האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

Israel Society for Physiology and Pharmacology

Annual Meeting הכנס השנתי

September 20th 2007

Ma'ale Hachamisha

PROGRAM & ABSTRACTS

הפקולטה למדעי החיים ע"ש מינה ואברד גודמן, אוניברסיטת בר-אילן, רמת-גן 52900

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האגודה הישראלית לפיזיולוגיה ופרמקולוגיה מודה לגופים הבאים שתמיכתם הנדיבה אפשרה קיום כנס זה

The Israeli Society for Physiology and Pharmacology wishes to acknowledge the following sponsors whose generous support has made this meeting possible

The Rector of Bar-Ilan University donated the prize for the student competition in the sum of $750. The award will be presented to the best student lecture and is intended to support active participation in international meetings.

The Magnes lecture prize was awarded to the plenary speaker by the Magnes Foundation.

Program Outline

8:30-9:30 Registration and Refreshments

9:30-11:10 Morning Sessions A-C

11:10-11:30 Coffee Break

11:30-12:30 Student Lecture Competition

12:30-13:30 Posters

13:30-14:30 Lunch

14:15-14:30 Business Meeting

14:30-16:00 Afternoon Sessions D-F

16:00-16:30 Coffee Break

16:30-17:30 The Magnes Memorial Lecture

Prof. Irwin B. Levitan

University of Pennsylvania, School of Medicine

Title: Roles of Ion Channel Regulatory Protein Complexes in Neuronal Physiology and Behaviour

17:30-17:45 Ceremony Awarding the Winners of the Poster and Student Lecture Competitions

Meeting Program

9:30-11:10 - MORNNING SESSION

A - Broken Heart and its Blood Supply (Hall A)
Chairperson: Moshe Flugelman, Technion - Israel Institute of Technology and Lady Davis Carmel Medical Center

9:30 Jonathan Leor, Tel-Aviv University and Sheba Medical Center:
Extracellular Matrix Modification and Tissue Scaffolding

9:55 Mizied Falah, MultiGene Vascular Systems Ltd. and Lady Davis Carmel Medical Center
Use of Endothelial Progenitor Cells (EPCs) for Tissue Engineering

10:20 Moshe Flugelman, Technion - Israel Institute of Technology and Lady Davis Carmel Medical Center
Use of Cells for Heart Failure: Update and Future Prospects

10:45 Keren Shapira, Technion - Israel Institute of Technology
Biocompatible Fibrin-Based Hydrogel for Cardiac Cell Therapy and Tissue Engineering

B - Drug Design (Hall B)
Chairperson: Nir Ben-Tal, Tel-Aviv University

9:30 Ehud Gazit, Tel-Aviv University
The Pre-Clinical Development of Drug Candidates for the Inhibition of Amyloid and Oligomer Formation

9:55 Abraham Nudelman, Bar-Ilan University
Novel Prodrugs of GABA and Typical Anti-Psychotic Agents Exhibit Antischizophrenic Efficacy with Diminished Extrapyramidal Effects

10:20 Yael Marantz, EPIX Pharmaceuticals
3D Structure Based Drug Discovery for GPCRs

10:45 Ronen Shemesh, Compugen Ltd.
Computational Discovery of Eight Novel Peptide Agonists for G-Protein Coupled Receptors

C – Stress (Hall C)
Chairperson: Marta Weinstock, The Hebrew University of Jerusalem

9:30 Marta Weinstock, The Hebrew University of Jerusalem
Gender Differences in the Effects of Prenatal Stress

9:55 Aron Weller, Bar-Ilan University
Effects of Early-Life Stress in Animal Models of Depression

10:20 Alon Chen, The Weizmann Institute of Science
Homeostatic Regulation of Neuroendocrine Stress by the CRF/Urocortin Peptide Family and their Receptors

10:45 Shai Shoham, Herzog Hospital
Stress-Induced Seizures: The Role of Interactions between Cholinergic and NPY-ergic Pathways

11:10-11:30 Coffee Break

11:30-12:30 Student Lecture Competition (Hall A)

11:30 Hodaya Dahan, College of Judea and Samaria and Bar-Ilan University
Neonatal Blockade of the CB1 Receptor: Further Support for Endocannabinoid-CB1 Deficiency as the Biological Basis of 'Non-Organic Failure-To-Thrive' in Infants

11:42 Inna Freikman, The Hebrew University of Jerusalem
NMR Studies of Oxidative Stress-Induced Changes of Membrane Phospholipids in Thalassemic RBC

11:54 Kseny Katsenelson, IDF Medical Corps
Hyperbaric Oxygen Pretreatment Reduces Decompression Sickness Incidence in Rats

12:06 Arieh Moussaieff, The Hebrew University of Jerusalem and College of Judea and Samaria
Is Incense a Pharmacological Tool for Spiritual Exaltation?

12:18 Gali Sela, Technion – Israel Institute of Technology
The Afterload Dependency of the Frank-Starling Law Reflects Cross-Bridge Dependent Regulation of Contraction

12:30-13:30 Posters

13:30-14:30 Lunch

14:15-14:30 Business Meeting (Hall A)

14:30-16:00 – AFTERNOON SESSION

D - Mitochondrial Biogenesis-Apoptosis, Transcription, Translation and Replication (Hall A)
Chairperson: Dan Mishmar, Ben-Gurion University of the Negev

14:30 Claes M. Gustafsson, Karolinska Institutet, Stockholm, Sweden
Regulation of Mitochondrial Transcription in Human Cells

14:55 Ann Saada (Reisch), The Hebrew University of Jerusalem
Disorders of Mitochondrial DNA Replication and Translation

15:20 Varda Shoshan-Barmatz, Ben-Gurion University of the Negev
Mitochondrial VDAC: The Gatekeeper of Cell Life and Death

15:45 Joseph Shlomai, The Hebrew University of Jerusalem
Redox-Mediated Regulation of Kinetoplast DNA Replication in Trypanosomatids

E - Fight for Sight, Physiology and Pharmacology Approaches (Hall B)
Chairperson: Elie Beit-Yannai, Ben-Gurion University of the Negev

14:30 Ahuva Dovrat, Technion - Israel Institute of Technology
Factors and Mechanisms Involved in Cataract Formation

14:55 Tamar Kadar, Israel Institute for Biological Research
Inhibition of Corneal Neovascularization Following Chemical Injury

15:20 Arieh S. Solomon, Tel-Aviv University
Congenital Glaucoma in Rabbit: A Model of Glaucoma Research

15:45 Anat Loewenstein, Tel-Aviv University
Toxicity, Pharmacokinetics and Penetration of Antiangiogenic Drugs

F – Neurodegeneration (Hall C)
Chairpersons: Rina Aharoni, The Weizmann Institute of Science and Tamir Ben-Hur, The Hebrew University of Jerusalem

14:30 Dan Frenkel, Tel-Aviv University
Immunology and Immunotherapy of Stroke

14:55 Alon Monsonego, Ben-Gurion University of the Negev
Immune Regulation and Neuronal Repair in the Progression of Alzheimer's Disease

15:20 Rina Aharoni, The Weizmann Institute of Science
The Potential for Neuroprotection and Neurogenesis in Multiple Sclerosis by an Immunomodulatory Treatment with Glatiramer Acetate

15:45 Tamir Ben-Hur, The Hebrew University of Jerusalem
Neural Stem Cell Therapy in Multiple Sclerosis

16:00-16:30 Coffee Break

16:30-17:30 The Magnes Memorial Lecture (Hall A)

Prof. Irwin B. Levitan

School of Medicine, University of Pennsylvania

Title: Roles of Ion Channel Regulatory Protein Complexes in Neuronal Physiology and Behaviour

17:30-17:45 Ceremony Awarding the Winners of the Poster and Student Lecture Competitions

Program at a glance

Session A (Hall A): **Broken Heart and its Blood Supply**		Session B (Hall B): **Drug Design**		Session C (Hall C): **Stress**	
Chairperson: **Moshe Flugelman**		Chairperson: **Nir Ben-Tal**		Chairperson: **Marta Weinstock**	
9:30	**Jonathan Leor** Extracellular Matrix Modification and Tissue Scaffolding	9:30	**Ehud Gazit** The Pre-Clinical Development of Drug Candidates for the Inhibition of Amyloid and Oligomer Formation	9:30	**Marta Weinstock** Gender Differences in the Effects of Prenatal Stress
9:55	**Mizied Falah** Use of Endothelial Progenitor Cells (EPCs) for Tissue Engineering	9:55	**Abraham Nudelman** Novel Prodrugs of GABA and Typical Anti-Psychotic Agents Exhibit Antischizophrenic Efficacy with Diminished Extrapyramidal Effects	9:55	**Aron Weller** Effects of Early-Life Stress in Animal Models of Depression
10:20	**Moshe Flugelman** Use of Cells for Heart Failure: Update and Future Prospects	10:20	**Yael Marantz** 3D Structure Based Drug Discovery for GPCRs	10:20	**Alon Chen** Homeostatic Regulation of Neuroendocrine Stress by the CRF/Urocortin Peptide Family and their Receptors
10:45	**Keren Shapira** Biocompatible Fibrin-Based Hydrogel for Cardiac Cell Therapy and Tissue Engineering	10:45	**Ronen Shemesh** Computational Discovery of Eight Novel Peptide Agonists for G-Protein Coupled Receptors	10:45	**Shai Shoham** Stress-Induced Seizures: The Role of Interactions between Cholinergic and NPY-ergic Pathways
Session D (Hall A): **Mitochondrial Biogenesis- Apoptosis, Transcription, Translation and Replication**		Session E (Hall B): **Fight for Sight, Physiology and Pharmacology Approaches**		Session F (Hall C): **Neurodegeneration**	
Chairperson: **Dan Mishmar**		Chairperson: **Elie Beit-Yannai**		Chairpersons: **Rina Aharoni and Tamir Ben-Hur**	
14:30	**Claes M. Gustafsson** Regulation of Mitochondrial Transcription in Human Cells	14:30	**Ahuva Dovrat** Factors and Mechanisms Involved in Cataract Formation	14:30	**Dan Frenkel** Immunology and Immunotherapy of Stroke
14:55	**Ann Saada (Reisch)** Disorders of Mitochondrial DNA Replication and Translation	14:55	**Tamar Kadar** Inhibition of Corneal Neovascularization Following Chemical Injury	14:55	**Alon Monsonego** Immune Regulation and Neuronal Repair in the Progression of Alzheimer's Disease
15:20	**Varda Shoshan-Barmatz** Mitochondrial VDAC: The Gatekeeper of Cell Life and Death	15:20	**Arieh S. Solomon** Congenital Glaucoma in Rabbit: A Model of Glaucoma Research	15:20	**Rina Aharoni** The Potential for Neuroprotection and Neurogenesis in Multiple Sclerosis by an Immunomodulatory Treatment with Glatiramer Acetate
15:45	**Joseph Shlomai** Redox-Mediated Regulation of Kinetoplast DNA Replication in Trypanosomatids	15:45	**Anat Loewenstein** Toxicity, Pharmacokinetics and Penetration of Antiangiogenic Drugs	15:45	**Tamir Ben-Hur** Neural Stem Cell Therapy in Multiple Sclerosis

Abstracts of Invited Presentations

Extracellular Matrix Modification and Tissue Scaffolding

Jonathan Leor
Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Hashomer, Israel.

Tissue engineering aims to create, repair and/or replace tissues and organs by using cells, scaffolds, biologically active molecules and physiologic signals. It is an interdisciplinary field that integrates aspects of engineering, chemistry, biology and medicine. One of the most challenging goals in the field of cardiovascular tissue engineering is the creation of a heart muscle patch. This presentation will describe the principles, achievements and challenges of achieving this ambitious goal of creating contractile heart muscle. In addition, the new strategy of in situ and injectable tissue engineering for myocardial repair and regeneration will be presented.

Use of Endothelial Progenitor Cells (EPCs) for Tissue Engineering

Mizied Falah and Moshe Flugelman

[1] MultiGene Vascular Systems Ltd. (MGVS) [2] Department of Cardiovascular Medicine, Lady Davis Carmel Medical Center, Haifa

EPCs circulating in the peripheral blood provide an alternative source for mature endothelial cells (ECs) that can be seeded onto the inner surface of synthetic grafts. Recently, MGVS Ltd had developed a bypass conduit composed of expanded Polytetrafluoroethylene (ePTFE) graft coated with ECs. ECs utilized in this graft are isolated from a short vein segment stripped from the patient's arm. The isolated cells are expanded ex-vivo, genetically modified and seeded onto the luminal surface of the synthetic grafts. Such grafts have prolonged patency due to the bio-compatible surface that confers long-term protection against thrombosis when they are exposed to blood flow. MGVS has attained FDA approval for clinical trials with this improved graft which will be implanted to bypass occluded arteries in the limb of patients who have peripheral arterial disease (PAD). As indicated, ECs seeded onto the graft are isolated from the patient's vein by surgical procedure. Such cells can also be derived from EPCs circulating in peripheral blood. The use of EPCs isolated from peripheral blood as a source for ECs will prevent the need for vein stripping, eliminating the risk involved in this surgical procedure. In this meeting we will present procedures showing the purification of EPCs from human peripheral blood samples and supply data showing their characterization and differentiation in vitro into the phenotype of mature endothelial cells. Our studies of seeding synthetic grafts with EPCs are also aimed to replace diseased vessels by tissue-engineered blood vessels.

Use of Cells for Heart Failure: Update and Future Prospects

Moshe Flugelman
Technion – Israel Institute of Technology and Department of Cardiovascular Medicine, Lady Davis Carmel Medical Center, Haifa, Israel

Morbidity and mortality attributed to diseases of the heart and blood vessels are growing worldwide because of changing lifestyles and increased longevity.
In affluent countries, many patients with cardiovascular syndromes do not benefit from current conventional therapies. Failure of current therapies including drugs, catheter-based, and surgical interventions are insufficient in millions of patients. Cell and gene therapy hold great promise for cardiovascular therapies. As most cardiovascular pathologies are confined to a specific organ and are associated with arterial occlusion or muscle damage, both may be theoretically amenable to local cell and gene therapies. Local delivery of genes or cells that can promote the formation of new blood vessels (*angiogenesis*) or lead to *tissue regeneration* should be achievable in the near future with expanding knowledge of cell physiology and with novel molecular technologies. Although thousands of patients were treated with cell and gene therapies to alleviate coronary and peripheral arterial occlusive syndromes, no overwhelming evidence of efficacy was demonstrated so far. Nevertheless, careful review of the work and of current thinking in the field leaves room for optimism that gene and cell therapy may reach the clinical setting in the near future, providing a new option for many patients. Use of stem cells in combination with gene transfer may be the most relevant approach to make these therapies happen.

Biocompatible Fibrin-Based Hydrogel for Cardiac Cell Therapy and Tissue Engineering

Keren Shapira[1], Manhal Habib[2], Lior Gepstein[2], and Dror Seliktar[1]

[1]Faculty of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa, Israel and [2] Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

There are currently no clinically treatments that can be applied to reverse the long-term remodeling associated with myocardial infarction (MI) and prevent the eventual need for whole heart transplantation. Cardiac cell therapy is a new treatment approach which aims to promote the formation of a new contractile cardiac tissue or improve the function of existing cardiac tissue by delivering healthy new cells into the damaged area of the heart. The overall objective of this work is to validate a biosynthetic hybrid biomaterial developed in our laboratory as a cell carrier for cardiac cell therapy. The injectable biomaterial is made from a PEGylated fibrinogen liquid precursor that can polymerize *in situ* using photopolymerization. This makes the material a good candidate for the application of injectable cardiac cell delivery. Moreover, the material properties of the PEGylated fibrinogen matrix can be precisely controlled to regulated cardiomyocyte phenotype and graft reorganization, while at the same time a biocompatible environment is maintained through the fibrin-like biological properties. Our hypothesis asserted that structural and compositional characteristics of a PEGylated fibrinogen matrix can regulate interactions between cardiomyocytes and the biomaterial leading to functional reorganization of the cells within the matrix and thus contribute to functional integration in cardiac cell grafting without impeding the natural contractility of the cardiac tissue. *In vitro* experiments using neonatal rat cardiomyocytes and PEGylated fibrinogen biomaterials were performed to investigate the relationship between material modulus and spontaneous contraction of cell within the construct. Video processing analysis algorithm was applied to video data from contracting constructs in order to characterize spontaneous contraction and to correlate temporal displacement patterns to the material composition and cell density, both of which can affect the matrix stiffness and cardiac remodeling. We also confirmed that cardiomyocyte-specific markers were present in the cells populating the construct. Staining for sarcomeric actin revealed large population of cardiomyocytes with typical striations in the scaffold after one week. Viability assessment was conducted in order to confirm that the different matrix compositions tested did not adversely effect the survival of the cells. Different drug treatments were also examined with the engineered tissue after 7 days in culture, including Isoproteneol, Carbamylcholine and Heptanol. In addition to the work on neonatal cardiomyocytes, feasibility experiments using human embryonic stem cell-derived cardiomyocytes were performed using the biosynthetic biomaterials. In vivo experiments were conducted in order to host integration of cell injections using the biomaterial carrier. The biomaterial was injected to the myocardium of adult fisher rats and polymerized *in situ* with and without syngeneic neonatal cardiomyocytes. The degrading implant was observed *in situ* after one month, accompanied by the formation of new blood vessels. Neonatal cells incorporated into the injected matrix became elongated and integrated with the host myocardium after 5 days. These promising preliminary results suggest that this new advance in injectable scaffold technology may open up new opportunities for myocardial regeneration procedures and provide innovative solutions for some of the most critical obstacles in cardiac cell transplantation.

The Pre-Clinical Development of Drug Candidates for the Inhibition of Amyloid and Oligomer Formation

Ehud Gazit
Department of Molecular Microbiology and Biotechnology,
George S. Wise Faculty of Life Sciences, Tel-Aviv University, Israel

Two key elements in the development of novel anti-amyloidogenic compounds are the targeting of the molecular-recognition modules and the inhibition of the transition of aggregating proteins into predominant β-sheet assemblies. We have suggested, based on experimental and bioinformatics analysis, that aromatic interactions may provide energetic contribution as well as order and directionality in the molecular-recognition and self-association processes that lead to the formation of these assemblies. This is in line with the well-known central role of aromatic-stacking interactions in self-assembly processes. Following this notion, we demonstrated that the diphenylalanine recognition motif of the Alzheimer's β-amyloid polypeptide self-assembles into ordered peptide nanotubes with a remarkable persistence length. Our model recently gained directed support from high-resolution X-ray and electron diffraction and solid-state NMR structures of amyloid fibrils as well as parameter-free models and molecular dynamics studies. We are currently using this notion, as well as a novel □-breakage strategy that was developed in our lab, for the development of novel inhibitors of the process of amyloid formation by utilizing hetero-aromatic interactions. Our lead compound is a novel chemical entity that inhibits the formation of β-amyloid oligomers in vitro and protects cultured cell and isolated cortical neurons from cytotoxic effect of β-amyloid aggregates. Chronic administration of the compound was shown safe and significantly effective in preventing memory impairment in this animal model as assayed by Morris Water Maze experiments. Taken together, our hypothesis provides a new approach to understand the self-assembly mechanism that governs amyloid formation and indicates possible ways to control this process.

Novel Prodrugs of GABA and Typical Anti-Psychotic Agents Exhibit Antischizophrenic Efficacy with Diminished Extrapyramidal Effects

Abraham Nudelman, Nava Shpaisman, Irit Gil-Ad, Igor Terasenko, Abraham Weizman, Kinneret Savitsky, Yona Geffen and Ada Rephaeli
Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel

Schizophrenia, a chronic disease that affects about 1% of the population, is primarily treated with typical and atypical neuroleptic drugs. The treatments are associated with undesirable side effects. Clinical studies have shown a GABAergic neuronal deficit in brains of schizophrenic patients. However, GABA is ineffective as a therapeutic agent, due to its inability to cross the blood brain barrier (BBB). In an attempt to design a new generation of neuroleptic drugs, we synthesized the phenothiazine esters **AN-168** and **AN-187** of GABA and perphenazine and fluphenazine, respective. Because of the greater lipophilicity of the esters than that of the parent molecules, it was expected that they may cross the BBB and be hydrolyzed in the brain, concomitantly releasing the phenothiazines and GABA. The activity of the esters was evaluated in rat models by: prolactin release, catalepsy and sedation, and antipsychotic efficacy. The level of circulatory plasma prolactin, as a marker for the blockade of central dopamine (DA) transmission, showed that the rats treated ip or po with the neuroleptics as well as with the esters was higher than in animals treated with vehicle, demonstrating that the esters were as effective DA receptor antagonists as perphenazine or fluphenazine and were orally bioavailable. Catalepsy as a manifestation of the extrapyramidal adverse effects of typical neuroleptics was studied and animals treated with **AN-168** showed minimal or no catalepsy whereas those treated with **AN-187** exhibited a very low cataleptic behavior. At the same time the parent perphenazine and fluphenazine induced high cataleptic behavior. The results suggest that the combination of dopaminergic-D2 antagonism and GABA agonistic activities displayed by the esters, resulted in a significant decrease of extrapyramidal side effects and preserved classical DA antagonistic activity. The neuroleptic efficacy was evaluated in the *D*-amphetamine–induced hyperactivity and motility model. In this model perphenazine administered po prior to *D*-amphetamine abolished the hyperactivity manifested by frequent climbing attempts and head movements, and induced sedation and catalepsy. In contrast, equimolar doses of **AN-168** reduced the wall-climbing attempts and head movements to non-induced control levels without sedation and with minimal or no catalepsy. It can be concluded that the novel ester **AN-168** is orally bio-available, crosses the BBB and displays GABAergic and neuroleptic activities with diminished catalepsy, thus, it constitutes a prototype of a new line of neuroleptics. A successful Phase I study with **AN-168** labeled as **BL-1020** was concluded and is currently undergoing Phase II testing.

3D Structure Based Drug Discovery for GPCRs

Y. Marantz, I. Sela and O. Kalid
Epix Pharmaceuticals Ltd., Ramat Gan, Israel

GPCR constitute a major family of drug targets involved in many physiological responses. Structure based drug discovery for the GPCR family has been limited by the existence of only one GPCR x-ray structure (i.e. bovine rhodopsin). In this presentation we will demonstrate our structure-based drug discovery process for GPCR targets which is based on a set of novel computational approaches, allowing discovery and development of drug candidates in very short time periods. This process starts with the PREDICT de novo modeling algorithm, continues with high-throughput structure-based in silico screening, which is then followed by integrated computational & medicinal-chemistry lead optimization.

Computational Discovery of Eight Novel Peptide Agonists for G-Protein Coupled Receptors

Ronen Shemesh
Compugen Ltd., Isarel

G-protein-coupled receptors (GPCRs) represent an important group of targets for pharmaceutical therapeutics. The completion of the human genome revealed a large number of putative GPCRs; however the identification of their natural ligands, and especially peptides, suffers from a low discovery rate, thus impeding development of therapeutics based on these potential drug targets.

In this report we describe a new computational method aimed at discovering novel GPCR peptide ligands encrypted in the human proteome. Hundreds of potential GPCR ligand peptides were predicted by machine learning related methods. In vitro screening of 33 of these peptides on a set of 152 selected GPCRs, which included a large group of designated orphan receptors, was conducted utilizing an intracellular calcium release measurement and a cAMP accumulation assay. The screening revealed eight novel peptides as potential agonists that specifically activated six different receptors, mostly in a dose dependent manner. While several peptides are agonists of previously characterized GPCRs, others showed a distinct stimulatory pattern targeted at previously designated orphan GPCRs.

Gender Differences in the Effects of Prenatal Stress

Marta Weinstock
Department of Pharmacology, The Hebrew University Medical Centre, Jerusalem, Israel

An increased incidence of anxiety, depression and attention deficits in children has been linked to psychological stress during pregnancy. We have found that subjection of pregnant Wistar rats to chronic variable stress on days 14-20 of gestation, when the foetal limbic and hypothalamic pituitary adrenal (HPA) axes develop, results in anxiogenic and depressive behaviour, learning and attention deficits in the offspring. The attainment of these effects depended on gender, intensity and duration of maternal stress and behaviour being tested. Learning deficits and reductions in hippocampal LTP were only seen in prenatally-stressed males, while anxiety, depression and increased response of the HPA axis to stress were more prevalent in females. Maternal stress increases corticosterone levels in both genders and decreases testosterone levels and brain aromatase activity in the male the foetal brain, and alters brain catecholamine activity to that in females. All the behavioural and hormonal alterations in the offspring listed above were prevented by maternal adrenalectomy. However, the increased anxiety and HPA axis activity, but not the learning deficits were restored by corticosterone injection that mimics the levels reached by maternal stress. The findings indicate that males and females differ in the sensitivity of developing brain areas to stress hormones.

Effects of Early-Life Stress in Animal Models of Depression

Aron Weller[1], *Yoram Braw*[1], *Rachel Maayan*[2], *Avi Weizman*[2]
[1] Department of Psychology, Bar-Ilan University, Ramat-Gan, Israel
[2] Tel-Aviv University, Tel-Aviv, Israel

Parental depression has been associated with impaired functioning of the hypothalamic-pituitary-adrenocortical axis (HPA) in the offspring. Therefore, we analyzed the activity of this axis in preweanling pups belonging to two animal models of depression (Wistar-Kyoto [WKY] and Flinders Sensitive Line [FSL] rats). In addition to a normal growing condition, a mild chronic stress condition (reduced bedding in postnatal days 2-9) was used in order to elucidate the possible interaction between hereditary vulnerability and early life stress. At postnatal day 17, two pups per litter were removed from the home cage, one was subjected to acute stress (exposure to an adult male rat) and the other was left undisturbed. Blood was collected and corticosterone was assayed by RIA. The results indicate that, while in normal growing conditions there were no differences between WKY and their Wistar strain controls, WKY pups, growing in the chronic stress condition, exhibited dramatically lower corticosterone. The WKY pups' hypo-secretion of corticosterone echoes studies in humans indicating that early stress can lead to a hypocortisolism, a condition with possible effects on brain development, ability to handle stress and health. Compared to Sprague-Dawley strain controls, FSL pups were more resilient to early stress (no difference in corticosterone was found as a result of the early chronic-stress). In addition, only Wistar/WKY rats showed an increase in corticosterone as a result of an acute stress, suggesting lower overall stress reactivity in the SD/FSL lines. The results provide insights into differences between the two models of depression and expand our knowledge on the interaction between hereditary factors and environmental conditions that lead to hypocortisolism in humans.

Homeostatic Regulation of Neuroendocrine Stress by the CRF/Urocortin Peptide Family and their Receptors

Alon Chen
Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Maintenance of homeostasis/allostasis in the presence of challenges requires numerous adaptive responses involving changes in the endocrine, central nervous and immune systems. Dysregulation of the stress response can have severe psychological and physiological consequences. The corticotropin releasing factor (CRF) peptide plays an important role in the regulation of the hypothalamic-pituitary-adrenal axis under basal and stress conditions by integrating the endocrine, autonomic and behavioral responses. CRF and the Urocortins are implicated in the control of arousal, anxiety, cognitive functions and appetite. I will describe the use of integrated molecular, biochemical, physiological and behavioral methods, focusing on transgenic mice as *in vivo* tools to study the physiological roles of novel proteins in this complex system. Identifying novel players and defining the mechanisms that govern the responses to stress will allow better understanding of stress-related disorders.

Stress-Induced Seizures: The Role of Interactions between Cholinergic and NPY-ergic Pathways

Shai Shoham
Research Department, Herzog Hospital, Jerusalem, Israel

According to self-report of epileptic patients, emotional stress is a prominent precipitant of seizures. Evidence for limbic involvement in both emotional stress and in seizures suggested the possibility that neural pathways activated by stress promote the emergence of electroencephalogram (EEG)-detected seizures in hippocampus and amygdala inducing a cascade of neuropathology and plasticity that lowers the threshold for subsequent seizures hence inducing epileptic seizures. In recent years it became evident that repeated seizures without the EEG correlate can be associated in human patients with emotional stress although unlike the case with epilepsy, there is no evidence for neuropathology ("non-epileptic seizures"). This suggested that the relationship between stress and seizures may encompass multiple types of interactions of stress-signals in limbic structures. Several pharmacological studies in animals yielded neuropathology-associated seizures. However, an animal model of "non-epileptic seizures" has not been found yet. A study of cholinergic neurotransmission in stress processes led to development of a transgenic mouse with overexpression of the acetylcholinesterase variant, AChE-R. In this transgenic mouse (TgR), seizures appear spontaneously in 30% of females after one year of age but not in males. Neuropathological analysis in female mice exhibiting repeated seizure episodes did not reveal cell loss in hippocampus or amygdala although intense astrocyte hypertrophy was observed in both regions. This suggested the possibility that these seizures might be prevented from becoming lethal to neurons by a moderating agent such as neuropeptide Y (NPY). NPY is a neurotransmitter that moderates stress reactions and suppresses seizures. We found decline in NPY innervation in the hippocampal stratum lacunosum moleculare and outer molecular layer of the dentate gyrus of TgR mice. The greatest decline in NPY innervation appeared in mice exhibiting seizures, suggesting the possibility that in nonepileptic seizures, a latent process may be a partial reduction in NPYergic neurotransmission resulting in reduced threshold for emotional activation of seizures but without deterioration to neuropathology. This model is of particular interest to exploration of human syndromes since more women exhibit non-epileptic seizures than men.

Regulation of Mitochondrial Transcription in Human Cells

Claes M. Gustafsson
Division of Metabolic Diseases, Karolinska Institutet, Stockholm, Sweden

Regulation of mammalian mtDNA expression is critical for altering oxidative phosphorylation capacity in response to physiological adaptation and disease. The basal machinery needed for mtDNA transcription initiation is molecularly. However, mitochondrial gene expression must also be modulated in response to altered demands for oxidative phosphorylation capacity. We will discuss the characterization of MTERF3 as a negative regulator of mtDNA transcription. MTERF3 is an essential gene, as homozygous knockout mouse embryos die in midgestation. Tissue-specific inactivation of MTERF3 in the heart causes aberrant mtDNA transcription and severe respiratory chain deficiency. MTERF3 binds the mtDNA promoter region and loss of MTERF3 leads to increased transcription initiation from both promoters. This increased mitochondrial transcription initiation causes transcriptional collision with decreased expression of critical promoter-distal tRNA genes, which, in turn, impair mitochondrial protein synthesis. MTERF3 is thus the first example of a mitochondrial protein that represses mammalian mtDNA transcription initiation *in vivo.*

Disorders of Mitochondrial DNA Replication and Translation

Ann Saada (Reisch)
Metabolic Disease Unit, Hadassah Medical Center, Jerusalem, Israel

Disorders of the mitochondrial OXPHOS system are estimated to occur in ca. 1:5,000 live births. They can be classified biochemically as either isolated or combined deficiencies in any of the five (I-IV) mitochondrial respiratory chain complexes (MRC).

The MRC consist of more than eighty five proteins of which only thirteen are encoded by the mitochondrial DNA (mtDNA) while the remaining are nuclear encoded. Complexes I, III,IV and V contain mtDNA encoded subunits while complex II is solely from nuclear origin. Apart from the MRC proteins, mtDNA also encode the mitochondrial t-RNA's and rRNA's. Thus combined OXPHOS deficiency may be caused by mutations either in the mtDNA or in one of the many components of the mitochondrial replication or translation machineries, which are all encoded by nuclear genes.

During the last two decades an increasing number of patients with combined deficiencies of MRC complexes I,III,IV and V but with normal complex II, have been diagnosed by biochemical means. The majority of these patients was of consanguineous origin and maternally inherited mtDNA mutations were ruled out. In a subgroup, decreased mtDNA copy number was detected (mtDNA depletion), while the remaining patients retained normal mtDNA abundance.

Recently, in a number of patients, the underlying cause of the combined OXPHOS defect has been identified and characterized; Mutations in genes involved in mitochondrial replication and maintenance were detected in patients with mtDNA depletion, mainly in genes encoding enzymes supplying the mitochondrial deoxyribonucleotide pool and in the mitochondrial polymerase, while patients with normal mtDNA harbored mutations affecting mitochondrial translation factors, or the mitochondrial ribosome.

The patho-mechanism of some of these defects has been subjected to more detailed studies. Thus the investigation of combined OXPHOS disorders, benefit not only the patients but also expand the basic knowledge of mitochondrial replication and translation.

Mitochondrial VDAC: The Gatekeeper of Cell Life and Death

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Mitochondria are not only central to basic life functions as the generators of cellular energy, but also act as the point for cellular decisions leading to apoptosis. In mitochondria-mediated apoptosis, pro-apoptotic proteins, normally located in the mitochondrial intermembranal space, are released to the cytosol. In this process, the voltage-dependent anion channel (VDAC) plays a central role. VDAC, located at the outer mitochondrial membrane, provides the passage for nucleotides, Ca^{2+} and other metabolites into and out of mitochondria while serving as a site for apoptotic signalling.

The role of VDAC in regulating cell life and death was investigated by silencing endogenous human VDAC1 (hVDAC1) expression using shRNA, controlling native and mutated murine VDAC1 expression levels, over-expressing anti-apoptotic proteins such as hexokinase-I (HK-I) and Bcl2, and using VDAC1-based peptides. We found that down-expression of hVDAC1 led to an inhibition of cell proliferation due to disrupted energy production in the cell. On the other hand, over-expression of native or mutated VDAC1 resulted in apoptotic cell death. We demonstrate that over-expression of HK-I and Bcl2 protects against apoptosis and that a single mutation in VDAC1 prevents this effect, suggesting that such protections relies on binding to VDAC1. In addition, our results indicate that oligomeric VDAC1 forms a protein-conducting channel for the passage of cytochrome *c*. Moreover, the VDAC1 N-terminus is required for the induction of apoptosis since cells expressing truncated VDAC1 were resistance to mitochondria-mediated apoptosis as induced by starusporine, curcumin or As_2O_3. Furthermore, using a SPR-based approach, a synthetic peptide representing the VDAC1 N-terminus was shown to specifically binds to HK-I and Bcl2. When expressed in cells overexpressing these proteins, the peptide prevented the protective effects of HK-I and Bcl2 in the face of staurosporine-induced apoptotic cell death. These results suggest that VDAC1 is a component of the apoptotic machinery, with its N-terminal region mediating this role, pointing to VDAC1 as a checkpoint for cell life and death.

Redox-Mediated Regulation of Kinetoplast DNA Replication in Trypanosomatids

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Kinetoplast DNA (kDNA) is the remarkable mitochondrial DNA of trypanosomatids. It consists of a few thousands DNA duplex DNA minicircles and few dozens maxicircles that are catenated topologically into a network. Replication of kDNA minicircles initiates at conserved origin sequences that are bound by UMSBP, a single-stranded sequence specific DNA binding protein that contains five CCHC-type zinc finger domains. Analyses of UMSBP activity in synchronized cell cultures of Crithidia fasciculata revealed that UMSBP activity is regulated in vivo through a cell cycle dependent control of the protein redox state. UMSBP activity cycles with the progress in the cell cycle, displaying peaks of activity during the S and G1-M phases of the cell cycle. Searching for an enzymatic mechanism that may function in the redox mediated regulation of UMSBP we have found that UMSBP reduction by tryparedoxin in vitro enabled its interaction with the replication origin, while the protein oxidation by the 2-Cys peroxiredoxin, tryparedoxin peroxidase, inhibits its binding to the DNA. Knocking down the two UMSBP encoding genes in Trypanosoma brucei (TbUMSBP1 & TbUMSBP2), by RNA interference (RNAi), results in the cells growth arrest and a significant decrease in the rate of minicircles replication initiation, implying a role for UMSBP during minicircles replication initiation. Moreover, these analyses have revealed that silencing of theUMSBP genes impairs the segregation of kDNA, as well as mitochondrial and nuclear mitosis and cell cytokinesis. The potential function of UMSBP in coordination of mitochondrial and nuclear S phases in trypanosomatids cells is discussed.

Factors and Mechanisms Involved in Cataract Formation

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Development of lens cataract involves a life-long accumulation of damage. There are several suspected factors which contribute to age-related damage in the eye lens including: (1) diseases, such as diabetes, (2) drugs, such as steroids and DL-propranolol, (3) medical treatment, such as hyperbaric oxygen, (4) environmental factors, such as UV-A, heat, electromagnetic radiation.

Purpose: Our study represents an effort to determine the effects of suspected cataractogenic factors on the eye lens, and measures to prevent lens damage.

Methods: The experiments are performed using a unique long-term lens organ culture system on bovine lenses . In our system it is possible to control amounts of the insult and monitor changes in lens optical quality with associated biochemical analysis. The system enables determination of the mechanisms of cataract formation.

Results: Using our system we have shown that UV-A, heat, high glucose concentrations, hyperbaric oxygen and electromagnetic radiation all cause damage to the eye lens. It takes some time for the lens to respond to the insult, which initially affects enzyme activities and only later affects lens optical quality. Changes in lens optics are correlated with protein (crystalline) modifications.

Conclusions: (1) The lenses can self-recover up to certain amount of damage. (2) Therapy is needed to prevent greater damage.

Inhibition of Corneal Neovascularization Following Chemical Injury

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Neovascularization (NV) of the cornea is a major public health problem. It is estimated that each year, 1.4 million patients in the US (4% of population) develop corneal NV following infectious, inflammatory, toxic or nutritional insults. The NV is associated with scar formation and loss of transparency, leading to visual impairments and blindness. The aim of the present study was to elucidate the pathological mechanism of NV in an experimental model of chemical burn, and to test the efficacy of various therapeutic modalities in ameliorating this pathology.

Chemical burn of cornea was performed in NZW rabbits, using sulfur mustard (SM) vapor. Corneal NV was evaluated by a clinical ocular examination, combined with morphometric analysis. Corneal thickness, as an indicative parameter for edema, was measured in vivo by pachymetry. Topical application of steroids (0.1% Dexamethasone sodium sulfate and 0.5% neomycin sulfate, Dexamycin®) was administered using different therapeutic regimes (x4/day). At the end of experiments eyes were taken for biochemical and histological evaluations.

Corneal NV following SM exposure developed, as early as two weeks after exposure, associated with chronic inflammation, prolonged impairment of corneal innervation and abnormal corneal epithelial phenotype. Steroid treatment delayed and attenuated the appearance of NV, when given during the first week. When treatment was applied symptomatically to corneas displaying NV, a significant regression in the angiogenic process was observed. Yet, the effect was temporal and angiogenesis reappeared when therapy was terminated. The potential use of growth factors, metalloproteinase inhibitors and anti-VEGF factors will be discussed.

Congenital Glaucoma in Rabbit : A Model of Glaucoma Research

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Glaucoma is still a challenge for the eye doctors and researchers. The research on new medications and methods of surgery is still very active . The model of research was and is still a dispute among scientists. Many animal models were created but a common point link all of them: the models are artificially created by acute obstruction of the aqueous outflow
and acute elevation of the intra ocular pressure. This is not identical to the open angle glaucoma (POAG) in human . The POAG is a gradually progressive disease with a gradual raise in the intra ocular pressure (IOP). The presented model of congenital glaucoma in rabbit is identical to human and is the only natural model of the disease in an animal model. There is known that beagles do suffer of glaucoma but they are not used in laboratory research.
We will present in our lecture the results of a long research on the glaucomatous rabbits.

Toxicity, Pharmacokinetics and Penetration of Antiangiogenic Drugs

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Background: Age related macular degeneration (AMD) is the most common cause of blindness in individuals 50 years of age or older. Recently, treatment with antiantiogenic drugs has been proven as effective in the treatment of choroidal neovascularization secondary to AMD. We investigated 1. The toxicity of Bevacizumab (Avastin), a full length antibody developed for the treatment of colorectal cancer to the rabbit retina; 2. The pharmacokinetics and serum bioavailability of intravitreal bevacizumab in the rabbit; 3. The penetration of this full length antibody (Bevacizumab= Avastin) to that of the fab molecule (Ranibizumab= Lucentis) which was developed specifically for intravitreal injection in the treatment of AMD.

Methods: Albino rabbits were intravitreally injected to one eye with bevacizumab 2.5 mg/0.05ml, and than with 1.25mg/0.05ml. Electrophysiological studies were performed to evaluate the toxicity to the retina of these drugs. Vitreous samples were taken at various time points, as were serum samples. Bevacizumab concentrations in the plasma and vitreous were determined by enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human IgG for capture and horseraddish peroxidase (HRP) conjugated rabbit anti-human IgG for detecting. Another group of albino rabbits were intravitreally injected to one eye with bevacizumab 200μg or with ranibizumab 500μg. Eyes were enucleated 1, 3, days, 1, 4 weeks after injection. Immunohistochemistry was performed.

Results: (1) Bevacizumab was found to be non toxic to the rabbit retina; (2) The mean vitreal concentration of bevacizumab decreased by 37%, 62%, 70% and 81% at the 1,2,4 and 6 weeks respectively. Mean vitreal concentration in the uninjected eye was 4.93 ng/ml, 4.36ng/ml, 1.06ng/ml and 0.41ng/ml at the 1,2,4,6 weeks respectively. Mean plasma concentrations of bevacizumab was 17.20 pg/ml, and 7.02 pg/ml at the 2 and 6 weeks respectively. Mean vitreal and plasma concentrations of the control rabbit was lower than the sensitivity of the assay; (3) Both ranibizumab and bevacizumab penetrated the retina of the albino rabbit. The penetration will be compared.

Conclusions: (1) Intravitreal bevacizumab is safe in the rabbit's eye; (2) The high intravitreal concentrations observed after 6 weeks demonstrates a lower than expected turnover of bevacizumab. The concentration of bevacizumab in the plasma and uninjected eye indicates systemic circulation; (3) Both ranibizumab and bevacizumab penetrated the retina of the albino rabbit.

Immunology and Immunotherapy of Stroke

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Stroke is one of the leading causes of death in the world, resulting in the death of approximately five million people per year. Ischemic stroke results from transient or permanent reduction in cerebral blood flow leading to ongoing neurotoxicity, post ischemic inflammation and apoptosis. Early inflammatory responses may aggravate the ischemic injury, while late responses may be important in recovery and repair. Since neuronal cell death following stroke might take several days, the prime goal of neuroprotection is to modulate the ischemic inflammation that facilitate further apoptotic death. Immunotherapeutic approaches in stroke to modulate cerebral inflammation may be effective either as a prophylactic therapy or treatment to reduce the severity of ischemic stroke.

Immune Regulation and Neuronal Repair in the Progression of Alzheimer's Disease

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Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder characterized by memory loss and severe cognitive decline. These clinical features are manifested morphologically by excessive accumulation of extracellular amyloid □-peptide (A-beta) in the brain parenchyma, particularly in the hippocampus and cerebral cortex, leading to neuronal loss. In addition, aggregates of A-beta that form neuritic plaques in the brain become toxic in that they trigger chronic glial activation and thereby interfere with normal brain function. Another hallmark of the disease, possibly secondary to A-beta neurotoxicity and glial activation, is the abnormal phosphorylation and aggregation of tau (which functions in microtubule assembly and stabilization) to form intracellular neurofibrillary tangles. It appears that regulation of the glial reaction to such neuronal insults turns is crucial for inducing mechanisms either of neuronal repair or of neuronal loss. Our approaches to characterizing the different types of glial activation and their contribution to neuronal loss or repair are based on new emerging characteristics of three biological systems: autoimmunity, brain-immune interactions, and neurogenesis. Our data demonstrate that A-beta-specific T cells are induced in AD and that their specificity and magnitude of activation depend primarily on HLA-DR alleles. Expression of IFN-□ in the brain, as observed during normal brain aging, is essential to promote migration of these A-beta reactive T cells to the parenchymal tissue in the hippocampus and subsequent interaction with brain-endogenous cells. In contrast to the injurious effects induced by chronic innate immune mechanisms involved in AD, T cells – or the cytokine they produce – can serve as key regulators of glial activation and differentiation, clearance of A-beta, neuroprotection and neuronal repair. The implications of these results in the context of immune system physiology and neuronal repair with age will be discussed.

The potential for Neuroprotection and Neurogenesis in Multiple Sclerosis by an Immunomodulatory Treatment with Glatiramer Acetate

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Multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), historically considered as genuine autoimmune inflammatory diseases, are now recognized as complex diseases involving oligodendrocyte, axonal and neuronal pathology. Degenerative processes initiated at disease onset and revealed when compensatory CNS resources are exhausted, are major determinants of the irreversible neuronal disability and disease progression. Novel treatment strategies thus aim to act within the CNS by inducing neuroprotection as well as by interfering with the autoimmune inflammation.

The immunomodulator Glatiramer acetate (GA, Copaxone®), an approved drug for the treatment of MS, has been shown to induce specific Th2/3 cells that penetrate the CNS, secreting in situ anti-inflammatory cytokines. However, the effect of GA is not restricted to anti-inflammation, as GA treatment resulted in the elevation of the neurotrophic factors BDNF, NT3 and NT4, which are important regulators of neuronal function and survival. The therapeutic significance of this effect was manifested in actual protection and preservation of the CNS in EAE-inflicted mice i.e. reduction in both neuronal and myelin damages, as demonstrated by scanning electron microscopy and immunohistological methods. Furthermore, detection of progenitor developmental markers incorporated with the proliferation marker BrdU revealed that GA augments CNS repair mechanisms as well. Hence, the three processes characteristic of neurogenesis, namely neuronal proliferation, migration and differentiation were elevated after GA treatment. The newborn neurons migrated through existing and dormant pathways, into injury sites in brain regions, which do not normally undergo neurogenesis, and differentiated to mature neuronal phenotype. GA treatment affected also myelin producing cells by inducing proliferation and differentiation towards the oligodendrocyte lineage.

Altogether, these findings suggest that immunomodulatory treatment may counteract neurodegenerative disease course drawing a direct linkage between immunomodulation, neuroprotection, neurogenesis and therapeutic activity in the CNS.

Neural Stem Cell Therapy in Multiple Sclerosis

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Neural (stem) cell transplantation has been proposed as a means of cell replacement therapy for diseases of the central nervous system (CNS). Various cell types may serve as a source of myelin-forming cells that remyelinate the CNS in demyelinating diseases such as multiple sclerosis (MS). Multipotential neural precursor cells (NPCs) that expand in floating spheres, and are (partially) committed to a glial fate, showed excellent remyelinating properties in a focal, chemically induced demyelinated lesion in the rat spinal cord. Following these results, we next examined the clinical value of NPC therapy in experimental autoimmune encephalomyelitis (EAE) models, which are more clinically relevant for multiple sclerosis (MS).

We found that the inflammatory process in the CNS of rodents with acute and chronic EAE attracted targeted migration of transplanted precursor cells exclusively into inflamed white matter but not into adjacent gray matter. The pro-inflammatory cytokines TNFα and IFNγ participated in the mobilization of NPCs in vitro and increased the expression of matrix metalloproteinases in the cells. Since the therapeutic value of transplanted stem cells is dependent on their ability to migrate to the multiple disease foci in the brain, the stem cell property of targeted migration towards the inflammatory process is highly important.

To develop non-invasive methods of tracing the graft in the brain, we transplanted magnetically labeled NPCs into EAE animals, and obtained high-resolution (microscopic) magnetic resonance (MR) images. Migration patterns on MRI correlated well with the corresponding histopathology. Human ESC-derived neural precursors responded to tissue signals in EAE, similar to rodent cells. Thus, MR cell tracking can be used to guide the design and optimization of successful (clinical) transplantation protocol.

We next examined the effects of neural sphere transplantation on the clinical and pathological course of EAE. Neural sphere transplantation attenuated the clinical severity of acute EAE in Lewis rats. Since acute EAE manifests with an inflammatory process without demyelination, we hypothesized that transplanted cells may exert their beneficial effect by attenuating the inflammatory process, rather than by cell replacement. Indeed, transplanted rats exhibited an attenuated inflammatory process in the CNS. The anti-inflammatory effect of transplanted neurospheres was further supported in the MOG35-55 -chronic EAE model in C57Bl/6 mice, where neural spheres transplantation attenuated significantly brain inflammation, reduced the degree of demyelination and of axonal pathology and attenuated the clinical course of disease. To further study the immunomodulatory properties of NPCs, we delivered them via the intravenous (IV) route. IV-injected NPCs did not enter the CNS but were transiently found in lymph nodes and spleen. IV injection of NPCs significantly inhibited EAE and reduced CNS inflammation as well as axonal injury and demyelination. Co-culture experiments showed that NPCs inhibited the activation and proliferation of lymph node-derived T cells in response to specific and to non-specific stimuli, and did not actively induce T-cell apoptosis. The relevance of NPC-LNC interactions *in vivo* was further demonstrated when LNCs obtained from IV NPC-treated mice exhibited poor encephalitogenicity upon transfer to naïve mice as compared to the severe EAE induced by LNCs from mice that were not injected with NPCs. Thus, IV administration of neural precursors inhibits EAE solely by a peripheral immunosuppressive effect involving a profound bystander inhibitory effect of NPCs on T cell activation and proliferation in the lymph nodes.

In conclusion, while (stem) cell transplantation was introduced as cell replacement therapy, neural precursor cells also exert an immunomodulatory effect that inhibits the autoimmune-inflammatory process. Cell therapy in MS should be optimized in such a way to utilize both regenerative and immunologic properties of the cells.

Roles of Ion Channel Regulatory Protein Complexes in Neuronal Physiology and Behavior

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Many ion channels are intimately associated with one or more auxiliary proteins that participate in the regulation of channel activity. While the molecular details of ion channel regulatory protein complexes have been widely studied, their physiological roles remain poorly understood. We have taken advantage of *Drosophila* genetics to explore the role of the Slowpoke channel binding protein Slob in the modulation of large conductance calcium-dependent potassium channel activity *in vivo*. Patch recordings from neurons in the brains of living flies reveal changes in macroscopic outward current in Slob null and Slob over-expression flies. These changes are consistent with the effects of Slob described previously in a heterologous expression system. Furthermore, *in vivo* single channel recordings demonstrate large changes in Slowpoke channel activity in Slob null and Slob over-expression flies. In addition, knock-out of Slob by either P-element mutagenesis or expression of transgenic RNAi leads to changes in fly feeding behavior. Our results provide evidence that an ion channel regulatory protein complex can modulate neuronal physiology, and ultimately behavior, in an intact organism.

Abstracts of Student Lecture Competition

Neonatal Blockade of the CB1 Receptor: Further Support for Endocannabinoid-CB1 Deficiency as the Biological Basis of 'Non-Organic Failure-To-Thrive' in Infants

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We have shown in previous studies that a single exposure to the cannabinoid CB1 receptor antagonist/inverse agonist rimonabant (SR141716) resulted in impaired milk suckling and severe growth failure. We further showed that the growth failure which is due to an inability to ingest maternal milk, does not result from a motivational deficiency, but from an (oral)-motor impairment. The similarities between the SR141716-treated mice and the symptoms which characterize the enigmatic "non-organic failure-to-thrive" (NOFTT), which appears in 2-4% of infants, spurred us to suggest neonatal CB1 blockade-induced growth failure as the first animal model for NOFTT. In the present work we performed additional experiments (using ICR mice) to establish a deficient endocannabinoid-CB1 receptor system as the biological basis for NOFTT.

Methods: Neonatal (ICR) mice were injected with SR141716 (10-20mg/kg) at several time interval after birth. Parameters including body weight, milk ingestion and body temperature were measured throughout the first 10 days of life.

Three studies were performed: (1) We studied the correlation between the immediacy of SR141716 administration to birth with the severity of its effect. (2) We allowed SR141716- and vehicle-treated pups to lick a mixture of milk/cream mixture from a dish ('lapping'), on each of the first 3 postnatal days. Successful food ingestion by the SR141716-treated pups would indicate that SR141716 indeed selectively impairs oral-motor strength required to suck milk from the maternal nipple, rather than the motivation and ability to ingest and assimilate food. (3) We raised mice in very small *vs* very large litters and thus established an undernourished state without SR141716. The pups were treated daily with THC (1-5 mg/kg) or 2AG (1 mg/kg-5 mg/kg) for the first 5 days of life.

Results: (1) Our findings suggest that as long as the pups were treated within 24 h of birth, the number of hours were not critical for the SR141716-induced effects. (2) The pups were able to ingest significant amounts of the milk/cream mixture from the first day of life; lapping within the first week of life has not been shown previously. Moreover, the SR141716-treated pups were able to ingest the mixture to the same degree as their vehicle-treated littermates. (3) Finally, whereas 5 mg/kg of 2AG did not improve weight gain consistently, 1 mg/kg significantly enhanced weight gain. THC injections had no effect on the growth curve.

We conclude from these observations that we have now solid evidence that endocannabinoid and/or CB1 receptor insufficiency underlies the enigmatic infant condition NOFTT and that cannabinoid-based treatment should be considered to improve food intake and weight gain in NOFTT infants.

NMR Studies of Oxidative Stress-Induced Changes of Membrane Phospholipids in Thalassemic RBC

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Changes in phospholipid asymmetry is one of the hallmarks of apoptosis of nucleated cells. Although mature anucleated RBC, do not undergo the classical apoptosis, upon trauma or aging they present changes in membrane asymmetry. These changes include phosphatidylserine (PS) externalization, which stimulates RBC phagocytosis and removal from the circulation. Oxidative stress is among the causes of PS externalization of RBC. In the β-hemoglobinopathies, β-thalassemia and sickle cell disease, although the primary defects are mutations in the globin genes, oxidative stress is thought to mediate part of the damage to the RBC, and particularly to its membrane, including PS externalization.

In the present study, we used NMR spectroscopy to analyze normal and thalassemic RBC with respect to oxidative stress. Using ^{1}H NMR, we demonstrated a higher lactate/pyruvate ratio in thalassemic RBC, confirming their state of oxidative stress. Using ^{31}P NMR, we then measured the membranal contents of various phospholipids and found more PC, but unexpectedly, less PS in thalassemic RBC than in normal RBC. The PS in RBC was increased by treatment with anti-oxidants and decreased by oxidants, while PC showed the opposite behavior, indicating correlation between PS and PC content and the oxidative status. NMR analysis of blood plasma obtained from normal and thalassemic donors indicated an increased PS and PC content in the latter plasma. In vitro incubation of RBC produced much higher PS in supernatants derived from thalassemic RBC compared with those of normal RBC. Anti-oxidants reduced the PS shedding from thalassemic RBC into their supernatants while oxidants increased the PS shedding by normal RBC.

RBC are known to shed membranous particles (termed also vesicles or microparticles) in vitro and in vivo during their physiologic and pathological senescence. We studied this point by purifying microparticles from plasma and RBC supernatants of normal and thalassemic donors, and measuring the PLs content in their lipophilic extracts by ^{31}P-NMR. We found that the PS content and its proportion out of the total PLs were higher in microparticles purified from thalassemic plasma (0.25±0.04 mM, 19% of the plasma total PS) or RBC supernatants than in normal plasma microparticles (0.045±0.06 mM, 9.5% of the plasma total PS) or supernatants. The results also show that although microparticles are enriched in PS compared to their intact RBC, the bulk of the shed PS is not associated with microparticles.

These results suggest that oxidative stress in RBC causes them to shed their PS and that the increase in PC levels maybe as a compensating mechanism. The pathological consequences of these phenomena on the survival of RBC in thalassemia warrants further study.

Hyperbaric Oxygen Pretreatment Reduces Decompression Sickness Incidence in Rats

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Our research hypothesis is that the number of bubbles evolving during decompression from a dive, and consequently the incidence and severity of decompression sickness (DCS), might be reduced by pretreatment with hyperbaric oxygen (HBO), because the inert gas in the gas micronuclei is thus replaced by oxygen, which would subsequently be consumed by the mitochondria. We have demonstrated that pretreatment with HBO in the transparent prawn reduced the number of bubbles which grow during decompression, probably by replacing the inert gas in the gas micronuclei with oxygen. Our present objective was to investigate whether our hypothesis holds for mammals too, and whether HBO pretreatment will reduce DCS in the rat .Male Sprague-Dawley rats weighing 256-307 g were pretreated with HBO at 101, 203, 304, 405 or 507 kPa for 20 min .We also tested shorter pretreatment for 5 or 10 min at 304 kPa. After pretreatment, rats were exposed to air at 1013 kPa for 33 min, followed by fast decompression (200 kPa/min). Control rats were exposed to the same pressure for 32min, without HBO pretreatment. On reaching atmospheric pressure, the rat was immediately placed in a cage rotating at ~3 m/min for 30 min. The animal's behavior enabled us to make an early diagnosis of DCS according to the following signs: walking difficulties, abnormal breathing pattern, forelimb and/or hind limb paralysis, rolling about the cage, convulsions, and death. Rats were examined again after two and 24 h. After 2 h, 65% of the control rats had suffered DCS (45% of them were dead), whereas 35% had no DCS. HBO pretreatment at 101 405 ,304 ,203 ,and 507 kPa for 20 min significantly reduced the incidence of DCS at 2 h to 45%, 45%, 40%, 40% and 35%, respectively. The 45% mortality rate in the control group after 24 h, was reduced in the pretreated groups to 10%, 25% 15% ,15% ,and 15%, respectively. HBO pretreatment at 304 kPa for 10 and 20 min reduced the incidence of DCS at 2 h to 45% and 40%, respectively. Mortality rate after 24 h was reduced to 15% in both groups. After HBO pretreatment at 304 kPa for 5 min, 65% of the rats suffered DCS, exactly as seen in the control group .HBO pretreatment for 20 min at 304, 405 or 507 kPa has a beneficial effect ,causing a significant reduction in the incidence of DCS in rats decompressed from 1013 kPa. HBO pretreatment at 304 kPa for 10 min is sufficient to reduce the incidence of DCS in rats decompressed from 1013 kPa; any treatment for less than 10 min was ineffective.

Is Incense a Pharmacological Tool for Spiritual Exaltation?

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Burning of *Boswellia* resin as incense has been part of religious and cultural ceremonies for millennia. We assumed that the spiritual exaltation at these ceremonies would be enhanced by possible effects of *Boswellia* constituents on the central nervous system.

Behavioral assays: We isolated a constituent of *Boswellia* resin, incensole acetate (IA), a diterpene, which showed activity in several standard tests in mice, namely the Porsolt forced swimming test, the elevated plus maze, locomotion in the open field test, cataleptic response in a ring test and body-temperature. In the Porsolt forced swimming test, a standard anti-depression assay, IA significantly reduced the immobility recorded over 9 mins in the treated mice. IA exerted a potent anxiolytic-like effect in the elevated plus maze assay, which is based on the preference of mice for the closed arms of a maze, apparently due to fear of open spaces. We also observed significant reduction of ambulation in the open field and immobility on the ring, as well as a marked hypothermic effect in IA treated mice.

TRPV3 activation: TRPV3 protein is highly expressed in epithelial cells of the skin and tongue; peripheral activation of TRPV3 causes a sensation of warmth. RNA encoding this channel has also been observed in brain, but its role there remains unknown. We now show that IA a potent activator of TRPV3 channels (EC_{50} = 16 μM) known so far. Hence, its use in religious ceremonies, by activation of TRPV3 channels, with the attendant sensation of warmth, may be an important factor in the overall profile of IA's activity and the concomitant feeling of exaltation. No effects of IA were noted on the related channels TRPV1, TRPV2, and TRPV4, or on 26 surveyed receptors, ion channels and transport proteins.

IA causes anxiolytic, antidepressive and hypothermic effects in $TRPV3^{+/+}$ mice, but not in $TRPV3^{-/-}$ mice, thus indicating its central effects.

C-Fos immunoreactivity: C-Fos is a product of an immediate–early gene and its presence serves as a marker of changes in neuronal activity. IA significantly increased c-Fos in the lateral septum, central nucleus of the amygdala and solitary nucleus, while significantly reducing c-Fos in the motor cortex, medial striatum and hippocampal CA3 region.

Conclusions: IA's biochemical and pharmacological effects provide a biological basis for a deeply rooted cultural and religious tradition. They also provide for the first time indications for a role of the TRPV3 in the brain.

The Afterload Dependency of the Frank-Starling Law Reflects Cross-Bridge Dependent Regulation of Contraction

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Despite the importance of the Frank Starling Law (FSL), its underlying mechanisms are not completely elucidated. The related force length relationship in the isolated fiber, which relates to the FSL, is regulated by a cooperativity mechanism, whereby the strong cross-bridges (XBs) affect the affinity of troponin for calcium and modulate new XB recruitment. Therefore, the afterload which also determines the number of strong Xbs, affects XB recruitment and force developemnt. The study aims to unveil the effects of the afterload on the FSL, and to elucidate manifestations of cellular mechanism at the whole heart level. The effects of the preload and afterload on cardiac mechanics were examined *in situ* in adult sheep (n=8) weighing 69.1±9.6Kg. Different afterloads were imposed by partial aortic occlusions. Transient inferior vena cava occlusions (IVCOs) were preformed at each steady afterload. External work (EW) and pressure time integral (PTI) were calculated for each beat during the IVCOs. A highly linear EW-PTI relationship (WPTiR) was found for all afterloads (R^2=0.98±0.02 in 54 data sets). A unique WPTiR was obtained during both the occlusion and the release phases of each IVCO, while two distinct EW-preload relationships were observed. The slope of the WPTiR was 34±2.8[mJ/mmHg/sec] at baselines and decreased by 0.91±0.53[mJ/mmHg/sec] per 1[mmHg ·min/L] increase in the total peripheral resistance, suggesting that the FSl is afterload dependent. This novel and consistent WPTiR represents a basic feature of cardiac control of contraction, that ties the Frank and Starling phenomena together. The observations yield better understanding of the FSL based on established cellular control of contraction

Abstracts of Posters

Poster # 1

Does Evolutionary Adaptation to Desert Conditions Affect Heat Acclimation Plasticity?

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Adaptations evolving throughout phylogenic time are evolutionary adaptations. Acclimation, in contrast is within life time response of an individual (phenotypic adaptation). Collectively, acclimation is the optimal outcome for the body needs in the specific acclimating environment. Upon acclimation to hot environment there is a positive correlation between acclimation temperature and several molecular responses such as the heat shock response (HSR), thus allowing protection to variety of damages in the organism. Heat Shock Proteins are the indispensable components of the HSR. A connection between the ability to adapt and the degree of polymorphism of HSPs has been documented.

Aims: To study HSPs levels and HSR dynamics before and after heat acclimation in desert and non desert rodents.

Materials and methods: We used one month acclimation at ambient temperature of 34°C protocol. Four animal species were tested: the diurnal *Psammomys obesus* and *Acomys russatus* and *the nocturnal Acomys cahirinus* and the laboratory rat (*Rattus novegicus*- Sabara Strain).

Physiological experiments: Measurements of colonic temperature and body weight during the acclimation period and during exposure to heat stress.

Protein Measurements: HSP90 & HSP70 were detected in the liver, brain and heart tissues via Western immunoblotting, and Two Dimensional Electrophoreses (2DE) and Mass Spectrometry. *mRNA* of *hsp70* was measured in the heart by qRT-PCR.

Results: Body temperatures of the diurnal desert species showed fewer changes in their body temperature than the nocturnal ones and the percentage of weight gain for the diurnal species was higher than that of the nocturnal. HSP70 and HSP90 levels increased after acclimation in all species. Exposure to heat stress elevated HSPs levels even further. However, this increase was faster in the acclimated nocturnal sp compared to the diurnal ones. HSP elevation (HSP70) was accompanied by elevated mRNA (heart, *P. obesus*). 2DE analyses has identified a larger number of peptide sequences assigned to HSP70 in the nocturnal sp. Likewise, 2DE analysis showed clear increase in the ATP synthase protein following heat acclimation.

Conclusion: Acclimation to heat in evolutionary adapted rodents to hot/arid environment differs from that of comfort dwellers. Body wgt and temperature are better regulated (than the nocturnal) while subjected to the acclimating protocol and the time-threshold for HSP synthesis (following heat stress) is delayed suggesting different hierarchy of compensatory responses. Furthermore-our results showed that these species have a narrower range of HSP70 spots after heat acclimation as compared to less genetically adapted species (nocturnal sp.). The increase in mitochondrial ATP synthase level of the heat acclimated individuals, in the face of decreased metabolic rate induced by heat acclimation raises questions regarding its activity level.

Poster # 2

Effect of L-Carnosine and Related Derivatives on the Release of Inflammatory and Oxidative Stress Mediators in BV2 Cells

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Chronic inflammation and oxidative stress are involved in the pathogenesis of neurodegenerative disorders like Alzheimer's disease (AD). Activated glial cells are a major source of inflammatory and oxidative stress mediators like cytokines, prostaglandins and nitric oxide (NO). It is well known that lipopolysaccharide (LPS, 5 ng/ml, an inflammatory inducer) increased NO synthesis in BV2 cells. L-carnosine is known as efficient endogenous anti-oxidant.

The aim of this study was to gain insight into the role of L-Carnosine and its related synthetic derivatives on oxidative stress and inflammation pathways in microglial cell line, BV2.

We have shown that L-Carnosine, up to 20 mM, did not alter basal NO synthesis in BV2 cells. L-Carnosine (10-20 mM) inhibited LPS- induced NO levels by 75% in BV2 cells. In addition we showed that N-Acetyl-L-Carnosine (NAC) (0.5-10 mM), a lipophilic synthetic derivative of L-Carnosine, inhibited LPS-induced NO synthesis in BV2 cells by 40% to 80%.

Our results suggest that L-Carnosine and NAC may play a protective role in oxidative stress process in the brain.

Further studies are needed aiming to understand the mechanism by which L-Carnosine and its derivates act and to use these compounds as a potential treatment strategy for neurodegenerative diseases.

Poster # 3

Voltage-Dependent Anion Channel-1-Based Peptides Interact with Hexokinase and Prevent its anti-Apoptotic Activity

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Apoptosis is regulated by different proteins some of which interact with the mitochondrial protein - VDAC. Along with its importance in regulating cellular energy metabolism, VDAC is also recognized as a key protein in mitochondria-mediated apoptosis, participating in the release of apoptotic proteins and interacting with the apoptotic proteins such as the pro-survival isoforms of hexokinase (HK-I and HK-II). Tumor cells exhibit a high rate of glycolysis and up to 100- higher expression levels of mitochondria-bound HK-I and HK-II.. Recently, we demonstrated that HK-I interacts directly with VDAC, preventing cytochrome c release, and that HK-I over-expression protects against staurosporine (STS)-induced apoptotic cell death. Moreover, by site-directed mutations, we defined VDAC1 residues, found in two cytoplasmic domains, involved in the interaction with HK-I. In this study, we use synthetic peptides corresponding to the proposed VDAC1 domains interacting with HK-I to interfere with HK-I and HK-II anti-apoptotic activity. Synthetic peptides corresponding to the VDAC1 N-terminal region and three cytosolic loops were revealed to bind to immobilized HK-I by Surface Plasmon Resonance technology in a specific manner. The same VDAC1-based peptides caused the detachment of mitochondria-bound HK-I from isolated rat brain mitochondria or tumor-derived mitochondria. Moreover, expression of the VDAC1-based peptides in cells over-expressing HK-I or HK-II prevented HK protection against STS-induced release of cytochrome *c* and cell death. These results suggest that HK over-expression in cancer cells promote tumor cell survival through its direct interaction with VDAC1, inhibiting cytochrome *c* release and thereby, apoptotic cell death. Moreover, these findings suggest that interference with the anti-apoptotic effect of HK-I by VDAC1-based peptides may be a practicable modality by which to potentiate the efficacy of conventional chemotherapeutic agents.

Poster # 4

Phospholipase D Mediates Hyperactivated Motility in Sperm Capacitation

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Introduction: Phospholipase D (PLD) catalyses the hydrolysis of phosphatidylcholine, to choline and phosphatidic acid (PA). It was recently demonstrated in our laboratory that Phospolipase D (PLD)-dependent actin polymerization is a necessary step in the cascade leading to capacitation, and it is known that capacitated sperm show hyperactivated motility. In the present study, we showed for the first time, correlation between PLD-dependent actin polymerization and hyperactivated motility during mouse and human sperm capacitation.

Materials: In vitro capacitation: Cauda epididymal mouse sperm were incubated in defined - capacitation medium for 90 min. Actin polymerization was measured by staining the F-actin with Phalloidin FITC, fluorescence intensity was quantified and analyzed using "Image - J" software. Sperms motility was determined by CASA using the IVOS device and manually by light microscope counting in camera sperm showing hyperactivated motility. Acrosome reaction was measured by PNA-FITC staining. IVF: metaphase II-arrested eggs and sperm were mixed and incubated for 24 hours. Fertilization rate was determined by counting the number of cleaved oocyte.

Results: Sperm incubated under capacitation conditions revealed a time dependent increase in actin polymerization followed by the development of hyperactivated motility. These two activities were blocked by the PLD inhibitor butan-1-ol but not by butan-2-ol, indicating the specificity of PLD inhibition. The activity of actin polymerization and motility in the inhibited cells, could be restored by adding PA, further indicating the involvement of PLD in these processes. Moreover, addition of exogenous PA or PLD to the cells, caused a rapid increase in actin polymerization, that was followed by a rise in the hyperactivated motility. The addition of Cytochalasin D (a known inhibitor of actin polymerization), blocked both actin polymerization and hyperactived motility during capacitation. These results showed that actin polymerization occurs prior to the hyperactivated motility. Therefore, we speculated that the development of hyperactivated motility depends on actin polymerization. To further support this notion, we showed that phorbol ester a known Protein kinase C activator, induces actin polymerization that was followed by an increase in hyperactivated motility. Other capacitation dependent activities like AR and IVF rate are also mediated by PLD activity.

Conclusions: In conclusion, we showed that PLD-dependent actin polymerization is a critical step in the development of hyperactivated motility during mouse and human sperm capacitation.

Poster # 5

Regulation of Inflammation in Microglial Cells: A Possible Role of Kinins

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Increasing evidence suggests that inflammatory mechanisms represent a crucial component in neurodegenerative diseases, such as Alzheimer's disease, and may significantly contribute to disease progression. Activated microglial cells are a major source of inflammatory mediators and neurotoxic factors such as prostaglandins (PGs), cytokines and nitric oxide (NO). Kinins are known as potent stimulators of the production and release of NO in different cells. However, recent reports suggest a possible neuroprotective role of bradykinin (BK) via microglial cells. The aim of the present study was to investigate the role of BK in the regulation of basal and lipopolysaccharide (LPS)-induced NO synthesis in microglial cell line, BV2, and to assess the involvement of each of the two BK receptors, B1 and B2 receptors, in this effect. Long-term exposure of cells to BK reduced basal NO release by 75% at a concentration of 10 nM, where as at 1 nM concentration, BK had little or no effect on basal NO production. B1 receptor agonist had the same effect of BK, 70% reduction of NO synthesis at a concentration of 10 nM and little effect at a concentration of 1 nM. BK also attenuated LPS-induced NO production by 30% and 50% at concentrations of 1 nM and 10 nM, respectively. These preliminary results imply a novel neuroprotective role of BK via attenuation of neurotoxic factors release from microglial cells under basal and inflammatory conditions.

Poster # 6

Activation Gating of Potassium Channel Pores is Controlled by Hydrophobic Interactions

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Potassium channels open and close their pores in response to changes in chemical or electrical potential. This process results from mechanical coupling of gating domain movements to pore opening and closing. Two intriguing questions are related to the intrinsic stability of the pore domain: if the pore domain of a K^+ channel is detached from its gating domain, will it stay closed or open? What factors determine the pore's conformational stability? Hints to the answers for these questions may be gained by considering two K^+ channel families, the voltage activated K^+ channel family (Kv) and the leak K_{2P} channel family that demonstrate opposing closed and open pore stability phenotypes, respectively. A chimeric channel protein in which the pore domain of the *Shaker* Kv channel was replaced with that of the KCNK0 leak channel resulted in substantial stabilization of the open channel conformation as compared to the wild-type *Shaker* channel. Multiple sequence alignment of both channel families reveled that while the activation gate region of Kv family members is spanned by hydrophobic amino acids, the corresponding region of the K_{2P} channels is enriched in glycine residues. Indeed, systematic replacement of activation gate hydrophobic residues of the *Shaker* Kv channel to the corresponding glycines of the KCNK0 leak channel resulted in dramatic open-state stabilization effects. The complementary experiments in which the glycine activation gate residues of the K_{2P} channel were replaced with the corresponding hydrophobic amino acids of the *Shaker* channel revealed dramatic closed state stabilization effects, as judged by both macroscopic and single channel measurements.

Taken together, our results for both channels are coherent and suggest a general role for hydrophobic interactions in determining the intrinsic conformational stability of the pore domain of K^+ channels.

Poster # 7

Lysophospholipids Modulate Voltage-Gated Calcium Channel Currents in Pituitary Cells

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Lysophospholipids (LPLs) are lipophilic molecules consisting of a hydrophilic head and a hydrophobic tail. LPLs containing a large hydrophilic head and a thin hydrophobic tail were defined by their molecular shape as cones. It was hypothesized that incorporation of cones into the outer leaflet of the phospholipid bilayer increases membrane curvature and membrane tension. Support for this hypothesis came from studies demonstrating that cone-shaped LPLs altered the gating of mechanosensitive ion channels. Previous studies demonstrated that voltage-gated calcium channels (VGCC) may also be modulated by alterations in membrane tension. We therefore examined the effects of the cone-shaped molecule, Lysophosphatidylchloline (LPC), on VGCC in pituitary cells.

Our main findings may be summarized as follows: (1) LPC (10-30 μM) differentially suppressed L-type and T-type calcium channel currents (I_L and I_T, respectively); the effects on I_T started after shorter delays and were more prominent than the effects on I_L. (2) The effects of LPC on I_L started after long delays (50-100 seconds), exhibited slow onset kinetics and were reversible only after washout with fatty acid free BSA (0.5 mg/ml). (3) The effects of LPC on I_L were both dose-dependent and voltage-dependent with a rightward shift in the activation curve of about 9 mV. (4) The effects of LPC on I_L were mimicked by lysophosphatidylinositol (LPI), a negatively charged cone-shaped lipophilic molecule. (5) The effects of LPC on I_L were not mimicked by a short chain LPC (C6:0) and by phosphatidylcholine (PC), a cylindrical-shaped lipophilic molecule. 6. The effects of LPC on I_L persisted after block of G-proteins with GDPβS (5mM) and after block of PKC with GF 109203X (5μM).

In summary, our results show that cone-shaped lipophilic molecules suppress VGCC in pituitary cells, and suggest that molecular shape is a determinant in this suppression. It is possible that partition of these molecules into the phospholipid bilayer alters membrane curvature and tension, thereby affecting calcium channel gating. Alternatively, it is possible that the effects results from perturbations in the lipid microenvironment of the channel proteins.

Poster # 8

The N-Terminal Domain of VDAC1 is Essential for Mitochondria-Mediated Apoptosis and Regulation by Anti-Apoptotic Proteins

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Apoptotic signalling to the mitochondria results in the efflux of apoptotic regulators from the intermembranal space to the cytosol, triggering caspase activation and cell destruction. Accumulating evidence indicates that the voltage-dependent anion channel (VDAC), the main interface between mitochondrial and cellular metabolism, plays a central role in mitochondria-mediated apoptosis. Here we demonstrate that the VDAC1 N-terminal amphipathic α-helix region is required for induction of apoptosis. Expression of Δ(1-26)mVDAC1, an N-terminal truncated form of murine VDAC1, in hVDAC1-shRNA cells possessing low levels of endogenous human VDAC1, revealed the N-terminal region to be required for the release of cytochrome *c* and apoptotic cell death. Furthermore, the VDAC1 N-terminal α-helix was shown to mediate the interaction of VDAC1 with the anti-apoptotic, pro-survival factors, hexokinase (HK)-I, HK-II and Bcl2. The interaction of the VDAC1 N-terminal region with purified rat brain HK-I and recombinant Bcl2(□23) was demonstrated using bilayer-reconstituted VDAC1 and real- time surface plasmon resonance. A synthetic peptide corresponding to the VDAC1 N-terminal region bound to HK and Bcl2, preventing their anti-apoptotic effects when expressed in cells overexpressing these proteins. These results, together with our finding of apoptosis-induced VDAC oligomerization, show VDAC1 to be a critical component of the apoptosis machinery and that its N-terminal region controls cytochrome *c* release and the regulation of apoptosis by anti-apoptotic proteins.

Poster # 9

Simulator Sickness in Helicopter Simulator

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Introduction: Flight simulators are safe and cost-effective training tools, however they may be associated with motion sickness-like symptoms. These symptoms have been reported to persist for hours to days following the training experience. The objectives of this study were to evaluate the IAF flight simulator and to measure symptoms 12-24 hours post training.

Methods: The simulator is a Helicopter Aircraft Weapon System Trainer (HAWST). Participants were experienced helicopter pilots. They filled out a pre-exposure simulator sickness questionnaire (courtesy of Kennedy RS et al) and a motion sickness history questionnaire. Training protocols included 3 sequential flights (1. low altitude daylight flight, 2. low altitude night-time flight, 3. instrument only flight). Questionnaires were filled after each training session and 12 and 24 hours after the training session.

Results: 98 subjects participated in this study. Among these aviators the average score after each session was 14-20.6 with high variability (maximal score 101). The highest scores were associated with a low altitude night time flight. Previous complaints of motion sickness during ship cruise, aircraft flight, simulator flight and theme park attractions ride, as well as general motion sickness sensitivity were associated with higher score of simulator sickness ($p<0.05$). 50 and 48 subjects (with scores similar to the larger group) were followed respectively 12 and 24 hours after training . Twelve hours after training 5 (10%) subjects reported significant symptoms (score ≥ 10).

Conclusion: Relatively high scores of simulator sickness were reported when training with this simulator, which may be partly due to the specific flight patterns which accentuate the visual limitations of the simulator. The simulator sickness score 12-24 hours post-training may have a role when evaluating post training activities.

Poster # 10

The Proliferative Potential of Carp (*Cyprinus carpio*) Cardiomyocytes

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Adult fish cardiomyocytes, in contrast to their mammalian counterparts, can proliferate after injury and contribute to the functional regeneration of the heart. In order to understand the mechanisms underlying this plasticity we performed studies on fish cardiomyocytes in culture. Most of the cells in culture had typical cardiomyocytes morphology and they began to contract after 3 days in culture. The cardiomyocytes maintained proliferation up to day 50 in culture. In contrast to mammalian cardiomyocytes in culture, the cells showed no fibroblast like properties. Furthermore, conditioned medium taken from this culture on days 3 and 4 could significantly stimulate the *in vitro* proliferation of neonatal rat cardiomyocytes. Histological survey of cardiac tissue following serious experimental injury showed complete regeneration without any fibroblastic regression. Understanding the proliferative potential of fish cardiomyocytes and how they differ from their mammalian counterparts may lead to manipulations that can enhance the regenerative potential in the mammalian heart.

Poster # 11

Gender Differences in the Response to Abdominal Compartment Syndrome in Rats

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Abdominal compartment syndrome (ACS) is defined as an increased intra-abdominal pressure (IAP > 20mmHg) in combination with single or multiple organ dysfunction which was not previously present. This condition affects multiple organ systems in a graded fashion. Early identification and abdominal decompression are essential in the management and treatment of this difficult situation, otherwise, it may lead to multiple organ failure and ultimately, death. Studies have demonstrated that estradiol, the predominant sex hormone in females, has protective effects on cardiovascular and hepatocellular functions after trauma-hemorrhage. The aims of the present study were to compare the hemodynamic and metabolic response to ACS in female and male rats, and to assess the survival rate differences between the two groups. After anesthesia and cannulations, a group of 32 male rats and 32 female rats were randomly divided into 4 groups: Group 1 (n = 8) sham-operated including canulation and insertion of a sterile balloon to the abdominal cavity. Group 2 (n = 8), IAP was increased to 10 mmHg. In group 3 (n = 8), IAP was increased to 20 mmHg, and in group 4 (n = 8), IAP was increased to 30 mmHg. The animals were observed for 4 hours. Increase in intraperitoneal pressure to 20 mmHg and 30 mmHg led to a decrease in mean arterial pressure (MAP) from 116.1 ± 3.9 to 51.8 ± 7.1 mmHg ($p<0.01$) and 33.3 ± 9.3 ($p<0.01$) mmHg respectively, in male rats. In female rats a similar increase of intra-abdominal pressure (IAP) led to a decrease in MAP from 93.7 ± 2.0 to 73.1 ± 6.3 mmHg ($p<0.05$) and 52.0 ± 3.4 ($p<0.05$) mmHg, respectively. Thus, the decrease in blood pressure that was observed in females was significantly lower than that observed in males. In males subjected to 20 or 30 mmHg IAP, glucose level was reduced in comparison to control rats, while in female rats blood glucose was elevated when they were subjected to 20 mmHg, but was reduced after they were subjected to 30 mmHg. Lactate level was also elevated in males subjected to 20 mmHg or 30 mmH IAP and in females subjected to 30 mmHg. In females subjected to 20 mmHg, lactate was elevated only after 2 hrs. The pH levels were reduced in both males and females subjected to 20 or 30 mmHg compared to control rats. However, the reduction in pH observed in females subjected to 20 mmHg was significantly lower than that observed in males ($p<0.05$). In addition, females that were subjected to IAP of 30 mmHg had significantly better ($p<0.05$) survival than males that were subjected to the same pressure. These results indicate that female rats preserved their blood pressure and their hemodynamic parameters better than male rats during intra-abdominal hypertension and therefore are better protected against lethal ACS than males.

Poster # 12

Toll-Like Receptor 4 (TLR4) and Macrophage Migration Inhibitory Factor (MIF) Expression in the Myocardium Following Ischemic Injury or LPS Injection

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Recent studies have indicated that MIF and TLR4 play an important role in the regulation of innate immune responses. Inflammation plays an important role in the pathology of ischemic heart disease and in myocardial contractile depression during septic shock. The inflammation in cardiovascular disease is associated with the activation of variety of cells including immune cells and cardiac myocytes, which secrete proinflammatory cytokine such as interleukin 1 beta (IL-1 β) and tumor necrosis factor alpha (TNF α). The goals of this study were to investigate the time course of myocardial dysfunction and cytokine expression in models of LPS induced sepsis and myocardial ischemia (MI) induced by left anterior descending coronary artery ligation (LAD), and to determine the changes in the myocardial gene and protein expression of TLR4 and MIF.

C57Bl male mice were challenged to sepsis by I.P. injection of LPS or were subjected to MI by LAD ligation. Cardiac function was measured by a micro-catheter tip pressure transducer. Cytokine expression was detected by ELISA. TLR4 and MIF mRNA expression was determined by quantitative RT-PCR, and protein expression using Western blot.

Myocardial levels of IL-1 β and TNF α increased significantly after MI or LPS injection reaching maximum at 4 hours. The IL-1 β and TNF α levels remained high in the MI group 72 hours after MI whereas in LPS injected group levels returned to baseline within this period. The decrease in hemodynamic function following LPS injection was transient, maximizing at 4 hours (78±11 mmHg, 65 % of baseline values). In MI however, hemodynamic function decreased to 67±8.5mmHg, 58 % of baseline values at 24 hours, $p<0.05$, remaining so for 72 hours. As cardiac function decreased, myocardial TLR4 and MIF mRNA and protein expression both increased These data suggest that in addition to their role in the innate immune response, TLR4 and MIF from a myocardial source, depress cardiac function in both ischemia and LPS injury.

Poster # 13

Indirect Detection of Phosphorus-31 Signals in Cells by 2D-Heteronuclear Methods

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Introduction: Indirect detection (ID) is a method of observing the signals of a less sensitive NMR nucleus through cross-polarization or spin-spin (J) coupling with a more sensitive nucleus. ^{1}H and ^{31}P are the most common nuclei used for observation of biochemical and metabolic processes *in vitro* and *in vivo*, and consequently, there might be some advantage in using these ID approaches for ^{31}P observation. Generally the only coupling between ^{1}H and ^{31}P is through the phosphate ester 3 bond couplings (JPOCH = 8-10 Hz). While this is a small coupling its presence is enough to obtain 31P spectra at proton sensitivity. However, difficulties arise in carrying out these experiments due to the interference of ^{1}H-^{1}H couplings with similar values. A means of overcoming these difficulties and to obtain ^{31}P data at higher sensitivity is to use heteronuclear multiple quantum correlation (HMQC) spectroscopy as the POCH fragment requires multiple quantum transitions. In this project we evaluate the use of ID 2D-heteronuclear methods for the detection of phosphate metabolites in live cells.

Material and Methods: HL-60 cells (acute myeloid leukemia) and A2780 cells (human ovarian carcinoma) were grown according to established procedures. Spectra of intact cells were determined using the gel perfusion method were cells are embedded in alginate beads (Cohen and Kaplan, Immunomethods 4:139, 1994). Cytoplasmic components were extracted according to Teleman et al (Anal. Biochem., 272:71, 1999). NMR spectra were obtained with Varian Inova 500. 1mM methylphosphonic acid was used as an internal standard for method development and optimization (at 10^{o}C, 1.1 ppm)

Results: ATP (50 mM) in D2O standard was used for method development and optimization. The chemical shift was stable under pH of 7.0 ±0.1 at temp. of 10ºC and

peak volume compared to the peak of methylphosphonic acid at constant concentration was reproducible. Chemical shifts of ^{1}H and ^{31}P correlate with the cross peak obtained by HMQC. Peak volume was found to be linear with concentration using the methylphosphonic (MeP) acid cross peak as an internal standard.

HMQC of cell extracts and perfused intact cells revealed that ^{1}H -^{31}P HMQC provides improved resolution and sensitivity compared to direct ^{31}P MR detection. The UDPS peak is resolved into UDP-glucose and UDP-galactose. Additional phosphosugars mono- and diesters (fructose 6 phosphate, fructose 1,6 bisphosphate, glucose 6 phosphate, glucose 1,6 bisphosphate) peaks are also resolved.

Conclusions: 1H -31P HMQC provides improved resolution and sensitivity compared to direct 31P MR detection. The UDPS peak is resolved into UDP-glucose and UDP-galactose. Additional phosphosugars mono- and diester peaks are also resolved. Spectra of A2780 and HL-60 intact cells are well resolved, reproducible and peak chemical shifts and relative volumes correlate well with cell extract spectra.

Poster # 14

Effect of Pacing Lead Position on Ischemic Left-Ventricle Functioning. Should We Change the Strategy of Lead Placement in Cardiac Resynchronization Therapy?

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Background: Although the benefit of Cardiac Resynchronization Therapy (CRT) in the treatment of heart failure is widely recognized, over 35% of the patients treated with CRT do not exhibit immediate functional improvement and long term Left Ventricular (LV) reverse remodeling. Most of these patients have ischemic heart disease. We suggest that current practice to pace the left lateral wall of the LV, regardless of the size and location of ischemic myocardial regions, may not be the optimal pacing treatment. We **hypothesize** that improvement in local and global myocardial functions will be obtained by pacing the weak ischemic regions, since pacing decreases the generated external work and energy consumption at the pacing site. This will decrease the workload of these weaker sites and increase the preload of the distal normal myocardium. The study **aims** to test the local and global short term effects of the opposing strategies: pacing at the lateral wall, which is usually the last activated site, as is done currently, or at the ischemic site.

Methods: Myocardial infarction was created in the anteroseptal region, in open chest anesthetized sheep (n=4), by ligation of a large marginal coronary artery. Pacing electrodes were placed in the anteroseptal (ischemic) region and the lateral (last activated) region before the ligation. Local and global LV functions were measured during: i) normal sinus activation (without pacing), ii) anteroseptal pacing or iii) lateral pacing. Cardiac function was assessed at baseline, before the coronary occlusion and after reaching a steady state with overt myocardial infarction. LV external work, global pressure-volume and local pressure-segmental length (PL) loops were measured by utilizing (Millar) pressure transducers in the LV and aorta, sonocrystals (Sonometrics), impedance catheter (CD Lycome) and aortic flowmeter (Transonics).

Results: *[Mean+STD];* Pacing on either site didn't significantly affect the global external work (EW), at baseline, although EW was slightly larger during anteroseptal pacing (1.5%, $p=.24$) relative to the lateral pacing. However, huge decrease in the anteroseptal work was observed during local pacing (-39%±16%, $p<.02$) at baseline. The anteroseptal infarction extended the local segment's end-diastolic length (+5%±4.7%, $p=.1$), decreased the local work (-61%±17%, $p<.002$) and yielded post-systolic shortening (7±5 fold increase, $p<.1$) where the ischemic region generated active work during diastole. Anteroseptal pacing presents three favorable effects; It significantly decreased the work of the ischemic region (-38%±16%, $p<.02$) while the lateral pacing had no marked affect on the ischemic region (-8%, $p>.55$). Moreover, pacing in the ischemic region diminished the post-systolic shortening work (in 3 out of 4 sheep: -44%±22%, $p<.05$), and thus improves the diastolic function. Interestingly, the reduction in the local ischemic anteroseptal work with even a slight improvement in the global function implies that the distal healthier regions generate more work.

Conclusions: Pacing in the ischemic region decreases the work generated by the ischemic region and conspicuously decreases the post-systolic work. Redistribution of the workload, by reducing the work of weaker areas and loading the healthier regions is feasible. Improving the balance between mechanical demands and energy supply and improving the cardiac diastolic function (less post systolic shortening work) may promote myocardial reverse remodeling and elongate patient survival.

Poster # 15

Uridine-5'-Triphosphate (UTP) Protects Against Hepatic Ischemic Injury in Mice

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Warm ischemia occurs in liver transplantation, trauma, shock and during partial hepatectomy. Liver failure is the most common cause of mortality. Adenosine 5'-triphosphate (ATP)-depletion from hepatic tissue following warm ischemia causes necrotic and apoptotic cell death. Uridine 5'-triphosphate (UTP) significantly reduced cardiomyocyte death induced by hypoxia via activating P2Y receptors. The role of pyrimidine nucleotides in the hypoxic liver has not been well explored.

The aim of this study was to investigate the role of UTP on the hepatic injury induced by ischemia in isolated mouse livers. Isolated mouse livers were randomly divided into five groups: (1) control group, perfused for the whole study period (105 minutes), (2) 30-minute perfusion followed by 90 minutes of ischemia; (3) like group 2, but with the perfusion of UTP (1μM), for 30 minutes before ischemia; (4) like group 2, but with the perfusion of suramin (200 μM), a P2Y antagonist, for 30 minutes before ischemia (5) like group 3 but with the simultaneous perfusion of suramin . Effluent liver enzyme levels, intrahepatic ATP content and caspase-3 activity were measured. Apoptotic cells were identified by morphological criteria, the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) fluorometric assay and immunohistochemistry for caspase-3.

Results: Post-ischemia, there was a statistically significant reduction in liver enzyme levels in the animals pretreated with UTP ($p<0.05$), the intrahepatic caspase-3 activity was significantly decreased ($p<0.05$) and the intrahepatic ATP content increased ($p<0.05$) compared to group 2 (ischemic untreated). The reduction in postischemic apoptotic hepatic injury in the UTP-treated groups was confirmed morphologically, by the significantly fewer apoptotic hepatocyte cells detected ($p< 0.05$); immunohistochemically, by the significantly weaker activation of caspase-3 compared to the ischemic untreated group 2 ($p< 0.05$); and by the TUNEL assay ($p< 0.05$). The administration of suramin (group 4) aggravated the apoptotic ischemic injury while the simultaneous perfusion of UTP and suramin induced ischemic changes as in group 2**.**

Conclusion: The administration of UTP before induction of ischemia attenuates the postischemic hepatocyte apoptosis and thereby minimize liver damage. Apoptotic hepatic injury seems to be mediated through caspase-3 activity. Inhibition of the release of endogenous UTP augments ischemic hepatic injury. These findings have important implications for the potential use of UTP in ischemic hepatic injury.

Poster # 16

Photosensitizable Cellular Targets

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The science of photobiostimulation includes phenomena such as, stimulation of wound healing or improving the fertilizing capability of sperm cells. These phenomena were connected to light induced reactive oxygen species (ROS) known to modulate biological processes.

As light must be absorbed by the cell in order to induce a chemical effect, determination of the photoabsorbing molecules and their sites is a key questions. The EPR spin trapping technique coupled with the probe, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), served us to answer these questions. We first studied wavelengths dependence of DMPO-OH production in cardiac and sperm cells. We found that the 400-500 nm range is responsible for most of the DMPO-OH spin adduct production. In addition, when comparing DMPO-OH generation in illuminated fractions of dialyzed cells homogenates, it was found that the photosensitizer molecule is small, water soluble, and active only at wavelengths below 500 nm. Therefore it was hypothesized that the flavins are the ones responsible for ROS formation. Since both mitochondria and membranes contain flavocytochrome, and flavin nucleotide we studied ROS generation in each of them. An increase in the DMPO-OH spin adduct attributed to •OH generation was measured in illuminated intact cells and isolated sperm plasma membranes. In intact cells the presence of the extracellular scavenger, BSA, decreased DMPO-OH adduct, therefore we believe that the extracellular membrane is responsible for ROS generation. By using the fluorescent probe, MitoTracker Red (MTR) we found that light causes an increase in ROS also in the mitochondria. These findings are of much importance for elaborating the mechanism of light-tissue interaction.

Poster # 17

Ranibizumab and Bevacizumab: Pharmacokinetic and Penetration Studies

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Purpose: (1) To determine the pharmacokinetics and serum bioavailability of intravitreal bevacizumab in the rabbit. (2) To compare retinal penetration of ranibizumab to bevacizumab.

Methods: (1) 12 albino rabbits were intravitreally injected to one eye with bevacizumab1.25mg/0.05ml. One rabbit served as intact control. Vitreous samples were taken 1, 2 ,4, 6 weeks after injection, 3 rabbits for each time point. Blood samples were taken 2 and 6 weeks after injection. Bevacizumab concentrations in the plasma and vitreous were determined by enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human IgG for capture and horseraddish peroxidase (HRP) conjugated rabbit anti-human IgG for detecting. (2) 12 albino rabbits were intravitreally injected to one eye with bevacizumab 200 μg or with ranibizumab 500 μg. Eyes were enucleated 1, 3, days, 1, 4 weeks after injection. Immunohistochemistry was performed.

Results: (1) The mean vitreal concentration of bevacizumab decreased by 37%, 62%, 70% and 81% at the 1,2,4 and 6 weeks respectively. Mean vitreal concentration in the uninjected eye was 4.93 ng/ml, 4.36ng/ml, 1.06ng/ml and 0.41ng/ml at the 1,2,4,6 weeks respectively. Mean plasma concentrations of bevacizumab was 17.20 pg/ml, and 7.02 pg/ml at the 2 and 6 weeks respectively. Mean vitreal and plasma concentrations of the control rabbit was lower than the sensitivity of the assay. (2) both ranibizumab and bevacizumab penetrated the retina of the albino rabbit. The penetration will be compared.

Conclusions: (1) The mean half time of bevacizumab in the rabbit eye was 10 days. The high intravitreal concentrations observed after 6 weeks demonstrates a lower than expected turnover of bevacizumab. The concentration of bevacizumab in the plasma and uninjected eye indicates systemic circulation. (2) Both ranibizumab and bevacizumab penetrated the retina of the albino rabbit, differences in penetration will be discussed.

Poster # 18

The Signal-Transduction Pathway of Direct Activation Of Ca^{2+} Uptake Into Sarcoplasmic Reticulum by Various Drugs

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The aim of this study was to examine the effect of adenosine, Cl-IB-MECA (A3 adenosine receptor agonist) and D-sotalol (antiarrhythmic drug class III), on calcium accumulation inside the sarcoplasmic reticulum (SR). All these drugs have been shown previously to prolong the action potential duration and the refractoriness of the heart. Chemical skinning of cultured rat myocardial cells with saponin compromised the barrier function of the cell membrane and thus permitted direct exposure of the SR. Mitochondrial calcium accumulation was negligible in the presence of sodium azide and ruthenium red. Incubation with 10 μM D-sotalol, 100 nM Cl-IB-MECA, increased the level of calcium accumulation within the SR. A similar effect was obtained after 24-48 hr incubation. Western blot analyses showed that Cl-IB-MECA induced phosphorylation of CaMKΠ while D-sotalol reduced it. On the other hand, D-sotalol induced phosphorylation of phospholamban (PLB). These results suggest that Cl-IB-MECA and D-sotalol interfere with two distinct mechanisms relating to the calcium accumulation into the SR.

Poster # 19

Reversible Pegylation Prolongs the Hypotensive Effect of Atrial Natriuretic Peptide

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Background: Natriuretic peptides (NP), including Atrial Natriuretic Peptide (ANP), induce potent natriuresis and vasodilation and thereby generate hypotension *in-vivo*. Despite intensive efforts, clinical application of NP as an anti-hypertensive agent is limited because of their short biological half-life and poor bio-availability. Recently, we have developed a strategy that facilitates slow release of peptides from PEG-peptide inactive conjugates, based on reversible pegylation. Peptides prepared by this approach undergo slow, spontaneous chemical hydrolysis at physiological conditions, releasing the native active peptide/protein drug from the inactive conjugates over prolonged periods.

Aims: The aims of this study were to synthesize reversible pegylated-ANP and compare its activity to that of ANP on systemic blood pressure.

Materials and Method*:* Experiments were performed on Wistar rats weighing 300 gr. Animal were anesthetized with Urethane. Following tracheotomy the two femoral veins and the right femoral artery were cannulated with a PE50 heparanized tube. Blood pressure (BP) was recorded from the right femoral artery using SP 844 pressure transducer. All Animals received an infusion of adrenalin (3.3μg/kg/min in 30 μl saline) throughout the experiment. Following 30 min of the adrenalin infusion the test compounds were administered by a bolus injection through the second femoral vein cannule in 100 μl saline.

Results: A PEG-chain of 30 kDa covalently linked to the α-amino side chain of ANP via MAL-Fmoc-OSu spacer was synthesized. This conjugate, PEG_{30}-Fmoc-ANP, undergoes hydrolysis under physiological conditions releasing native ANP at a rate of 0.78±0.03□g ANP per 10μg covalently linked ANP per day. Bolus administration of native ANP (3 or 100 μg/kg) yields a short, transitory effect in lowering BP, reaching a maximum at 2 min and then returned to control values after 12 to 25 min. In contrast, administration of PEG_{30}-Fmoc-ANP (96±7 □g covalently linked ANP/kg) lowered BP following a lag period of 50 min, and maintained low BP for a period exceeding 60 min. Saline or PEG_{30}-Fmoc-Alanine were not effective in lowering BP in Wistar rats.

Conclusions: These results show that PEG_{30}-Fmoc-ANP is a reversible pegylated pro-drug derivative that facilitates prolonged BP lowering effect in rats and may be considered as a candidate for development into an anti-hypertensive drug.

Poster # 20

Activation of PKC-Alpha and Delta in Sperm Capacitation and Acrosome Reaction

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Introduction: The PKC family of serin/threonine kinase, function as key proteins in cell signaling and in the control of many cellular processes.Several studies support the possible involvement of PKC in the acrosome reaction (AR). Adding PMA (specific PKC activator) to capacitated spermatozoa, induced the occurance of the AR. This effect was blocked by adding PKC inhibitors. In addition, it seems that PKC is involved in the regulation of sperm motility, specially in hyperactivated motility.

Aim: Investigation the distribution and activation of PKC alpha and delta isoformes during capacitation and AR of bovine sperm.

Materials and methods: In vitro capacitation was performed by incubation of bovine sperm in defined-capacitation medium for 4h. The AR was induced by adding calcium ionophore at the end of capacitation. PKC isoformes were detected by anti PKC-alpha, anti p-PKC alpha and anti PKC delta. The antibodies incubated with nitrocellulose membranes contains whole cell lysate or subcellular fractions: head, tail, membrane and cytosol. For immunocytochemistry – cells were spread on glass slides and stained with anti PKC alpha or anti PKC delta (red) and with pissum-FITC for AR detection (green).

Results: PKC alpha is already phosphorylated at the beginning of the capacitation. The quantity and phosphorylation of PKC alpha decrease during capacitation and AR.Immunocytochemical analysis revealed that at the beginning of the capacitaion PKC alpha is located at the top of the head and at the end of the capacitation the enzyme is localized at the post acrosomal region and in the tail. Aftr the AR, it appears in the post acrosome and in the tail.Cellular fructionation analysis show that the main quantity of PKC alpha at the beginning of capacitaion is in the cytosol,while after 4 h of capacitation, its quantity in cytosol is decreased and after the AR, it disappeared from the cytosol and translocate to the head and tail. In addition, PKC alpha is located at the sperm plasma membrane during capacitation and AR.

PKC delta shows no change in quantity or phosphorylation during capacitation and AR. It localized exclusively at the sperm's head and tail.

Conclusions: The quantity and phosphorylation of PKC alpha decrease during capacitation and AR, whereas PKC delta remains without change.

Poster # 21

The Role of Monoamine Oxidase Subtypes in Striatal Metabolism of Dopamine Produced from L-DOPA in the Rat

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The central drug treatment for Parkinson's disease (PD) is L-3,4-dihydroxyphenylalanine (L-DOPA). The MAO-B inhibitor drugs, rasagiline and selegiline, are effective adjunct treatments to L-DOPA, however the precise role of chronic MAO-B inhibition in L-DOPA derived DA metabolism still needs to be clarified.

In brains of patients suffering from advanced PD there is pronounced degeneration of serotonergic and noradrenergic, as well as dopaminergic neurons, but despite that L-DOPA still produces dopaminergic effects, albeit with problems such as fluctuations in response, dystonia and dyskinesia. We have used a model of serotonergic as well as dopaminergic depletion in the rat, and have investigated the striatal metabolism of L-DOPA-derived DA by MAO subtypes in this model system.

Following dopaminergic denervation with 6-hydroxydopamine (6-OHDA) alone or with additional serotonergic denervation with 5,7-dihydroxytryptamine (5,7-DHT) in rats, striatal MAO activity and GFAP expression were investigated. Neither MAO-A nor MAO-B activity was significantly affected by 6-OHDA lesion alone, however there was a significant increase (137.5± 0.06 %, $p<0.01$ for comparison with sham-operated tissue) in MAO-B activity in the striatum lesioned with both 6-OHDA and 5,7-DHT, with no significant change in MAO-A activity. The increase in MAO-B activity following the combined lesion was associated with a significant increase in GFAP expression (391.13 ± 0.059%, $p<0.01$ for comparison with sham-operated tissue).

Using microdialysis technique, the effect of chronic MAO-A and MAO-B inhibition in rats bearing both dopaminergic and serotonergic lesions was investigated. Rats were treated daily for 2 weeks with either saline, clorgyline (1mg/kg sc, MAO-A inhibitor) or rasagiline (0.05mg/kg sc). On the 14^{th} day, striatal microdialysates were collected following a single systemic injection of L-DOPA (25mg/kg) plus carbidopa (6 mg/kg). Striatal dopamine levels were about 20 fold higher in the clorgyline group compared to controls. Rasagilne also increased DA levels significantly (about two fold). Reduction in DA metabolites (dihydroxyphenylacetic acid, homovanillic acid)) levels compared to saline was more significant in the clorgyline treatment group than the rasagiline group.

These results indicate that most of the MAO-A activity in striatum is not located in either dopaminergic or serotonergic axonal varicosities. In addition, MOA-B, possibly in glia, plays an important role in the metabolism of L-DOPA-derived DA in parkinsonian brains.

Poster # 22

ARTS Structure and Expression in Rat Cortical Neurons

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The pro-apoptotic protein ARTS is a member of the filament-forming septin family, but being derived by alternate splicing from the H5/PNUTL2/hcdcrel2a/2b gene, it differs in its C-terminal structure from other products of this gene, and from other septins.

ARTS promotes apoptosis which is induced by a variety of proapoptotic stimulators. In human cells of peripheral tissue origin, ARTS is located in the mitochondria and translocates to the nucleus when apoptosis occurs. In rat cortical neurons, however, ARTS is localized in both cytoplasmic and nuclear compartments, even in the absence of apoptotic stimulation.

Like other mitochondrial proapoptotic proteins such as Smac/Diablo and Omi/H2ra, ARTS induces apoptosis through binding to and antagonizing IAPs (inhibitors of apoptosis proteins). Upon apoptotic stimuli, ARTS is released from mitochondria, binds XIAP and decreases its levels. As a result, caspase inhibition is removed and apoptosis can be executed.

ARTS is strongly expressed in the brain and may play an important role in the nervous system. The ARTS protein in cells of peripheral origin has a molecular size on gel electrophoresis of 32kDa in human and 28kDa in rat. however ARTS in human brain tissue has a molecular size of 28 kDa. Similarly, ARTS in rat brain tissue has a molecular size of about 22 kDa, lacking its N-terminal region, as opposed to 28 kDa in rat primary cardiomyocytes. ARTS is widely expressed in rat brain, with highest levels in cortex and hippocampus.

In order to learn more about ARTS and its physiological role, we have sequenced the ARTS cDNA from rat brain. Alignment between the amino acids sequence of human and rat ARTS showed 82.8% identity.

The effect of several pro-apoptotic stimulati were tested on rat E18 cortical primary neuronal cultures, for their effect on the expression of ARTS and active caspase-3 (as an apoptosis indicator). Neurons were grown in Neurobasal medium plus B-27 additives. Removal of B-27 at DIV 6 induced apoptosis.

Western blot analysis showed an increase in ARTS protein expression in cells which were incubated 24h in medium without B27. High concentrations of glutamate (100μM to 50mM) also induced apoptosis. At 50mM, glutamate increased expression of ARTS and active caspase-3 ($P<0.05$).

ARTS may be involved in neuronal apoptosis, but its precise role is still unclear.

Poster # 23

Retina Expresses a Novel Variant of the Ryanodine Receptor

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Calcium released from intracellular stores via the ryanodine receptor (RyR) mediates a variety of signaling processes. We previously showed that retina expresses the three known types of ryanodine receptor (RyR), but retinal membrane preparations exhibit unique characteristics, such as Ca^{2+}-independent [^{3}H]ryanodine-binding and inhibition by caffeine. We have heretofore suggested that the major retinal RyR isoform is novel. The present study aimed to identify this receptor isoform and to localize RyR in mammalian retina. Immunoblotting with specific and pan-antibodies showed that the major retinal RyR has a mobility like that of RyR2 or RyR3. Real time PCR revealed that the major type is RyR2, and RT-PCR followed by sequencing showed a transcript that encodes a protein with about 99% identity to RyR2, yet lacking two regions of 7 and 12 amino acids and including an additional insertion of 8 amino acids. An antibody against RyR2 localized this type to somas and primary dendrites of most retinal neurons. An antibody against RyR1 localized RyR to most somas, but also revealed staining in photoreceptor outer segments, concentrated on the disk membranes at their rim. The ryanodine-binding properties and the electrophoretic mobility of RyR from the outer segments were similar to those of whole retinal preparation. The results thus identify a novel variant of RyR2 which can contribute to regulating photoreceptor Ca^{2+} concentrations. The restricted localization of the outer segment RyR to the disk rim suggests that its activation mechanism involves a coupling between retinal RyR and the cGMP-gated channel.