectured that the differences in Na^+, K^+-ATPase activity in BD could result partially from genetic variations in Na^+, K^+-ATPase α isoforms. This hypothesis was addressed by a comprehensive study of 13 tagged SNPs (Single Nucleotide Polymorphism) across the Na^+, K^+-ATPase $\alpha1$ (ATP1A1), $\alpha2$ (ATP1A2) and $\alpha3$ (ATP1A3) isoform genes (identified using HapMap data and the Haploview algorithm) in DSMIV bipolar in a family-based study. A total of 126 probands were examined and DNA was obtained from parents and probands. Implementation of PBAT, a family-based association test, revealed a significant association with bipolar disorder in six single SNPs ($\alpha1$:rs805078; $\alpha2$:rs2070704, rs1016732, rs2854248 and rs2295623; $\alpha3$:rs919390) in the three genes of Na^+, K^+-ATPase α isoforms. Haplotype analysis of the $\alpha2$ isoforms isoform (*ATP1A2* gene) showed a significant association with two loci haplotypes with BD (rs2295623: rs2070704; global p value=0.0198, following a permutation test). This study demonstrates for the first time that SNPs polymorphisms in Na^+, K^+-ATPase are associated with BD and suggest the involvement of this enzyme in the etiology of this disease.

Poster #14

Apoptosis Induction is Associated with VDAC1 Oligomerization

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Mitochondria are essential for cell survival, providing sources of cellular energy, as well as lie at the heart of apoptotic regulation. Mitochondria-mediated apoptosis results in the efflux of a number of potential apoptotic regulators, such as cytochrome *c*, to the cytosol, triggering the caspases cascade and cell destruction. There is substantial evidence suggesting that the voltage-dependent anion channel-1 (VDAC1) is a critical player in apoptosis by regulating the release of apoptogenic proteins from mitochondria in mammalian cells (e.g cytochrome *c*). However, the VDAC1 pore diameter is about 3 nm, too small for protein transport. Therefore, we propose that a mega pore is created between the VDAC monomers allowing cytochrome *c* release. The precise mechanism regulating cytochrome *c* release remains unknown, and the molecular architecture of the cytochrome *c*-conducting channel has also to be determined.

Here, we demonstrate that induction of apoptosis by various stimuli (i.e. staurosporine, cisplatin, curcumin, As_2O_3, etoposide, TNF-α) induces VDAC1 oligomerization (dimers to multimers). Moreover, a direct relationship between VDAC oligomerization and apoptosis is reflected in the linear correlation between the extent of apoptosis and the level of VDAC oligomerization. Apoptosis induction dramatically enhances VDAC1 oligomerization regardless of cell type used, demonstrating that this phenomena is not a cell type specific. In addition, cell death induced by VDAC1 over-expression also results in highly enhanced VDAC1 oligomerization. These findings support our original proposal that oligomeric VDAC1 forms a structure which mediates the release of cytochrome *c*. We propose that VDAC1 oligomerization is a dynamic process in which apoptosis induction shifts VDAC1 equilibrium towards oligomerization, forming a large pore allowing the release of apoptogenic proteins, such as cytochrome *c*.

Poster #15

Regulation of Blood Glutamate Levels by Stress

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Since rats submitted to a closed head injury (CHI) display a spontaneous decrease of their blood Glutamate (Glu) levels, we have investigated the mechanisms by which this phenomenon takes place. First, we observed that the anesthesia of naive rats or the catherization of their tail artery does not affect blood Glu levels. Reasoning that the spontaneous decrease of blood Glu levels after CHI could be part of a stress response and the activation of the hypothalamo-pituitary-adrenal pathway, we observed that neither corticosterone nor noradrenaline administered to naïve rats modifies their blood Glu levels. In contrast, adrenaline, isoproterenol, a β2-selective adrenoreceptor agonist, metaprolol, a β1-selective antagonist, corticotropin-releasing factor and adrenocorticotrophic hormone caused a significant blood Glu decrease. We concluded that stress induces a decrease of blood Glu levels via in part the activation of the β2-adrenoreceptor. While propranolol, a non selective β-antagonist, did not modify blood Glu levels, it prevented the spontaneous decrease of blood Glu observed after CHI and prevented the spontaneous neurological improvement normally observed after 24 hours. Thus, the spontaneous neurological recovery after CHI is related at least in part to a stress-induced sympathetic discharge that causes a decrease of blood Glu.

Poster #16

Implication of Exercise Training on Cardiomyopathy and Catecholamine-Dependent Polymorphic Ventricular Tachycardia (CPVT) in Calsequestrin Deficienct Mice

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CPVT is a lethal ventricular arrhythmia evoked by physical or emotional stress. Recessively inherited CPVT is caused by either missense or null-allele mutations in the cardiac calsequestrin (CASQ2) gene. It was suggested that defects in CASQ2 causing protein deficiency, impair Ca^{2+} uptake to the sarcoplasmic reticulum and Ca^{2+}-dependent inhibition of ryanodine channels, leading to diastolic Ca^{2+} leak, after-depolarizations and arrhythmia.

To examine the effect of exercise training on left ventricular remodeling and arrhythmia, CASQ2 knockout ($CASQ^{DE9}$) mice and wild-type controls underwent echocardiography and heart rhythm telemetry before and after 6 weeks training protocol using treadmill exercise. RT-PCR was used to measure the expression of A and B-type natriuretic peptide genes (ANP and BNP).

Left ventricular fractional shortening was impaired in $CASQ^{DE9}$ (35±3% vs 41±8% in controls, $p<0.05$) but improved after training (44±5% and 51±3 in $CASQ^{DE9}$ and control mice, respectively, p=NS). The exercise tolerance was 16±1 min in $CASQ^{DE9}$ mice vs 29±2 in controls, $p<0.01$, but improved in trained animals (26±2 vs 30±3 min, respectively, p=NS). The hearts of $CASQ^{DE9}$ mice had higher basal expression of the BNP gene, but ANP was not significantly different from controls.

After training the expression of both natriuretic peptide genes was markedly decreased in $CASQ^{DE9}$ and controls. Exercise training was not associated with a change in CPVT severity but appeared to decrease the prevalence of ventricular arrhythmia during stress. We conclude that in $CASQ^{DE9}$ mice, recapitulating the phenotype of human CPVT, exercise training is beneficial and could offer a strategy for prophylactic and therapeutic interventions.

Poster #17

Angiogenic and Protective Effects of Nerve Growth Factor (NGF) in Brain Capillary Endothelial Cells Involves Transient Activation of Erk1/2 Signaling Pathway

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NGF angiogenic effects are expressed by proliferation and migration of endothelial cells, sprouting of capillaries from aortic rings and enhanced formation of small arteries in the quail chorioallantoic membrane assays. In the present study, we characterized NGF-induced TrkA-mediated activation of Erk1/2 pathway in relation to proliferation, migration and protection of endothelial cells towards ischemic insult. NGF at the concentration of 10 ng/ml rapidly and transiently induced (5-10 min) TrkA receptor autophosphorylation as well as downstream Erk1/2 phosphorylation. This temporal transient effect is opposite to the NGF-induced sustained signaling in neuronal cells. Pretreatment of the cells for 1 h with TrkA antagonist, K252a (100 nM) or Erk1/2 inhibitor PD98059 (20 microM) blocked NGF-induced angiogenic effects. Exposure of the endothelial cells to a mild 8 hours ischemic insult achieved by deprivation of oxygen, glucose and serum followed by 18 hours reoxygenation caused 70% cell death. Concomitant exposure of the cells to the ischemic insult and 10 ng/ml NGF conferred 50% protection from cell death. NGF-induced protection was blocked by K252a and PD98059 thus, suggesting an important role of TrkA/Erk pathway in endothelial cells survival. These findings propose an important protective role of NGF in the pathophysiology of brain capillary endothelium under ischemic insults.

Acknowledgement

SL is supported by "Levi Eshkol" fellowship in biomedicine and biomedical engineering from the Israeli Ministry of Science, Culture and Sport.

Poster #18

Identification and Pharmacological Characterization of Novel Nerve Growth Factor Isoform from *Echis carinatus sochureki* Snake Venom

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Snake venom proteins play a variety of roles in biomedicine. Due to their high potency, specificity and selectivity, snake venom proteins are used in diagnostic kits, lead compounds or vaccines and they serve as excellent tools to study biological systems. Snake venoms NGF (svNGF) are an alternative richest source of NGF after male mouse submaxillary gland (mNGF) and may provide novel tools to study NGF receptors (TrkA and $p75^{NTR}$) structure and activity.

The major purpose of the present study was to isolate and identify NGF isoforms from North Indian snake *Echis carinatus sochureki* venom. The venom was separated by high pressure liquid chromatography into 26 protein fractions. Using a PC12 cell neuronal bioassay, two NGF-like bioactive, non-toxic fractions, named ECS10 and ECS13 were identified. These fractions, similar to mNGF, are characterized by molecular weight of 26 kDa. ECS10 and ECS13 show similar potency of 30 ng/ml but lower potency and similar efficacy compared to mNGF. The NGF-like bioactivity of ECS10 and ECS13 were completely abolished in the presence of 100 nM of NGF/TrkA receptor antagonist K252a. The ECS13 induced progressive neurotropic effects resulted with stable neuronal differentiation after seven days of treatment. In contrast, the ECS10 induced fast and transient neurotropic effects during the first two days of treatment.

These svNGFs isoforms are promising agonists to study TrkA receptors properties and functions.

Acknowledgement

SL is supported by "Levi Eshkol" fellowship in biomedicine and biomedical engineering from the Israeli Ministry of Science, Culture and Sport.

Poster #19

Calcipotriol-Based Compound for Topical Treatment of Psoriasis: Synthesis, Anti-Proliferative Activity and Metabolic Studies

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Vitamin D_3 analogs are useful for the treatment of hyper-proliferative skin diseases, such as psoriasis and are involved in essential cell regulatory processes, such as proliferation and differentiation in different cell types, including cancer cells. Calcipotriol (CPT), a synthetic side chain analog of vitamin D_3, was developed for treating psoriasis. CPT is as potent as vitamin D_3 in its ability to inhibit proliferation and induce differentiation, but skin irritation and burning are well known side effects, which limit its topical extended use. New putative agent, BGP-013, based on CPT structure, was synthesized in our lab, as a potential candidate for the treatment of psoriasis. Evaluation of its viability and cellular internalization was carried out. BGP-013 displayed a significant anti-proliferative activity, relatively to CPT, in several human normal cell lines (HaCaT - immortalized keratinocytes and HDF - dermal fibroblasts). In addition, BGP-013 demonstrated a higher epidermal cells' internalization in comparison to the parent molecule. Metabolic studies in HaCaT cells and rat skin showed that BGP-13 is actually one of the natural CPT metabolites that may use as quencher of singlet oxygen. The above results are indicating the potential of BGP-013 to serve as a topical agent for the treatment of psoriasis.

Poster #20

ZnT-1, an Endogenous Inhibitor of the L-type Calcium Channel: Molecular Mechanism

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Background: The L-type calcium channels (LTCC), one of the main routes for calcium entry into cardiomyocytes, are involved in various aspects of cardiac function. Modulations of LTCC activity are observed in various cardiac pathologies such as ischemia\reperfusion and atrial fibrillation. Recently we showed that ZnT-1, a membrane protein, inhibits the LTCC without altering its expression. The protein exists in the heart and it expression increased in rat atria following acute rapid pacing as well as in the atria of AF patients. In this study we investigated the molecular mechanism of ZnT-1 induced inhibition of the LTCC, with special emphasis on its interaction with the regulatory β-subunit of the LTCC.

Methods and Results: Co-expressing ZnT-1 with the LTCC in *Xenopus* oocytes led to substantial inhibition of the LTCC current with no apparent shift in the current-voltage relationship (59.5 ± 3.2 % of controls for normalized peak current, $P < 0.01$). In either absence or excess of the β-subunit, ZnT-1 did not inhibit the LTCC current in *Xenopus* oocytes (104 ± 1% and 100 ± 1% of the normalized peak current, respectively). Direct interaction between ZnT-1 and the LTCC β-subunit was demonstrated in HEK293 cells by co-immunoprecipitation of ZnT-1-myc and the β-subunit using anti- β-subunit and anti-myc antibodies. Finally, using biotinylation experiment, we demonstrated that co expression of LTCC with ZnT-1 led to a significant reduction in the surface but not in the total expression of the LTCC α-subunit (105 ± 6 % of control for total expression and 47.1 ± 5.8 % of control for surface expression, n=5; p<0.01). The ZnT-1 reduced surface expression of the LTCC α subunit was further substantiated utilizing Total Internal Reflection Microscopy (TIRFM). The TIRFM results confirmed the correlation between the expressions of ZnT-1 and the level of the reduction in the surface expression of α_1-subunit.

Conclusion: The interaction between the LTCC β-subunit and ZnT-1 is an essential component in the mechanism underlying the ZnT-1 induced inhibition of the LTCC probably due to its interfering with the translocation of the LTCC α_1 subunit to the surface membrane. This mechanism may serve as a novel drug target for modulation of LTCC function in the diseased myocardium.

Poster #21

Oligomeric VDAC1 as the Functional Unit in Apoptosis: Dominant Negative VDAC1 Mutants

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The voltage-dependent anion channel (VDAC) plays a central role in apoptosis, participating in the release of apoptogenic factors, such as cytochrome *c*. The diameter of the VDAC1 pore in its high conductance state is about 2.6-3.0 nm, sufficient to move nucleotides and small molecules but insufficient to pass a folded protein, like cytochrome *c* (12 kDa). The involvement of a VDAC1 oligomeric unit in the release of cyochrome *c* and, hence, in apoptosis, has been proposed in our previous studies.

Here, we expressed a fusion protein consisting of native VDAC1 (wt) and E72Q-VDAC1 monomers covalently-linked in tandem and studied the capacity of ruthenium red (RuR, known to bind to VDAC and prevent apoptosis)-insensitive mutant to protect against apoptosis, as induced by various stimuli.

Functional 70 kDa fusion proteins, Wt–WtVDAC1, E72Q-Wt-, Wt–E72Q- and E72Q–E72Q-VDAC1 were constructed and successfully expressed in T-REx-293 cells. As with monomeric VDAC1 over-expression, the Wt–Wt fusion protein induced apoptotic cell death that was prevented by ruthenium red. No anti-apoptotic effect of RuR was observed in cells expressing monomeric E72Q-VDAC1 or an E72Q-containing chimeric protein, although endogenous VDAC1 was present in these cells. RuR-mediated protection against apoptosis as induced by staurosporine (STS) was also observed in T-REx-293 cells expressing monomeric and Wt-Wt VDAC1 but not in cells expressing monomeric E72Q-VDAC1 or an E72Q-containing chimera. These results indicate that E72Q-VDAC1 possesses a dominant negative effect and implies that VDAC1 molecules are capable of intermolecular interactions to form a homo-oligomer, creating a protein-conducting channel that mediates the release of pro-apoptotic proteins. These finding strongly support the concept of a role for VDAC1 in mitochondria-mediated apoptosis.

Poster #22

Cannabidiol (CBD) Ameliorates Cognitive Impairments Associated with a Model of Chronic Liver Disease in Mice

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Background: Hepatic encephalopathy (HE) is a major neuropsychiatric complication of both acute and chronic liver failure, but its pathogenesis is still unknown. It has been suggested that the cognitive deficits characterizing this state result, at least in part, from an inflammatory response in the brain. Cannabidiol (CBD) is a non-psychoactive ingredient of the plant *Cannabis sativa* known for its anti-inflammatory properties. Also, its structure resembles that of resveratrol, which is found in red wine and has anti-inflammatory activity. Resveratrol has also been shown to decrease liver oxidative stress in a model of chronic liver disease induced by ligation of the bile duct in rats (BDL). On the basis of these findings, we hypothesized that CBD may have therapeutic potential in chronic liver disease through anti-inflammatory actions.

Methods: Female Sabra mice were subjected to ligation of the bile duct (BDL). Sham operated animals were used as controls. Three weeks post-surgery, animals receiving either vehicle or 5mg/kg CBD daily were evaluated for cognitive and motor function using the eight arm maze and the open field tests, respectively. The animals were sacrificed and their hippocampi were analyzed for mRNA levels of brain derived neurotrophic factor (BDNF) by RT-PCR analysis, while their striata were analyzed for mRNA levels of the dopamine D_2 receptor.

Results: BDNF expression in the hippocampus and D_2 expression in the striatum decreased two-fold and 1.5-fold, respectively, in BDL mice after 3 weeks, and were fully normalized by CBD. Cognitive function was significantly impaired and activity in the open field decreased two-fold in BDL mice and both were partially restored by CBD.

Conclusion: These results indicate that CBD improves cognitive function by elevating the level of BDNF, which is a neurotrophic factor involved in synaptic plasticity and contributes to normal learning. The decrease in BDNF may result from neuroinflammation. In the striatum, the normalization of D_2 receptor expression may explain the improvement in motor function since this receptor is involved in the control of movement. Further studies are required in order to elucidate the action of CBD by using antagonists of potential pathways of its activity, such as the serotonergic and the adenosine systems.

Poster #23

Adenylyl Cyclase (AC) Isoform Inhibition and the Mechanism of Mood Stabilizers

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Background: Lithium salts (Li), valproate (VPA) and carbamezapine (CBZ) are mood-stabilizing drugs used to treat bipolar disorder, however their molecular mechanism of action is still unknown. All three drugs were reported to have an inhibitory effect on adenylyl cyclase (AC) activity, which is now known to have nine membrane-bound isoforms (AC1-AC9). Our recent study demonstrated that AC5 is the most Li-inhibitable. The present study aimed to investigate whether Li, CBZ and VPA exhibit differential effect on the various AC isoforms. Since we found that a therapeutically-relevant Li concentration preferentially inhibited AC5 activity both when the enzyme was stimulated by forskolin, a direct activator of AC, and when the enzyme was stimulated via the D1 receptor we also studied whether, as previously reported for total AC activity, Li's inhibition of AC5 is due to competition with Mg^{2+} ions, and whether AC5 knockout mice exhibit lithium-like behavior in models of depression and mania.

Methods: The effect of Li, CBZ and VPA on forskolin- or D1 agonist-stimulated ACs activity was studied in COS7 cells transfected with each of the AC isoforms (1-9) with or without the D1 dopaminergic receptor. The effect of Mg^{2+} ions on Li's inhibition of AC5 was studied in isolated membranes from COS7 cells expressing AC5 and the D1 receptor with 1, 3 or 10 mM Mg^{2+}. AC5 KO mice were tested for Li-like behavior in the Porsolt forced-swim test model of depression and in the amphetamine-induced hyperactivity model of mania.

Results: Similarly to our above mentioned findings with Li, CBZ specifically inhibited forskolin-stimulated AC1 and AC5. When stimulated by the D1 agonist SKF-82958 all AC isoforms were significantly inhibited, the most inhibitable isoforms being AC5 and AC7. *In silico* investigation raises the possibility that CBZ preferentially attaches either to the P-site or to the catechol estrogens (CE) site of AC5. VPA did not affect any of the AC isoforms stimulated either by forskolin or via the D1 receptor. Ten mM $Mg2^{+}$ reduced Li's inhibition of AC5 to 30% compared with the ~100% inhibition obtained with 1 and 3 mM. AC5 knockout mice behaved in the Porsolt forced-swim test (FST) similarly to antidepressants- or Li-treated wildtype littermates but did not differ from the wildtypes in the amphetamine-induced hyperactivity test.

Discussion: Li, CBZ and VPA seem to attenuate the increase in cAMP levels via different mechanisms. Li competes with essential Mg^{2+} ions of AC5, CBZ apparently competes for P-site or CE-site binding, and VPA activates phosphdiesterase. The *in vitro* along with the behavioral findings of this study suggest that AC5 is involved in the antidepressant effect of both Li and CBZ. AC5 is particularly localized in brain dopaminergic regions therefore our results suggest the involvement of dopamine and dopaminergic regions in the antidepressant effect of mood stabilizers. Search for novel specific AC5 inhibitors and investigating their effect in the antidepressant-predictive FST may lead to the development of new antidepressant drugs.

Poster #24

ZnT-1, a novel regulator of T- type calcium channels, mediating a crosstalk between T-type and L-type calcium channels

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Background: ZnT-1 is a ubiquitous transmembrane protein that was studied in the past mainly in the context of zinc metabolism. Recent findings marked ZnT-1 as endogenous inhibitor of L-type Calcium Channels (LTCC) and as a potent activator of Raf-1 in the Ras-ERK signaling pathway. These published results highlight the importance of ZnT-1 as regulatory protein in vital cellular functions other than zinc homeostasis. Recently, we demonstrated that ZnT-1 inhibits LTCC function by direct binding to the LTCC β subunit, acting as a scaffold protein interfering with the α subunit trafficking to the plasma membrane. In addition, we found that ZnT-1 expression in the heart is sensitive to physiological and pathophysiological stimuli such as electrical pacing and ischemia\reperfusion. In the present study we explore the regulatory effects of ZnT-1 on the activity of T-type Calcium Channels (TTCC) that have important functions in various tissues, and are known to co express with the LTCC in many cells.

Methods and Results: Two electrode voltage clamp recordings in *Xenopus* oocytes revealed that the TTCC current is markedly enhanced in the presence of ZnT-1 (peak current being 167.95±9.27 % of control, $p<0.005$, 4 independent experiments with 30 oocytes in each group), in contrast, to the observed inhibitory effect of ZnT-1 on LTCC function. Overexpression of dominant negative Raf-1 totally abolished the augmentation of the TTCC current by ZnT-1 (peak current being 103±4.1 % of control, $p=0.37$, 2 independent experiments with 25 oocytes in each group). In addition, we found that in the presence of the β subunit of the LTCC, ZnT-1 loses its ability to increase the TTCC current (peak current being 95.8±10.5 % of control, $p=0.43$,3 independent experiments with 54 oocytes in each group). Finely, co expression of LTCC, TTCC and ZnT-1 led to preferential inhibition of the LTCC while no augmentation of the TTCC was observed under these conditions.

Conclusion: The current work showed that ZnT-1 inversely regulates TTCC and LTCC function. The ZnT-1 induced augmentation of the TTCC involves activation of the ERK-MAPK signaling pathway; whereas the LTCC β subunit interaction with ZnT-1 mediating crosstalk between LTCC and TTCC. These findings may indicate a key role for ZnT-1 as a regulator of cellular calcium homeostasis.

Poster #25

INO-8875, a Highly-Selective A1 Adenosine Receptor Agonist: Evaluation of Chronotropic, Dromotropic and Hemodynamic Effects in Rats

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Background: Although adenosine is the most potent known pharmacologic inhibitor of AV nodal conduction, its therapeutic utility is limited to the acute treatment of SVT owing to its extremely short physiological half-life and hemodynamic side-effect profile. The purpose of the present study was to compare the cardiovascular profiles of two novel adenosine 1 (A1) receptor agonists intended for slowing of AV nodal conduction. CV-510 has demonstrated negative dromotropic clinical effect with minimal chronotropic and hemodynamic activity. INO-8875 (Ki < 1 nM; A1/A2a receptor binding affinity > 10,000) has recently entered human studies.

Methods and Results: Mechanically-ventilated rats were instrumented with bipolar mini-hook electrodes inserted via a minimal lateral thoracotomy. Pacing and recording hooks were placed on the high and low RA, respectively, whereas a third pair recorded from the RV. INO-8875 (1-50 mg/Kg) and CVT-510 (20 and 50 mg/Kg) injected IV over 20 min induced dose-dependent slowing of AV nodal conduction and heart rate that were linearly related, although INO-8875 activity was significantly more pronounced on AV nodal conduction (regression slopes of INO-8875/CV-510 = 0.42/0.27, $p < 0.05$). At the highest doses, INO-8875 and CVT-510 increased the RR interval to 165±12 % and 180±4 % of control, respectively (n=5, $p<0.01$ for both), and prolonged the minimal 1:1 AV conduction cycle to 131±78 % of control (*n*=5, $p<0.05$) and 119±5 % (*n*=4, $P<0.05$), respectively. INO-8875 exhibited a greater duration of action, lasting up to 2.5 hours post-dosing, whereas the effects of CV-510 dissipated over 1 hour ($p<0.01$). Under constant atrial pacing (150 ms cycle length) INO-8875 (50 mg/Kg) did not reduce the carotid arterial blood pressure (*n*=4).

Conclusions: In rats, INO-8875 exhibits increased dromotropic activity and greater duration of action compared to CV-510. INO-8875 does not reduce the arterial blood pressure up to the maximal tested dose of the present study (50 mg/Kg). Based upon the above data, INO-8875 may have future role in acute and sub-acute clinical situations that need AV conduction slowing such as AF or A flutter/tachycardia.

Poster #26

Carvacrol Is a Novel Inhibitor of *Drosophila* TRPL and Mammalian TRPM7 Channels

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Ion channels from the superfamily of the Transient Receptor Potential (TRP) channels are universal biological sensors that detect changes in the environment in response to a myriad of stimuli including cold or hot temperatures, natural chemical compounds and mechanical stimuli. Most of the research on modulators of TRP channels was focused on the heat-activated TRPV1 and cold-activated TRPM8 channel which are activated by capsaicin and menthol respectively. We investigated the effects of known activators of the TRPV3 channel on non-thermo TRP channels. Carvacrol, the main ingredient of the essential oil of Oregano (*Origanum vulgare*), was identified as an inhibitor of both, *Drosophila* TRP and TRPL channels, as well as mammalian TRPM7 channels. Application of carvacrol caused a significant reduction of currents mediated by these channels, both in-vivo and in-vitro. Carvacrol caused a dramatic reduction in light induced currents in Drosophila photoreceptor cells. In Drosophila S2 cells expressing the TRPL channel and human HEK-293 cells expressing the TRPM7 channel application of carvacrol caused a reversible inhibition of TRP channel mediated currents. Since TRPM7 was shown to be critically involved in neurotransmitter release (Brauchi et al., PNAS, 2008, 8304-8) the effect of carvacrol was also investigated in a primary culture of hippocampal CA1-CA3 neurons expressing TRPM7. Again, carvacrol inhibited vesicle release by a fixed number of single regular spikes. In addition we showed that carvacrol inhibits phospholipase C (PLC) but its inhibition of TRP channels is not mediated via PLC and the effect is independent of open channel block. Giving the importance of the *Drosophila* photoreceptor cell as a model for TRP channels and the involvement of TRPM7 in critical physiological and pathophysiological processes these findings provide a novel pharmacological tool for investigating and understanding these processes.

Poster #27

Targeting the Voltage Sensor of Kv7 Channels: Structural and Chemical Strategies to Cure Hyperexcitability Disorders

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Voltage-sensitive ion channels play crucial roles in brain and cardiac excitability. They are endowed with two main structural modules, the voltage sensor domain (VSD) and the pore domain. Mutations of ion channel genes in humans lead to severe inherited neurological, cardiovascular or metabolic disorders, called 'channelopathies'. To cure these diseases, only the pore and gate regions of the channels have been targeted so far with drugs acting as channel blockers or openers. In contrast, the voltage-sensing domain was practically not exploited for these therapeutic purposes. We recently designed a series of novel diphenylamine carboxylate derivatives to generate powerful Kv7.2 channel openers and blockers. The carboxylate moiety was derivatized into amides or esters and linked to various alkyl and ether chains. All the compounds act from the outside and not from inside the cell. We found that aromatic ring deactivators (electron-withdrawing groups) like nitro groups (NO_2) or halogens (Cl) increase the drug potency and that a terminal hydroxyl group in the ether and alkyl chain is absolutely required to generate a Kv7.2 channel opener. Openers like the compound NH29, robustly increased Kv7.2 K^+ currents, causing a hyperpolarizing shift of channel activation. In sensory dorsal root ganglion and hippocampal neurons, the opener hyperpolarized the membrane potential and depressed evoked spike discharge. NH29 also dampened hippocampal glutamate and GABA release by inhibiting spontaneous excitatory and inhibitory post-synaptic currents. In vivo, these openers exhibited anti-convulsant and anti-neuropathic pain activities. To identify the residues involved in the action of NH29 we performed site directed mutagenesis in the homomeric Kv7.2 channel, expressed in CHO cells and checked the potency of NH29 using the whole-cell patch-clamp technique. Our data indicate that the site of the Kv7.2 opener compound maps to the VSD of the Kv7.2 subunit at residues, which are located at the interface between the S1-S2 and S4 helices. Notably, we found that the S4 mutant residues L197G, R198A, R201A, R207W and R214W are significantly less sensitive to the activating effect of NH29 compared to wild-type Kv7.2 channels. Interestingly, our data indicate that NH29 does not act on the binding site of a well-known opener retigabine at the cytoplasmic parts of S5 and S6 near the gate. Docking experiments suggest that the nitro functionalities of NH29 acts as a nucleophile/H-bonding acceptor and interacts with the guanidinium group/H-bonding donor from arginine residues in the S4 helix (R207). In all, our data suggest that the new Kv7.2 channel openers are gating modifiers, which interact with the externally accessible surface of the VSD at the groove formed by the interface between S4 helix and S1-S2, thereby stabilizing the VSD in the activated conformation. Our research strategy is expected to provide new therapeutic modalities for the treatment of major excitability disorders like epilepsy, migraine or neuropathic pain.

Poster #28

Cannabinoid CB1 Receptor Antagonist Manipulation at Birth and May Be Associated with ADHD

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Background: Attention Deficit Hyperactivity Disorder (ADHD) is a condition that becomes apparent in some children in the preschool and early school years. It is estimated that between 3-5 percent of children have ADHD in USA, and 5-10 percent in the Israel. Attention Deficit Hyperactivity Disorder is characterized by inattention, impulsivity and hyperactivity. A familiar feature of ADHD is the response to psychostimulants such as methylphenidate (Ritalin) and D-amphetamine (Adderall). In ADHD patients, low doses of stimulants produce beneficial behavioral effects by reducing excess motor activity and enhancing concentration. Although ADHD has been known for over 80 years, the etiological and risk factors for ADHD are still unclear. Chadapin and colleagues (2005) found that low birth weight is one of the most important predictive factors of ADHD. Thus low birth weight infants are commonly found to have both cognitive and behavioral problems in childhood. In a series of studies performed in neonatal mice we have demonstrated that the cannabinoid CB1 receptor is critically important for the initiation of the suckling response (Fride et al., 2001; 2003; 2007). Non organic failure-to-thrive (NOFTT) is defined as an abnormally low weight and/or height for age without a known organic cause. Children who suffered from NOFTT are thought to display behavioral and cognitive dysfunctions in later years. Recent data from our laboratory suggest that an oral motor deficiency, which is similar to symptoms of non organic failure to thrive in infants with NOFTT, develop cognitive and behavioral abnormalities at later stages of development. Now we propose that a deficient ECBR (Endocannabinoid-CB-receptor) system may comprise a risk factor for ADHD.
Methods: In order to investigate the effects of neonatal exposure to cannabinoid CB1 receptor blockade, male and female pups were administered a single injection of SR141617 (rimonabant, 5 or 10 mg/kg), within 24 hours of birth. At two months of age, mice were tested in an assay for pre-pulse inhibition of the acoustic startle response. At the age of 16 weeks the same mice were tested for motor activity in an open field, immobility on an elevated ring and for anxiety in the 'plus-maze' assay.
Results: We found a significant reduction in the acoustic startle response in the female mice treated with 10 mg/kg, and decreased performance in the PPI test at all doses. To the 5 mg/kg dose, mice responded in a sex-dependent manner: only male mice showed a decrease in PPI and increase in ASR while females only showed a trend. Both male and females displayed significant hyperactivity and decreased ring-immobility. In the plus maze, both males and females spend more time in the open arms than in the closed arms, suggesting decreased vulnerability to anxiety-provoking situations.
Conclusions: we have demonstrated here that neonatal manipulation of the cannabinoid CB1 receptor precipitates symptom of ADHD at adulthood. We conclude that at least a subgroup of ADHD may be caused by a developmental deficiency of the Endocannabinoid system.

Poster #29

Imaging of Hexokinase-GFP as an Indicator of Apoptosis Induction

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Recent studies, from our and other groups, demonstrated that the mitochondrial bound isoforms of hexokinase (HK-I and HK-II) can prevent cytochrome *c* release from the mitochondria and thus apoptosis by binding to the voltage-dependent anion channel (VDAC). In cancer cells, HK is at the apex of the glycolytic pathway that provides the energy and metabolic intermediates required by the biosynthetic pathways of such demanding cells. Because of its dual role in bioenergetics and cell death, the HK-mitochondria interaction has been considered as a target for anti-cancer drugs in recent years.

In this study, we have used HK-I-GFP and HK-II-GFP in order to monitor, by confocal fluorescence microscopy, the relationship between the interaction of HK with VDAC and HK protection against apoptosis. While in cells expressing native VDAC1, HK-I-GFP fluorescence is punctuated and co-localized with MitoTracker (a mitochondria marker), in cells expressing E202Q-mVDAC1 mutant, which does not confer HK protection against cell death, HK-I-GFP fluorescence was diffused in the cytosol rather than co-localized with mitochondria. These results indicate that indeed, HK-I does not bind to the mutated VDAC1 in agreement with its inability to confer protection against cell death. Similarly, HK-I-GFP fluorescence in cells expressing VDAC1-based peptides, found to prevent HK protection against cell death, was diffused throughout the cytosol. Thus, conditions and treatments that detach mitochondrial-bound HK-GFP can be reflected in converting the HK-GFP punctuated fluorescence to diffuse in the cytosol.

Since one of the major hallmarks of tumor cells is their relative resistance against cell death, owing to over-expression of anti-apoptotic proteins of the Bcl2 family and HK, as mediated via interaction with VDAC1, displacing HK from its binding site in VDAC1 can, therefore, serve to guide development of a new selective approach for cancer therapy.

Poster #30

Diverse Regulation of Neuronal G protein Gated K^+ Channel (GIRK), GIRK1 and GIRK2 by Gα and Gβγ

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G protein activated K^+ channels (GIRK, Kir3) mediate postsynaptic inhibitory effects of neurotransmitters by direct binding of Gbg following activation of $G_{i/o}$ proteins via numerous G protein coupled receptors. The main neuronal GIRK channel is composed of GIRK1 and GIRK2 heteromers. These channels express in various regions of the brain such as the hippocampus and the cerebellum. Unlike GIRK1, GIRK2 can also form function homomeric channels predominantly expressed in the substantia nigra.

Using *in vitro* protein interaction studies, we show that purified Gβγ enhances the binding of the whole cytosolic domain of GIRK1 to Ga_{i3}^{GDP} or Ga_{i3}^{GTP}. This enhancement was not observed with GIRK2, suggesting a diverse modulation of these channels by Gα and Gβγ.

To explore the functional implication of these differences we expressed homomeric GIRK1* (using a pore mutant that forms functional homomers) and GIRK2 channels in *Xenopus* oocytes. GIRK channels were activated by agonist application via a coexpressed G protein couple receptor (GPCR), by coexpression of Gβγ (whole-cell experiments), or by application of purified Gβγ to excised membrane patches. In addition, we utilized two $G\alpha_{i3}$ mutants, “constitutively active” $G\alpha_{i3}$Q204L ("QL"; poor GTPase) and “constitutively inactive” $G\alpha_{i3}$G203A ("GA") which forms a stable complex with Gβγ, simulating the GTP/GDP bound state. GIRK2 behaved like a “classical” Gβγ effector, showing very low basal activity and strong Gβγ- dependent activation, while Gα expression was without effect. GIRK1*, on the other hand, exhibited larger basal currents and low response to Gβγ. Furthermore, at high expression levels GIRK1* could not be activated by coexpressed Gβγ whilst retaining activated by agonist via a GPCR. In addition, in excised patches GIRK1* homomers displayed a reveres correlation between the basal activity and the Gβγ evoked currents. $G\alpha_{i3}$GA, a “constitutively inactive” mutant, restored the ability of Gβγ to activate GIRK1* (in whole cells as well as excised patches), indicating that $G\alpha_i$, probably as $G\alpha_i\beta\gamma$ heterotrimer, regulates the Gβγ gating of GIRK. Ga_{i3}QL elicited no effect.

These results suggest a specific role for GIRK1 as the scaffold for Gαβγ within GIRK-G protein signaling complex, and imply that the $G\alpha_{i/o}\beta\gamma$ heterotrimers are not only precursors of free Gβγ, but also active regulators of GIRK gating.

Poster #31

The Activity of K_{2P} Potassium Leak Channels is Regulated by the Membrane Potential

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Potassium K_{2P} channels carry leak or 'background'currents that are mostly time- and voltage-independent. By influencing the cell membrane resting potential and resistance, leak currents shape the duration, frequency and amplitude of action potentials, and therefore, modulate cell responsiveness and excitability.

We have previously shown that changes in cell holding potential regulate the activity of K_{2P} channels. In this work we studied the mechanism by which K_{2P} channels, expressed in *Xenopus laevis* oocytes, sense changes in membrane holding potential. We used the human K_{2P}2.1 (KCNK2, TREK1) and *Drosophila* KCNK0 as model channels. Holding the oocyte at a negative membrane potential (-80 mV) for 2-5 minutes decreased KCNK0 currents by approximately 5-fold, while increasing K_{2P}2.1 currents by approximately 5-fold. In contrast, holding the oocyte at a depolarized potential (-20 mV) increased KCNK0 currents by approximately 3-fold, while decreasing K_{2P}2.1 currents by approximately 4.0-fold. Over expressing the *Xenopus* voltage-sensor-containing phosphoinositide phosphatase (VSP) had only a minor effect on K_{2P} channels activity. Conversely, application of a G-protein activator, a G-protein inhibitor or a phospholipase C inhibitor eliminated all voltage-related current fluctuations. Moreover, mutating K_{2P}2.1 phosphorylation sites abolished the channel's voltage sensitivity. We thus hypothesize that the activity of specific protein kinases is regulated by the membrane potential through modulation of G-proteins activity. This, in turn, is expected to modulate the activity of membrane proteins and the biophysical properties of excitable systems.

Poster #32

The Anthelmintic Drug, Ivermectin, Stabilizes a Closed Channel Conformation of a Chimeric Cys-loop Receptor

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Ivermectin (IVM) is an antiparasitic drug widely administered to cattle to treat against intestinal worms. The major molecular targets of IVM are invertebrate glutamate-activated chloride channels (GluClα/β receptors). These pentameric channels belong to the Cys-loop receptor superfamily. At nM concentrations, IVM irreversibly activates the heteromeric GluClα/βRs by acting as an agonist that dissociates very slowly. As such, IVM enables continuous inflow of Cl^- ions across the postsynaptic membrane and it sustains hyperpolarization in peripheral motor interneuronic synapses. This suppresses transmission of neuronal impulses and ultimately leads to paralysis and death of the worm. Long-term IVM usage has already caused an environmental damage because residual drug is excreted in the feces of the cattle. Insects, which are adversely affected by the drug, no longer degrade the cattle's dung that consequently decomposes very slowly and causes persistent contamination of underground water.
With μM concentrations, IVM can activate and/or potentiate vertebrate glycine-, GABA- and acetylcholine-gated ion channels that also belong to the Cys-loop receptor superfamily. The finding that IVM activates the glycine receptor even in the presence of the competitive antagonist, strychnine, indicates that IVM does not bind in the neurotransmitter-binding pocket(s). Yet, nothing is known about the region where IVM binds, its relation to the neurotransmitter-binding pocket(s) and how it irreversibly stabilizes the gate of the pore in an open state.

Here, the effect of IVM was examined on a chimeric α7-GluClβR, which has the extracellular acetylcholine-binding domain of the α7 nicotinic acetylcholine receptor (nAChR) and the pore domain of the GluClβR. Since a homomeric GluClR that consists only of β subunits cannot be activated by IVM while the α7-nAChR is potentiated by IVM, it was initially assumed that the chimeric α7-GluClβR would be potentiated as well. Unexpectedly, application of IVM (5 μM) for 10 or 4 sec prior to application of the agonist (ACh) led to strong inhibition of the response (~90%). This inhibition occurred even when a 10-sec-long washout was performed in between the IVM and ACh applications. Kinetic analysis of currents' decline after activation (i.e., desensitization) shows that addition of IVM to the ACh-containing solution, during desensitization, moderately accelerates the desensitization of the α7-GluClβR. Also, following desensitization in the presence of IVM, the remaining steady-state currents were ~55% of those measured in the presence of ACh alone.

We conclude that IVM exerts its inhibitory effect by stabilizing predominantly the resting state of the α7-GluClβR, but it can also stabilize the desensitized state of the chimeric receptor. Since the major structural differences between the IVM-activated/potentiated Cys-loop receptors and the IVM-inhibited α7-GluClβR reside at the interface between the neurotransmitter-binding domain and the pore domain, we suggest that IVM binds at this interface.

Poster #33

Non Stationary Fluctuation Analysis of Calcium in Secretory Vesicles

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Calcium ions have several important functions in synaptic communication. They are directly involved in the triggering of secretory vesicle content release by exocytosis. These vesicles however, are too small for confocal optical analysis. Sea urchin unfertilized eggs have a much larger vesicle size and hence have been found to be suitable and convenient for studying calcium dependent exocytosis.

Previous work has shown that sea urchin vesicles contain a high calcium concentration. Fluorescent confocal microscopy demonstrated well defined calcium dynamics.

Here we analyzed the calcium fluctuations. Due to a bleaching process we used non-stationary stochastic analysis. It showed that the variance of the calcium signal is larger than the mean. This indicates that the variance is much larger than the expected for a random Poisson process. We speculate that this extra-variance analysis can contribute to the understanding of the processes that control intracellular calcium. We found that, as expected, the highest levels of calcium induced fluorescence were in the center of the secretory vesicle. The Fano factor (variance\mean fluorescence) is the lowest in the center and increases as one approaches the edges of the vesicle. The highest extra-variance is at the edges of the secretory vesicle. Due do this extra variance, we can speculate that there is an interaction between the calcium in the vesicle and the calcium in the cytosol. Moreover, we speculate that the secretory vesicle takes part in the regulation of cytosolic calcium concentration.

Poster #34

The Role of the Cysteine Residues of VDAC1 in Cell Growth and Apoptosis

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Mitochondria play a critical role in ATP synthesis, modulation of cell redox status, osmotic regulation, pH control, calcium ions homeostasis and cell signaling. Mitochondria also play a central role in the regulation of apoptosis. During transduction of an apoptotic signal into the cell, an alteration in the mitochondrial permeability transition occurs causing the release of apoptogenic proteins, such as cytochrome *c* and apoptosis inducing factor. Evidence indicates that voltage-dependent anion channel (VDAC) allows communication between the mitochondria and the cytosol and regulated apoptotic cell death by participating in the release of cytochrome *c* from the mitochondria and interacting with apoptosis regulating proteins. Although VDAC1-3D structure has yet to be determined, it is agreed that VDAC1 consists of a β-barrel composed of 13-16 β-strands and an α-helix at the N-terminal. Rat VDAC1 contains two cysteine residues at position 127 and 232, that according to most proposed VDAC1 topology models at least one of these cysteine residues is facing the hydrophilic pore.
To verify the involvement of the VDAC1 cysteines in its functions in cell growth and apoptosis, we construct a mutated rat VDAC1 in which the two cysteines C-127 and C-232 were replaced by alanine. We found that the cysteine-less VDAC1 expressed in human cell lines is localized to mitochondria and can restore cell growth of cells silenced for endogenous VDAC1 expression. Moreover, as over-expression of native VDAC1, the cysteine-less VDAC1 induced apoptotic cell death and underwent VDAC1 oligomerization upon apoptosis induction. The results suggest that the two cysteines are not necessary for VDAC1 oligomerization and apoptosis as induced by over-expression of VDAC1. These findings indicate that the cysteine residues of VDAC1 do not interfere with VDAC1 role in cell growth or apoptosis.

Poster #35

The Orientation of Amino Acid Side Chains in the Activation Gate of a Cys-loop Receptor Is Conserved During Channel Gating

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Neurons regulate the propagation of chemo-electric signals throughout the nervous system by opening and closing ion channels, a process known as gating. Here, the orientation of amino acids comprising the activation gate of a Cys-loop receptor was probed during channel gating. To this end, we have substituted histidine residues at the bottom (most intracellular) end of the pore-lining segments and measured inhibition of ionic flow by externally applied Zn^{2+} ions. In a previous study (Paas et al., PNAS 2005), it was shown that application of Zn^{2+} before or after receptor activation (channel opening) results in stabilization of a closed-blocked or an open-blocked state, respectively. Here, short-term exposure of the open-blocked states to a washout solution devoid of an agonist and Zn^{2+} leads to 'complete' deactivation as reflected by the capacity of the channels to be fully re-activated, both in terms of activation kinetics and activation amplitude. In contrast, deactivation of the open-blocked state by omitting the agonist and keeping Zn^{2+} in the wash solution results in the stabilization of a large portion of the channel population in a nonconductive state, which could hardly be re-activated.

Direct radioligand binding studies show that Zn^{2+} does not affect the binding affinity of the receptor to its agonists, acetylcholine and nicotine. These binding results indicate that when Zn^{2+} blocks the pore and stabilizes it in an open-blocked conformation, washout in the presence of Zn^{2+} ions completely removes acetylcholine from its binding pockets.

Conclusively, washout of ACh from open-blocked channels with Zn^{2+}-containing physiological solution allows the activation gate to shut on the Zn^{2+} ion and to tightly trap it. Zn^{2+} trapping inside the gate is only feasible if the His side chains point towards the axis of the permeation pathway when the channel gate closes. In line with the experimental results, computer-assisted structural modeling shows that upon channel closure, the His residues get closer to the axis of ion conduction and adjacent histidines get closet to one another. This process enables tighter accommodation of a Zn^{2+} ion compared to Zn^{2+} binding within the open gate.

Poster #36

Computational Analysis of Voltage-Gated Potassium-Channels

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Voltage-gated potassium channels, such as Kv1.2, are composed of four identical monomers, each containing two domains: the voltage-sensing domain (VSD) and the pore domain (PD). It is thought that changes in the electrostatic potential across the membrane affect the pore opening through four conserved arginine residues in the VSD. We analyzed the fluctuations of the amino acids of Kv1.2 in order to further understand the conformational changes that occur in the VSD and the PD during the transition between the open and closed states of this channel. To this end, we used a model-structure of the entire channel, which was built based on an X-ray structure. The model-structure was analyzed using elastic network models.

We identified several hinges, and suggested motions of rigid elements within each domain, and motions of the domains with respect to each other. The results are consistent with experimental data and with previous elastic model calculations of the pore domain of other potassium channels. Interestingly, the analysis revealed correlation between the fluctuations of the selectivity filter, located in the external region of the channel, and the internal parts of the PD and the VSD. Moreover, differences in the hinges and the dynamic couplings observed in the monomer and the tetramer suggest that the voltage sensing motion occurs independently within each monomer, whereas gating is a cooperative motion of all four monomers.

Poster #37

Direct Analysis of Cooperativity in Multi-Subunit Allosteric Proteins

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Allosteric regulation of protein function is a fundamental phenomenon of major importance in many cellular processes. Such regulation is often achieved by ligand-induced conformational changes in multimeric proteins that may give rise to cooperativity in protein function. At the heart of allosteric mechanisms offered to account for such phenomenon, involving either concerted or sequential conformational transitions, are changes in inter-subunit interactions along the ligation pathway of the protein. Structure-function analysis of such homo-oligomeric proteins by means of mutagenesis, while providing valuable indirect information regarding (allosteric) mechanisms of action, does not define the contribution of individual subunits nor interactions thereof to cooperativity in protein function, since any point mutation introduced into homo-oligomeric proteins will be present in all subunits. Here, we present a general strategy for direct analysis of cooperativity in multi-subunit proteins that combines measurement of the effects on protein function of all possible combinations of mutated subunits, introduced in the framework of a functional oligomeric protein containing tandem-linked subunits, with analysis of the hierarchy of inter-subunit interactions, assessed using high-order double-mutant cycle coupling analysis. We show that the pattern of high-order inter-subunit coupling can serve as a discriminative criterion for defining the concerted *versus* sequential conformational transitions underlying protein function. This strategy was applied to the particular case of the voltage-activated potassium channel protein (Kv) to provide compelling evidence for a concerted all-or-none activation gate opening of the Kv channel pore domain. As such, this is the first direct and detailed analysis of cooperativity in the function of any allosteric protein.