

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

ISRAEL SOCIETY FOR PHYSIOLOGY
AND PHARMACOLOGY



Annual Meeting הכנס השנתי

September 18th 2006

Tel-Aviv University
The Sackler Faculty of Medicine

PROGRAM & ABSTRACTS

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

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האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

ISRAEL SOCIETY FOR PHYSIOLOGY AND PHARMACOLOGY

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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.

Program Outline

8:30-9:30 Registration and Refreshments

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

11:10-11:30 Coffee Break

11:30-13:10 Parallel Sessions D-F

13:10-15:00 Lunch+Posters

14:45-15:00 Business Meeting

15:00-16:00 The Magnes memorial lecture

Prof. Hermann Steller

Howard Hughes Medical Institute, The Rockefeller University

**Title: Death by design: Mechanism
and regulation of apoptosis**

Opening address on Jonathan Magnes by: Prof. Asher Ilani

16:00-17:00 Student Lecture Competition

Meeting Program

9:30-11:10 - FIRST SESSION

A - Environmental Physiology
Chairpersons: Prof. Michal Horowitz, The Hebrew University of Jerusalem and Noga Kronfeld-Schor, Tel-Aviv University
Meyerbaum Hall

- 9:30 Noam Meiri, Agriculture Research Organization, The Volcani Center
Molecular correlates of thermal control establishment: A non-declarative consolidation mechanism
- 9:55 Noga Kronfeld-Schor, Tel-Aviv University
Keeping body mass constant under fluctuating food availability in golden spiny mice
- 10:20 Yehuda Arieli, Israel Naval Medical Institute
Hyperoxic Preconditioning and Heat Acclimation: Does it have to do with CNS oxygen toxicity?
- 10:45 Naama Shein, The Hebrew University of Jerusalem
Heat acclimation as a model for self-induced neuroprotection

B - Normal and Abnormal Brain Excitability
Chairpersons: Eran Gilat, The Israeli Institute for Biological Research and Yoel Yaari, The Hebrew University of Jerusalem
Lola Hall

- 9:30 Vered Givant-Horwitz, The Israeli Institute for Biological Research
Alterations in rat brain mRNA levels following sarin-induced status epilepticus
- 9:55 Alon Friedman, Ben-Gurion University of the Negev
Interactions within the neurovascular unit during epileptogenesis
- 10:20 Yitzhak Schiller, Technion, Haifa
Network dynamics during the development and maintenance of seizures in Pilocarpine and Picrotoxin treated rats
- 10:45 Yoel Yaari, The Hebrew University of Jerusalem
Plasticity of the low-threshold calcium current in acquired epilepsy

C - Calcium Homeostasis

Chairperson: Varda Shoshan-Barmatz, Ben-Gurion University of the Negev

Dolphy Hall

- 9:30 Nathan Dascal, Tel-Aviv University
Complex modulation of L-type Ca^{2+} channels by auxiliary subunits and calcium
- 9:55 Yoram Etzion, Ben-Gurion University of the Negev
Crosstalk between L-type calcium channels and ZnT-1, a new player in rate-dependent cardiac electrical remodeling
- 10:20 Tally Naveh-Many, The Hebrew University of Jerusalem
Regulation of parathyroid hormone (PTH) gene expression: The sensing and response to serum calcium and phosphate
- 10:45 Micha Spira, The Hebrew University of Jerusalem
Calcium activated PLA2, PKC and calpain converge to generate the transformation of a cut axonal end into a motile growth cone
- 11:10-11:30 **Coffee break**

11:30-13:10 - SECOND SESSION

D - Hormonal Control of Feeding

Chairpersons: Avy Susswein and Aron Weller, Bar-Ilan University

Meyerboum Hall

- 11:30 Ester Fride, College of Judea and Samaria, Ariel
The endocannabinoid system in the regulation of feeding: Important for adults, critical for newborns
- 11:55 Itay Hurwitz, Bar-Ilan University
Control of feeding in Aplysia with steady-state access to food
- 12:20 Aron Weller, Bar-Ilan University
Examination of the emergence of obesity from birth to adulthood in the OLETF rat model
- 12:45 Yosefa Avraham, Hadassah Medical School, Jerusalem
Models of anorexia

E - Vascular Reactivity and Blood Pressure
Chairperson: Ehud Grossman, Sheba Medical Center
Lola Hall

- 11:30: Zaid Abassi, Rambam Medical Center
Influence of renin-angiotensin-aldosterone system and other vasoactive substances on the vasculature
- 11:55 Yehonatan Sharabi, Sheba Medical Center
Neurohormonal influences on the vascular tree
- 12:20 Michael Shechter, Sheba Medical Center
Endothelial function under elevated blood pressure
- 12:45 Ehud Grossman, Sheba Medical Center
Clinical application of vascular biology on elevated blood pressure

F - Circadian Rhythms
Chairperson: Nava Zisapel, Tel-Aviv University
Dolphy Hall

- 11:30 Oren Froy, The Hebrew University of Jerusalem
The biological clock, food and nutrition
- 11:55 Guy Bloch, The Hebrew University of Jerusalem
Molecular underpinnings of socially modulated plasticity in circadian rhythms in the honey bee *Apis mellifera*
- 12:20 Yoav Gothilf, Tel-Aviv University
Functional development of the circadian clock: A study using the zebrafish model
- 12:45 Tali Gorfine and Nava Zisapel, Tel-Aviv University
The effects of melatonin on behavior and brain activity in humans: Fmri studies

13:10-15:00 Lunch + Posters

14:45-15:00 Business meeting

15:00-16:00 Magnes memorial lecture

**In the memory of Jonathan Magnes: Prof. Asher Ilani,
The Hebrew University, Jerusalem**

Prof. Hermann Steller

Howard Hughes Medical Institute, The Rockefeller University

Title: Death by design: Mechanism and regulation of apoptosis

16:00-17:30 Student Lecture Competition

Program at a glance

Session A (Meyerboum Hall): New Environmental Physiology		Session B (Lola Hall): Normal and Abnormal Brain Excitability		Session C (Dolphy Hall): Calcium Homeostasis	
Chairpersons: Prof. Michal Horowitz and Noga Kronfeld-Schor		Chairperson: Eran Gilat and Yoel Yaari		Chairpersons: Varda Shoshan-Barmatz	
9:30	Noam Meiri Molecular correlates of thermal control establishment: A non-declarative consolidation mechanism	9:30	Vered Givant-Horwitz Alterations in rat brain mRNA levels following sarin-induced status epilepticus	9:30	Nathan Dascal Complex modulation of L-type Ca ²⁺ channels by auxiliary subunits and calcium
9:55	Noga Kronfeld-Schor Keeping body mass constant under fluctuation food availability in golden spiny mice	9:55	Alon Friedman Interactions within the neurovascular unit during epileptogenesis	9:55	Yoram Etzion Cross-talk between ZnT-1 and L-type Calcium channels: A new player in rate dependent cardiac electrical remodeling
10:20	Yehuda Arieli Hyperoxic Preconditioning and Heat Acclimation: Does it have to do with CNS oxygen toxicity?	10:20	Yitzhak Schiller Network dynamics leading to epileptic seizures in the pilocarpine treated rat	10:20	Tally Naveh-Many Regulation of parathyroid hormone (PTH) gene expression: The sensing and response to serum calcium and phosphate
10:45	Naama Shein Heat acclimation as a model for self induced neuroprotection	10:45	Yoel Yaari Plasticity of the low-threshold calcium current in epilepsy	10:45	Micha Spira Orchestrated restructuring of neurons after axotomy: The role of calcium-activated PLA2, PKC and calpain

Session D (Meyerboum Hall): Hormonal Control of Feeding		Session E (Lola Hall): Vascular Reactivity and Blood Pressure		Session F (Dolphy Hall): Circadian Rhythms	
Chairpersons: Avy Susswein and Aron Weller		Chairpersons: Ehud Grossman		Chairpersons: Nava Zisapel	
11:30	Ester Fride The endocannabinoid system in the regulation of feeding: Important for adults, critical for newborns	11:30	Zaid Abassi Influence of renin, angiotensin and vaso peptides on the vascular tree	11:30	Oren Froy The biological clock, food and nutrition
11:55	Itay Hurwitz Control of feeding in Aplysia with steady-state access to food	11:55	Yehonatan Sharabi Neurohormonal influences on blood vessels	11:55	Guy Bloch From social organization to brain clock gene expression in the honey bee
12:20	Aron Weller Examination of the emergence of obesity from birth to adulthood in the OLETF rat model	12:20	Michael Shechter Endothelial function under elevated blood pressure	12:20	Yoav Gothilf Functional development of the circadian clock: A study using the zebrafish model
12:45	Yosefa Avraham Models of Anorexia	12:45	Ehud Grossman Clinical application of vascular biology to treat elevated blood pressure	12:45	Tali Gorfine and Nava Zisapel Circadian modulation of brain activation patterns in humans: Role of melatonin

Abstracts of Invited Presentations

Molecular correlates of thermal control establishment: A non-declarative consolidation mechanism

Adi Katz, Galya Labunskay, Sharon Tirosh, Noam Meiri

Institute of Animal Science, Agriculture Research Organization, The Volcani Center,
Bet Dagan

Although there is some knowledge about the molecular pathways involved in declarative memory storage, other mechanisms underlying information on different neuronal plasticity-dependent memory systems such as the ability to adjust to changes in environmental temperature, and the induction of thermotolerance remain unknown. In this talk I will describe molecular correlates of thermal conditioning in chicks. Neuroanatomically, body temperature is balanced by the preoptic anterior hypothalamus (PO/AH) and controlled by thermo-sensitive neurons. Thermal-conditioning cause a plastic change in the ratio between thermo-sensitive neurons and innate PO/AH cells, reducing the number of temperature sensitive cells from 40% to 29%. In this project mRNA fingerprinting was used to identify the proteins which are involved in thermal adaptation in 3-day-old chicks. Fifteen genes were induced, among which were: NADH dehydrogenase, protocadherin, anolase α , 14-3-3 ϵ and R-Ras3. The role of each of these genes is potentially interesting and requires detailed evaluation but, nevertheless, since the working hypothesis assumes neuronal remodeling, we concentrated on the role of R-Ras3/(M-Ras). This gene is uniquely expressed in the brain, its physiological role has not been described previously, and it might play a pivotal role in neuronal plasticity. R-Ras3 was induced after both heat and cold conditioning. To improve our understanding of thermal adaptation related signal transduction we screened for changes in the expression of neurotrophic factors and transcription factors which were implicated with the Ras gene family, and found that the expression of BDNF but not NT3 or NGF are induced during heat conditioning. Among the transcription factor the only one that its expression was altered during temperature conditioning was jun. Taken together, these results correlate the BDNF - R-Ras3 – jun pathway with thermal- adaptation-related hypothalamic plasticity.

**Keeping body mass constant under fluctuating food availability
in golden spiny mice**

N. Kronfeld-Schor

Department of Zoology, Tel-Aviv University

Golden spiny mice (*Acomys russatus*) are omnivorous desert rodents that do not store food, and must therefore employ physiological means to cope with food shortage. We studied the physiological means used by golden spiny mice for coping with food restriction, and the mechanism by which food consumption may influence metabolic rates. We found that when kept under 50% food restriction, these rodents use two different behavioral strategies: 1. Increasing activity level and searching for food, thus increasing energy expenditure and losing mass rapidly, 2. Decreasing activity level and conserving energy, thereby defending their body mass. The use of these two strategies to cope with food shortage may be of evolutionary advantage, since it allows a more flexible reaction to food restriction at the population level. We also studied the physiological means for decreasing energy expenditure, and the role of hypothalamic neuropeptides in regulating the response of golden spiny mice to food restriction. We found that leptin levels, heart rate, body temperature and oxygen consumption dropped significantly after 24 hr of food restriction. Hypothalamic neuropeptides also showed a significant response after 24 hr: mRNA levels of AGRP and NPY increased significantly, while those of POMC increased only after long-term food restriction, and those of MC4-R and Ob-R did not change. Leptin replacement during food restriction (mouse leptin, R&D, using Alzet osmotic minipumps model 1002), which started 3 days before restriction onset, did not cause a dramatic change the response of golden spiny mice to food restriction.

Hyperoxic Preconditioning and Heat Acclimation: Does it have to do with CNS oxygen toxicity?

Yehuda Arieli

Israel Naval Medical Institute, Haifa

Background: The beneficial use of oxygen in diving and hyperbaric medicine may be accompanied by hazardous central nervous system oxygen toxicity (CNS-OT). Reactive oxygen species (ROS) are a major factor in the generation of CNS-OT. In stressful situations, such as hyperoxia, the balance between ROS production and scavenging is impaired by their increased generation. Extensive effort has therefore been directed to find means of protection against CNS-OT. Long-term heat acclimation is known to provide cross-tolerance to various forms of stress in different organs including the brain. Additionally, preconditioning to high [O₂] is known to provide protection to the lungs. The current review demonstrates our recent findings in the rat.

General Methods: Following heat- acclimation to 32°C or hyperbaric O₂ (HBO) preconditioning rats were exposed to oxygen at 608 kPa, and EEG was recorded continuously until the appearance of the first electrical discharge. Immediately thereafter, rats were sacrificed and samples were taken from brain, blood, and heart for biochemical investigation.

Results: *a. Heat Acclimation:* Latency to CNS-OT was twice as long in the heat-acclimated rat. This protective effect was studied for two weeks during deacclimation. This was associated with elevation of HSP-72 and Cu/ZnSOD. *b. HBO Preconditioning:* Time to CNS-OT was significantly increased from 9.6 to 15.9 min following PC. The activity of catalase and glutathione-peroxidase was significantly increased in 22 and 30% (respectively), while the activity of G6PD GST was significantly decreased in 37.3 and 30% (respectively).

Conclusions: We conclude that the protection afforded by heat acclimation is caused in part by the over- expression of HSP-72 and Cu/ZnSOD. Preconditioning to HBO, at 202 kPa provides protection against CNS-OT. The mechanism by which this protection is induced probably involves changes in the activity of ROS scavenging enzymes. The role played by HSP72 in providing protection against CNC-OT remains to be further investigated.

Heat acclimation as a model for self-induced neuroprotection

Naama A. Shein^{1,2}, Michal Horowitz² and Esther Shohami¹

Departments of ¹Pharmacology and ²Physiology, The Hebrew University of Jerusalem

Heat acclimation (HA) is a conserved adaptive response to exposure to moderately high ambient temperature. The process engulfs the development of a new physiological phenotype, resulting from both post-translational modifications as well as genomic responses. An inseparable outcome of HA is that adjusting to one stressor can, in addition to evolving primary adaptations, add to the amount of adjustment to additional stressors including ischemia, hyperoxia and as we have shown, traumatic brain injury. Using a rodent model of closed head injury (CHI), we have established HA as an effective mediator of neuroprotection. By examining several post-injury end-points, it was demonstrated that HA animals display faster and better functional recovery as well as reduced secondary tissue damage. HA is therefore a unique model in terms of providing insight into physiologically-induced neuroprotection. However, although the overall beneficial effect was evident, the molecular mechanisms underlying it remained unclear. Our studies provide an in depth examination of the effects of HA on distinct molecular entities which are known to play a role in either adaptation to challenging surroundings or in CHI patho-physiology. Findings to date indicate that HA leads to the potentiation of endogenous protective mechanisms, including low molecular antioxidants and basal anti-inflammatory capacity, expression of neurotrophic factors, hypoxia inducible factor and erythropoietin receptor, as well as signaling via the latter. Furthermore, the attenuation of detrimental post-injury processes such as microglial activation and early proinflammatory cytokine transcription also occurs. Unraveling the physiological mechanisms of tolerance development could have profound implications for treatment in the setting of brain trauma, since future intervention strategies may be aimed at enhancing endogenous protective ability in a physiological-mimetic manner.

Alterations in rat brain mRNA levels following sarin-induced status epilepticus

V. Givant-Horwitz, I. Rabinovitz, J. Kapon and E. Gilat

Department of Pharmacology, Israel Institute for Biological Research

Ness-Ziona

Convulsions are common features of organophosphates (OP) intoxication. The initiation and the early phase of OP-induced seizures are predominated by cholinergic mechanisms and the propagation and the maintenance are mainly non-cholinergic.

In the present study, the changes in mRNA levels of acetylcholinesterase (AChE), choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD) 65 and 67, bax and bcl-2 were measured in rat hippocampus, 2h and 24h post sarin exposure, using RT-PCR. The effects of antidotal treatments including TMB-4 and atropine (TA) 1min post sarin, alone or with the addition of immediate (1min post sarin) or delayed (15 min post sarin) midazolam were evaluated as well.

Two hours following sarin exposure, the hippocampal mRNA levels of all the genes, except for GAD67, were significantly reduced in convulsive untreated animals. At 24h post sarin, significant increase in hippocampal mRNA levels of ChAT, GAD67 and bax, together with significant loss of body weight and poor physical conditions, were observed in the untreated rats. Similar results were obtained in rats treated with TA alone. When immediate midazolam was added, mRNA levels and clinical conditions were as in naïve animals. However, if midazolam was delayed, mRNA levels and clinical conditions varied.

These findings imply that in the hippocampus, 24h post sarin exposure, there are requirements for acetylcholine (ACh) and gamma-butyric acid (GABA) synthesis, and possible activation of apoptosis cascade, reflected by the increase in mRNA levels of ChAT, GAD67 and bax respectively. Similar indications for ACh and GABA requirements were obtained when inadequate treatment, TA alone, was used. Overall, these findings may lead to the development of novel postponed therapeutic protocols for cases of insufficient early treatments.

Network dynamics during the development and maintenance of seizures in Pilocarpine and Picrotoxin treated rats

Adi Cymerblit1 and Yitzhak Schiller, MD PhD.

Department of Physiology, Technion Medical School and Department of Neurology, Rambam Medical Center, Haifa

Rationale: Seizures are associated with hyper-synchronous electrical activity in the EEG recordings. However, the network dynamics at the single neuron level during the development and maintenance of seizures remains largely unknown. In this study we studied the firing behavior of single neurons, and monitored synchronization between the different recorded neurons during developing and ongoing seizures evoked by pilocarpine and picrotoxin.

Methods: Multi-electrode extra-cellular recordings of single units and local field potentials were performed from the hippocampus of anesthetized (urethane) and awake rats under control conditions and after administration of pilocarpine and picrotoxin. The two convulsants were administered either by systemic (I.P.) or local (intra-hippocampal) injection. In addition simultaneous intracellular whole-cell recordings were performed.

Results: Immediately after administration of pilocarpine and picrotoxin, prior to initiation of clinical or electrographic seizures, the frequency of spikes decreased, and synchronization between neurons, as measured by the cross-correlogram, markedly diminished or disappeared all together. In contrast after clinical and electrographic seizures initiated the frequency of spikes gradually increased, neurons tended to fire in bursts and the inter-neuron synchronization markedly increased. The cross-correlograms during the clinical seizure revealed higher values and wider time-windows as compared to control conditions. Similar results were obtained in anesthetized and awake rats; in systemic (I.P.) and local intrahippocampal administration of the convulsants, and with pilocarpine and picrotoxin. Simultaneous intracellular whole-cell recording from anesthetized rats demonstrated the intracellular correlate of developing seizures.

Conclusion: In this study we showed that during the development of pharmacologically induced seizures synchronization of firing between different neurons in the hippocampus decreased dramatically, while only later during clinical seizures inter-neuronal synchronization gradually increased. These findings may promote our understanding of the network dynamics responsible for seizure initiation and maintenance. Moreover, in future it may serve as the basis for detection of impending seizures in human patients.

Plasticity of the low-threshold calcium current in acquired epilepsy

Yoel Yaari

Department of Physiology, Hebrew University School of Medicine

A single episode of status epilepticus (SE) induced in rodents by the convulsant pilocarpine, produces, after a latent period of two or more weeks, a chronic epileptic condition. In its neuropathological, electroencephalographic and behavioral features, this condition is reminiscent of human temporal lobe epilepsy (TLE). During the latent period of epileptogenesis (within 3 days after SE), most rat CA1 pyramidal cells that normally fire in a regular pattern acquire low-threshold bursting behavior, generating high-frequency clusters of 3-5 spikes as their minimal response to depolarizing stimuli. A Ni²⁺-sensitive T-type Ca²⁺ current (I_{CaT}), shown to be upregulated after SE, plays a critical role in intrinsic burst generation in most cases. The I_{CaT} driving low-threshold bursting is located in the apical dendrites and is recruited by backpropagating somatic spikes. Of the three identified α_1 subunits generating I_{CaT} (Ca_v3.1, Ca_v3.2 and Ca_v3.3), only Ca_v3.2 (α_{1H}) is upregulated in SE-experienced CA1 pyramidal cells, implicating this channel subunit in the de novo emergence of intrinsic bursting. Intriguingly, knockout mice lacking Ca_v3.2 develop acute SE after pilocarpine injection just like their wild type counterparts, but are significantly less likely to undergo epileptogenesis and acquire TLE. Together, these findings identify Ca_v3.2 as a potential target for pharmacological and molecular treatments aimed at halting epileptogenesis during its latent phase.

Complex modulation of L-type Ca²⁺ channels by auxiliary subunits and calcium

Nathan Dascal

Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University

L-type Ca²⁺ channels are crucial for the contraction of cardiac and smooth muscle and play an important role in the regulation of excitability, growth and other functions in neurons. The main, pore-forming subunit of these channels, α_{1C} , is a large protein encoded by a multiexon gene (more than 50 exons). Alternative splicing gives rise to many isoforms that appear to be highly tissue-specific. However, the exact exon selection preferences in different tissues and the physiological consequences of isoform variability in α_{1C} are poorly understood. Especially relevant for this study are two isoforms: long-NT α_{1C} , with a 46-amino acid (aa) initial segment encoded by exon 1a, and a short-NT α_{1C} containing a 16-aa initial segment encoded by the alternative exon 1. The long-NT α_{1C} is found mainly in the heart but also in the brain, whereas short-NT isoforms prevails in smooth muscle and brain. We have previously discovered that the initial segment of the long-NT α_{1C} is an inhibitory module that reduces channel opening at all voltages, and its removal (by deletion) greatly increases Ca²⁺ or Ba²⁺ currents via heterologously expressed L-type channels, and reduces the enhancement of currents caused by coexpression of the channel's β subunit, Ca_v β . Using a series of mutants and chimeras of α_{1C} , we demonstrated that the only the cardiac but not the brain/smooth muscle isoform possess that NT inhibitory module. By measurements of channel currents, surface expression and single-channel properties, we found that the NT inhibitory module exclusively regulates a single function of the Ca_v β subunit: the increase in the channel open probability, P_o. The NT also regulates to some extent the voltage-dependent inactivation of the L-type channel, but the initial segment does not appear to be involved in this function. We and others found that the NT of α_{1C} contains binding sites for calmodulin (CaM) and for the neuronal Ca²⁺-binding protein 1, CaBP1, but the function of this sites is not known. Normally, CaBP1 counteracts the acceleration of CaM-dependent channel inactivation caused by Ca²⁺ influx. Deletions in NT appear to remove the effect of CaBP1. However, we found that they actually only shift the voltage dependency of components of Ca-dependent inactivation. It remains to be investigated how the NT regulates the different forms of inactivation in the L-type Ca²⁺ channel, and how this regulation is related to CaM and CaBP1.

Crosstalk between L-type calcium channels and ZnT-1, a new player in rate-dependent cardiac electrical remodeling

*Ofer Beharier**¹, Yoram Etzion^{#1}, Amos Katz[§], Hani Friedman**, Nir Tenbosh**, Saar Zacharish**, Sergiy Bereza[#], Uri Goshen** and Arie Moran***

******Department of Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva; **#**Cardiac Arrhythmia Research Laboratory & Internal Medicine Department E, Soroka University Medical Center, Beer-Sheva; **§**Cardiology Department Barzilai Medical Center, Ashqelon

Crosstalk between two membrane transport systems is an established mechanism underlying regulation. In this study, we investigated the interaction between ZnT-1, a putative plasma membrane zinc transporter, and L-type voltage-dependent calcium channels (LTCC). In the atrium of the myocardium decreased activity of the LTCC is a dominant feature of patients with atrial fibrillation. The trigger for this inhibition has been attributed to the rapid firing rates and consequent calcium overload in the atrial cardiomyocytes. However, the underlying mechanism of LTCC inhibition is still to be elucidated. Here, we showed that the expression of ZnT-1 inhibits the activity of L-type channels during electrical remodeling induced by rapid pacing. (i) Direct manipulations of ZnT-1 expression in cultured cardiomyocytes either by ZnT-1 overexpression or by ZnT-1 silencing with siRNA, decreased or enhanced, respectively, the barium influx through the LTCC. Co-expression of ZnT-1 with LTCC in *Xenopus oocytes* decreased whole cell barium current through LTCC. (iii) Rapid pacing of cultured cardiomyocytes (4 h, 100 ms cycle) increased ZnT-1 protein expression and inhibited the voltage-dependent divalent cation influx through the LTCC. (iv) Atrial pacing of anesthetized adult rats (4 h, 50 ms cycle) led to a significant increase in atrial ZnT-1 protein expression in parallel with the typical decrease of the refractory period in the atria. Taken together, these findings demonstrate that crosstalk between ZnT-1 and the L-type calcium channels may underlie atrial response to rapid pacing, suggesting that ZnT-1 is a significant participant in rate dependent cardiac electrical remodeling.

Regulation of parathyroid hormone (PTH) gene expression: The sensing and response to serum calcium and phosphate

Tally Naveh-Many

Minerva Center for Calcium and Bone Metabolism, Nephrology Services,
Hadassah – The Hebrew University of Jerusalem, Medical Center

Parathyroid hormone (PTH) regulates serum calcium (Ca^{2+}) and phosphate (P) levels and bone strength. Ca^{2+} and P in turn regulate PTH gene expression, PTH secretion and parathyroid cell proliferation. Extracellular Ca^{2+} concentrations are sensed by a parathyroid G-protein coupled 7 transmembrane calcium sensing receptor (CaR). The CaR activates a signal transduction pathway that mediates the response of the parathyroid cell to extracellular Ca^{2+} . We have shown that the regulation of PTH gene expression by serum Ca^{2+} and P is post-transcriptional due to changes in mRNA stability. This regulation is mediated by the binding of parathyroid cytosolic *trans* acting factors to a defined *cis* instability element in the PTH mRNA. A low serum Ca^{2+} results in increased PTH mRNA levels in vivo in the rat, increased protein-PTH mRNA binding and increased PTH mRNA stability. A low P has the opposite effects. We have identified two *trans* acting proteins, AU rich binding factor 1 (AUF1) and Up stream of N-*ras* (Unr) that bind a specific conserved *cis* element in the PTH mRNA 3'- untranslated region (UTR). The *cis* element is both necessary and sufficient for the regulation of PTH gene expression by Ca^{2+} and P. PTH mRNA decay involves endo- and exo-ribonucleases which sequentially cleave the PTH mRNA. The degradation machinery that cleaves PTH mRNA is differentially recruited to the PTH mRNA in response to changes in Ca^{2+} and P, thus determining PTH mRNA stability and levels.

Calcium activated PLA2, PKC and calpain converge to generate the transformation of a cut axonal end into a motile growth cone

Micha E. Spira and Hadas Erez

Department of Neurobiology. Institute of Life Sciences. The Hebrew University of Jerusalem

Growth cone formation is a critical step in the cascade of events leading to regeneration after injury. On line confocal imaging of the free intracellular calcium concentration ($[Ca^{2+}]_i$), microtubules, the actin network, anterograde and retrograde axonal transport, complemented by pharmacological perturbations, electron microscopy and electrophysiology revealed that: the activation of the calcium-dependent cysteine protease- calpain, calcium-activated protein kinases and phosphates, and calcium-activated phospholipase A2 converge in time and space to activate different molecular and cellular cascades that are necessary to orchestrate the complex structural and functional reorganization of the cut axonal end into a motile GC. In the presentation we will describe the cellular transformation of a differentiated axonal segment into a motile growth cone (GC) and the role of $[Ca^{2+}]_i$ in orchestrating the cascade.

The endocannabinoid system in the regulation of feeding: Important for adults, critical for newborns*

Ester Fride

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The potential of the marihuana (*Cannabis sativa*) plant to stimulate appetite has been known for many years. In recent years, the receptor mechanisms by which the active cannabis component Δ^9 -tetrahydrocannabinol (THC) and the endogenous cannabinoids ('endocannabinoids') regulate appetite, food intake and body weight, have been explored. Thus activation of the cannabinoid CB₁ receptor enhances appetite and food intake, while blockade of the CB₁ receptor does the opposite, resulting in appetite- and weight-loss. In a series of clinical trials, the CB₁ receptor antagonist/inverse agonist SR141716 (rimonabant), successfully reduced body weight in obese subjects without serious side effects. Based on these findings we have developed two mouse models which use CB₁ receptor antagonism in order to reduce appetite and weight gain:

A) CB₁ receptor antagonism to prevent antidepressant-induced weight gain: Long-term treatment with antidepressants induces, after an initial reduction in body weight, undesirable weight gain. This in turn, puts the patient at risk for conditions such as coronary heart disease and treatment noncompliance. We have explored co-treatment of antidepressants with CB₁ receptor blockade (with rimonabant). Since endocannabinoids and the CB₁ receptor are also involved in mood regulation, we also investigated whether rimonabant interfered with the activity of the antidepressants. Acutely, female (Sabra) mice received rimonabant (5 mg/kg) prior to the antidepressants fluoxetine (20 mg/kg) or desipramine (15 mg/kg), after which they were tested in the 'forced swim test' for antidepressant effects. Chronically, rimonabant (2 mg/kg) and desipramine (5 mg/kg) were injected daily for 3 months. Body weight and performance in the forced swimming assay were assessed throughout treatment. Results: 1. Rimonabant did not interfere with the 'anti-depressant' effects of desipramine or fluoxetine. 2. The initial weight loss in desipramine-treated mice was reversed after several weeks of treatment, developing into significantly greater weight gain compared to controls. Rimonabant and desipramine together, prevented the weight gain induced by desipramine alone while the antidepressant effect of desipramine or of desipramine+rimonabant was preserved throughout chronic treatment. Thus, we suggest that rimonabant may be used as an adjunct to anti-depressant treatment with the aim to prevent excessive weight gain.

B) CB₁ receptor antagonism in newborns as a model for 'non-organic failure-to-thrive' (NOFTT): Based on our finding that endocannabinoids are present in maternal milk, while CB₁ receptors were found in newborn brain tissue, we have demonstrated that blockade of CB₁ receptors (with rimonabant) within 24 h of birth in mice, dramatically interferes with the ability to suckle milk and hence to develop and thrive; co-injection with a CB₁ receptor agonist prevented the devastating effect on the pups. Subsequently we demonstrated that the adverse effect of CB₁ receptor blockade can be ascribed to a selective oral-motor defect, similar to that seen in human infants with NOFTT. Thus we conclude that impaired functioning of the Endocannabinoid-CB₁ receptor system may constitute the organic basis of NOFTT. These findings open new doors to the development of novel therapeutic strategies to treat NOFTT.

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Control of feeding in *Aplysia* with steady-state access to food

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The patterning of feeding and the quantity eaten in *Aplysia californica* with *ad libitum* food access cannot be explained by the effects of three variables previously shown to control the patterning of consummatory feeding responses and the quantity eaten in animals hand-fed individual meals. Feeding in *ad libitum* conditions is regulated primarily by varying the time between feeding bouts, rather than by modulating bout lengths or the efficacy of consummatory movements within a bout. *Aplysia* with steady-state food access are in a newly characterized feeding state in which they are relatively unresponsive to food. They eat very little (1-4% of the time), and the quantity eaten is unrelated to the quantity of food in the anterior gut. The steady-state can be maintained by the presence of food, even if animals do not contact food. The chemosensory rhinophores signal the presence of food that maintains the steady-state. Up to 24 hours without food is needed for animals to recover from the inhibition of feeding by steady-state presence of food. Recovery from the steady state is partially governed by post-ingestion stimuli, as shown by a faster recovery in animals that have not been in contact with food. Inhibition of feeding during the steady-state is mediated in part via humoral factors, since bathing the cerebral and buccal ganglia in hemolymph from animals in the steady state inhibits the ability to elicit buccal motor programs via a cholinomimetic thought to simulate stimulation of the lips with food. After food deprivation that is sufficiently long so that the steady-state decays, animals eat a large meal whose size and dynamics are consistent with regulation via the three variables previously identified. This large meal is modulated by pheromones secreted by conspecifics even in sexually immature *Aplysia*.

Examination of the emergence of obesity from birth to adulthood in the OLETF rat model

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OLETF rats do not express functional cholecystokinin type 1 receptors and represent a well-recognized model of obesity that has been lately used to study the neurobiology of obesity. Adult OLETF males are hyperphagic, obese and eventually become diabetic. To examine the early origins of obesity in this model, we measured body-weight, intake from lactation and during independent ingestion, fat-pad distribution, leptin levels, and central NPY/POMC mRNA expression in OLETF and LETO (control) males from postnatal day 1 until young adulthood day 65. Compared to controls, OLETF rats 1) were significantly heavier beginning at birth, 2) ate more in independent ingestion tests, 3) spent more time nursing, showing increased initiative to start nursing episodes and increased weight gain after nursing, 4) gained weight more dramatically from the third postnatal week, accumulating significantly more retroperitoneal and inguinal white adipose tissues from the post weaning period and on and more epididymal white fat only in adulthood, 5) displayed increased leptin levels from weaning. 6) analysis of the mRNA of NPY (orexigenic peptide) and POMC (anorexigenic peptide) in hypothalamic brain sections showed that POMC was up-regulated in the arcuate nucleus from day 23 and on and NPY was up-regulated as early as PND 15 and 23 in the dorsal motor nucleus of OLETF pups, while arcuate NPY levels were not different from controls. The results suggest that OLETF rats present phenotypical pre-obese characteristics. The elevated NPY gene expression in the dorsal motor hypothalamus during the early period of development may play an etiological role in the hyperphagia and obesity of OLETF rats.

Models of anorexia

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Anorexia nervosa (AN) is a potentially life threatening eating disorder of unknown origin for which no effective drug treatment is currently available. It occurs predominantly in women in adolescence and is characterized by severe low weight, cognitive distortions about body shape and weight and amenorrhea. AN is the leading cause of death from a psychiatric disorder among women. It begins with “harmless” attempts at dieting which gets out of control. The patients think themselves to be too fat even when severely underweight. Understanding the interaction between severe reduction of body weight and cognitive function may lead to new strategies for the treatment of anorexia.

Development of an animal model (AM) has been difficult since its etiology of AN is multifactorial and involves a complex of environmental, genetic, cultural and social factors. Furthermore, no animal will starve to death when food is available. Three kinds of animal models have been developed in our laboratory: Diet Restriction (DR), Activity Wheel and Separation Stress, each of which mimics some aspects of the human disease. DR may benefit or impair animal cognition or motor performance depending on the degree of restriction. DR to 60% improved maze performance whereas 40% DR impaired it and was associated with high mortality. Our hypothesis is that part of the disease is caused by a deficiency in essential diet-derived nutrients responsible for brain activity. We have studied the effects of tyrosine as a catecholamine precursor, and endocannabinoids, derived from essential fatty acids of the n-6 series, as neuromodulators. We have found that severe DR impaired the adrenergic, cholinergic, serotonergic and opiate systems while tyrosine almost reversed the effects.

A major system known to be involved in the regulation of appetite and feeding behavior is the endocannabinoid system. DR to levels between 60% and 40% for 12 days lowered the levels of the endocannabinoid 2-arachidonoylglycerol (2-AG), in the hypothalamus and hippocampus, whereas full short time starvation elevated 2-AG levels. It seems possible that these time-dependent variations of 2-AG levels may be of importance as a general coping strategy by animals during periods of starvation.

We have investigated the effect of very low doses (without cannabinomimetic side effects) of the synthetic cannabinoids Δ^8 -THC, anandamide and 2-arachidonoylglycerol-ether (noladin) on food consumption, cognitive function, and neurotransmitter levels in mice as potential therapeutic effect in anorexia.

Tyrosine or EC supplementation to the 40% DR mice improved cognitive function and brain neurotransmitters *without increasing body weight*. Such a strategy might break the vicious cycle in initiating treatment in patients with AN. Patients sometimes will not respond to supportive and psychological treatment before there is nutritional rehabilitation, which they so strenuously resist because of the inevitable weight gain. DR was also found to increase brain enkephalin and dynorphin-like immunoreactivity. Such involvement of the opiate and EC systems might help understand *what "pleasure" anorectic patients might get in the place of eating*. Following these results we are currently undertaking clinical trials of ECs and tyrosine in the treatment of AN.

Effects of renin-angiotensin-aldosterone system and other vasoactive substances on the vasculature

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Vasoconstrictor neuro-humoral systems play a key role in the pathophysiology of hypertension, a growing medical problem in the western world with significant morbidity and mortality. Understanding the pathophysiologic mechanisms of this disease and its complications is therefore imperative for the development of new therapies. Pathologic activation of the circulatory and local renin angiotensin aldosterone system (RAAS) has deleterious effects on the vasculature, heart, and the kidneys. Indeed, ACE inhibitors and angiotensin blockers effectively reduce high blood pressure and exert cardio- and reno-protective actions. Moreover, the second active component of the RAAS, i.e., aldosterone has an important role in the pathophysiology of cardiovascular diseases, including hypertension and CHF.

The mechanisms underlying the adverse effects of aldosterone include induction of fibrosis, inflammation, and oxidative stress, and it has been recently shown that eplerenone and aldactone, aldosterone antagonists, improved cardiovascular function and survival rates and reduced blood pressure, and myocardial infarction most likely by reducing oxidative stress and atherogenesis. While the role of the RAAS and the sympathetic nervous system in the pathogenesis of cardiovascular diseases is well established, the contribution of the endothelin (ET) system has not been thoroughly studied. The biological effects of endothelin-1 on its target organs are mediated by two receptors: ETA and ETB. It is widely accepted that the vascular, cardiac, and renal adverse effects of ET-1 are mediated by ETA, while activation of ETB receptors leads to beneficial effects such as attenuation of the vascular and cardiac hypertrophic effects of ET-1 as well as the vasodilatory action of this peptide. Several experimental and clinical studies have revealed that ET-1 antagonists are beneficial therapeutic agents for the treatment of several cardiovascular diseases, including pulmonary hypertension, CHF, and cancer.

Neurohormonal influences on the vascular tree

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The vascular-tree function is critical for normal tissue perfusion. In order to transfer blood effectively, blood vessels should react to physical and biological forces. To do so, blood vessels are under continuous regulation of several neurohormonal systems which affect, among other things, the arterial tone, diameter, and biological properties such as inflammation and coagulation. These systems, detailed below, have direct effect and mutual influence that collectively ensure intact circulation.

The central nervous system plays an important role with regards to blood vessels tone, primarily through the sympathetic nervous system (SNS). The SNS itself is the principal regulator of the arterial pressure and is under close inter-relation with the baroreflex apparatus, the renin-angiotensin system and paracrine-vasoactive substances such as endothelin and nitric oxide. Additional neurohormonal systems that play a role in blood pressure regulation include adrenomedullin and calcitonin-gene related peptide.

In pathologic processes, such as hypertension, diabetes and hyperlipidemia, structural changes take place which eventually compromise the vascular physical and biological potency. This vascular remodeling in turn is the basis for future atherosclerotic-cardiovascular endpoints.

Endothelial function under elevated blood pressure

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Endothelial dysfunction is a systemic disorder and a key variable in the pathogenesis of atherosclerosis and its complications. Endothelial dysfunction reflects a vascular phenotype prone to atherogenesis and may therefore serve as a marker of inherent atherosclerotic risk. It is well known that flow-mediated endothelium-dependent vasodilation (FMD) is impaired in forearm vasculature of hypertensive patients, accounting for an increase in vascular resistance and vascular structural changes. Factors that impair endothelial function are believed to be associated with increased arterial stiffness. The large conduit vessels possess a significant muscular component, which is partially under the control of the endothelium. Therefore, changes in endothelial function can alter the mechanical properties of the large arteries and result in increased stiffness. Prior studies have demonstrated that endothelial function may contribute significantly to measure of central conduit vessel stiffness given the significant correlations between FMD and proximal aortic compliance, central pulse pressure, and central systolic pressure. In a subset of 1096 participants in the Framingham Heart Study FMD was inversely related to systolic blood pressure. Nevertheless, other studies indicate that endothelial dysfunction may contribute to the pathogenesis of essential hypertension by offsetting the balance between vasodilator and vasoconstrictor forces on vasculature. This abnormality, probably multifactorial, is mainly related to decreased nitric oxide (NO) bioavailability that may follow a reduction of NO synthesis and/or its increased inactivation by oxidative stress. According to the response-to-injury theory, the currently prevailing pathogenic concept of atherosclerosis, endothelial dysfunction is both an initial and integral part of the inflammatory-proliferative disease process in response to cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and smoking. Endothelial cell dysfunction is, therefore, both a consequence and a mediator of the pathophysiologic action of cardiovascular risk factors. Thus, endothelial dysfunction, which is characterized by reduced FMD and proinflammatory, proliferative, and procoagulatory properties may be considered the initial modification, present in patients with essential hypertension and other cardiovascular risk factors, that promotes the coronary and extracoronary atherosclerosis.

Measures of arterial stiffness correlate significantly with those of endothelial function. An increase in large conduit vessel stiffness may represent either a cause or consequence of endothelial dysfunction and may explain why elevated pulse pressure is a new cardiovascular risk factor. Brachial artery vasoreactivity testing (BRT) is a non-invasive technique used to evaluate FMD, an endothelium-dependent function.

Our group has recently demonstrated a significant inverse correlation between systolic blood pressure or pulse pressure and FMD in 1038 consecutive healthy subjects, suggesting a potential mechanism whereby elevated pulse pressure contributes to cardiovascular disease. As outlined by previous studies, this link might relate to a decrease in NO bioavailability, resulting in both impairment in endothelial function and increased arterial stiffness. Therefore, it is not surprising to find that increased pulse pressure and endothelial dysfunction often coexist in hypertensive patients. Based on clinical data, long-term follow-up is warranted to elucidate the incidence of cardiovascular disease in subjects with endothelial dysfunction induced hypertension and hypertension induced endothelial dysfunction.

Clinical application of vascular biology on elevated blood pressure

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Hypertension is one of the leading risk factors for cardiovascular (CV) morbidity and mortality. Lowering blood pressure can reduce the risk of CV diseases. Several mechanisms are involved in maintaining normal blood pressure, among them the most known are the sympathetic nervous system, the renin angiotensin system, the balance between vasoactive and vasodepressor peptide, and salt and volume. Over activity of some pathways and under activity of others may cause hypertension. Agents that interfere with these altered mechanisms may reduce blood pressure and reduce the risk of CV disease. However, blocking one pathway leads to over activation of the alternative pathways leading to attenuation of blood pressure reduction. It seems that not all pathways contribute equally to end organ damage. Blocking the sympathetic nervous system by either alpha or beta blockers lower blood pressure but is less effective in preventing congestive heart failure or stroke. Blocking the renin angiotensin system seems to confer benefit beyond the expected from lowering blood pressure. Vasopeptide modulation was tried in hypertensive patients but still not available in practice. Volume depletion is still the gold standard in hypertension and should be involved in most regimens. Drugs that improve insulin resistance may lower blood pressure but are not included in the armamentarium of blood pressure lowering agents. The complexity of the mechanism involved in the pathogenesis of hypertension justifies using various agents that act on different mechanisms and thereby enabling lowering blood pressure effectively.

The biological clock, food, and nutrition

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Robust biological rhythms have been shown to affect life span. Biological clocks can be entrained by two feeding regimens, restricted feeding (RF) and caloric restriction (CR). RF restricts the time of food availability, whereas CR restricts the amount of calories with temporal food consumption. CR is known to retard aging and extend life span of animals via yet unknown pathways. We hypothesize that resetting the biological clock could be one possible mechanism by which CR extends life span. As it is experimentally difficult to uncouple calorie reduction from temporal food consumption, we took advantage of the transgenic mice α MUPA over-expressing a serine protease implicated in brain development and plasticity that exhibit spontaneously reduced eating and increased life span. Quantitative real-time PCR analysis revealed that α MUPA mice exhibit robust expression of the clock genes *mPer1*, *mPer2*, *mClock*, and *mCry1*, but not *mBmal1* in the liver. We also found changes in the circadian amplitude and/or phase of clock-controlled output systems, such as feeding behavior and body temperature. A change in the light/dark regimen led to modified clock gene expression and abrogated circadian patterns of food intake in WT and α MUPA mice. Consequently, food consumption of WT mice increased, whereas that of α MUPA remained the same, indicating that reduced food intake occurs upstream and independently of the biological clock. Thus, we surmise that CR could lead to pronounced and synchronized biological rhythms. As the biological clock controls mitochondrial, hormonal, and physiological parameters, system synchronicity could lead to extended life span.

Molecular underpinnings of socially modulated plasticity in circadian rhythms in the honey bee *Apis mellifera*

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Keywords: honey bee, circadian rhythms, division of labor, plasticity, clock gene

Honey bee larvae require constant care and young “nurse” bees work around the clock with no circadian rhythms to provide it; older foragers have strong circadian rhythms that are used for sun compass navigation, dance communication, and for timing visits to flowers. To explore the molecular bases of this naturally-occurring behavioral plasticity, we cloned orthologues for *Drosophila* and mammals clock genes and measured (with real time-PCR) their mRNA levels in whole brains of foragers and nurses entrained in a 12 hrs light: 12 hrs dark (LD), illumination regime and collected in LD or in constant darkness. The honey bee genome encodes only a mammalian-type *Cry* and does not contain an ortholog to *dTim*, an indispensable component of the *Drosophila* clock. Brain *amPer* and *amCry* mRNA levels vary profoundly during the day in foragers but not in nurses under both LD and DD illumination regimes. However, bees that nursed brood with no circadian rhythms in the colony manifested significant circadian rhythms when removed from the colony and monitored individually. These results suggest that some important features of the honey bee clockwork are more similar to mammals than to *Drosophila*. Task-related plasticity in circadian rhythms depends on the social context and is mediated by altered pattern of brain clock gene expression. These findings link social interactions between individual bees (division of labor) to intricate molecular processes in the brain.

Functional development of the circadian clock: A study using the zebrafish model

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The zebrafish pineal gland is a photoreceptive organ containing an intrinsic central circadian oscillator which drives daily rhythms of the melatonin hormonal signal. Rhythms of gene expression in the pineal gland are the earliest detected circadian rhythms in zebrafish; appearing as early as the second and third day of development. Light exposure is mandatory for the development of the pineal circadian clock. Light induces the expression of *Period2 (Per2)* through a light-responsive enhancer in the *Per2* promoter. And, knockdown of PER2 abolishes rhythms. Thus, light-induced *Per2* expression is an important event in the development of the pineal circadian clock.

Interestingly, light treatments at early developmental stages, prior to pineal gland formation, also facilitated the development of the pineal circadian clock. These results suggest that early embryonic cells possess independent circadian oscillators. Light exposure entrains or synchronizes these oscillators and the 24-h rhythm is then maintained throughout development. The implication is that the circadian oscillator is maintained during a period of rapid proliferation and, remarkably, differentiation, that take place during development.

The effects of melatonin on behavior and brain activity in humans: Fmri studies

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Melatonin, the hormone produced nocturnally by the pineal gland, is an endogenous regulator of the sleep-wake cycle but its mode of action is largely unknown. The effects of melatonin on human brain activities and their relation to subjective measurements of sleepiness were studied in randomized, double-blind, placebo controlled functional magnetic resonance imaging (fMRI) studies performed in the afternoon (namely when it is not present endogenously) and evening (with relation to endogenous levels of salivary melatonin). Melatonin given in the afternoon, reduced task related activity in the rostro-medial aspect of the occipital cortex and in the auditory cortex compared to placebo; these effects were associated with increased fatigue. In addition, melatonin enhanced the activation in the left parahippocampus. These changes resembled changes in brain activities seen during actual sleep but not after sleep deprivation.

In the evening, subjects with elevated endogenous melatonin levels reported greater subjective fatigue and their brain activation patterns partly resembled those of subjects given exogenous melatonin in the afternoon. Melatonin administered in the evening had no behavioral effect and little effects on activation in the rostro-medial aspect of the occipital cortex and the auditory cortex. However, hippocampal activation was reduced with melatonin in subjects with initial high endogenous melatonin levels in the evening. Altogether these studies demonstrate a major role for melatonin in circadian sleep regulation and modulations of brain activity in sleep-related brain areas. Furthermore, an effect of melatonin on hippocampal activity is demonstrated which may be important in sleep associated memory consolidation processes.

Death by design: Mechanism and regulation of apoptosis

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Essentially all animal cells have the ability to kill themselves by activating a gene-encoded cell suicide program. The execution of this self-destruction program leads to a morphologically distinct form of cell death termed apoptosis. Apoptosis plays an important role in sculpting the developing organism and eliminating unwanted and potentially dangerous cells throughout life. The decision of whether a particular cell will live or die is tightly regulated by many different signals originating both from within the cell and from its environment, and abnormal regulation of apoptosis is associated with a variety of diseases, including cancer, autoimmune diseases, stroke and neurodegenerative disorders. The overall goal of our research is to elucidate the precise mechanism by which cells undergo apoptosis and how this process is regulated by diverse signaling pathways.

In the past, most of our work utilized a highly accessible model organism, the fruit fly *Drosophila*, which offers unique advantages for the discovery of novel cell death genes using powerful genetic techniques. From surveying a large fraction of the *Drosophila* genome for genes that are required for programmed cell death, three apoptotic activators, termed *reaper*, *head involution defective (hid)*, and *grim* were identified. All three genes are necessary and sufficient for the activation of apoptosis in *Drosophila*. Significantly, *reaper*, *hid* and *grim* are all transcriptionally regulated by a variety of death-inducing stimuli and act as integrators for relaying different apoptotic signals to the core death program.

Reaper, Hid and Grim kill by inhibiting the anti-apoptotic activity of inhibitor of apoptosis proteins (IAPs). IAPs can block cell death by inhibiting caspases, a family of proteases that are key executioners of apoptosis. The active forms of Reaper, Hid and Grim can bind to IAPs and prevent them from caspase inhibition. Reaper-family proteins can also promote auto-ubiquitination and self-destruction of IAPs, thereby irreversibly removing a key “brake” on death. These results suggest a novel strategy for the selective elimination of tumor cells that express elevated IAP protein levels.

Since the mechanism of apoptosis has been conserved in evolution from worms to insects to man, knowledge gained from studying cell death in *Drosophila* is likely to apply to mammalian systems as well. In order to test this directly, we have initiated several mammalian cell death projects to study the regulation of apoptosis in the mouse, and the contribution of abnormal regulation of apoptosis to human diseases. For example, we have generated mice that lack a mammalian IAP-antagonist termed ARTS. These mice display elevated XIAP protein in certain tissues and have defects in the caspase-mediated elimination of bulk cytoplasm during spermiogenesis, resulting in male sterility. Furthermore, ARTS-deficient mice develop spontaneous tumors, in particular hematopoietic malignancies. Remarkably, these mice have increased numbers of stem cells at a very young age, well before the onset of any malignancies. These observations suggest that loss of ARTS promotes tumorigenesis by increasing the number of normal stem cells through reduced cell death. Therefore, loss of ARTS may be a defining property in the transition from a normal stem cell to a cancer stem cell.

Abstracts of Student Lecture Competition

Propargylamine derivatives provide therapeutic efficacy in *in Vitro* and *in Vivo* experimental models of heart diseases

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Based on the premise that neurons and cardiac myocytes share similar mechanisms of cell death, we tested the hypothesis that the neuroprotective propargylamine derivatives (of which rasagiline was recently approved for Parkinsonian patients), TVP1022 (N-propargyl-1-S-aminoindan) and propargylamine, can provide protection against myocardial damage and death caused by several provocative stimuli, including volume-overload and those mimicking myocardial ischemia. The hypothesis was tested in H9c2 cardiac cell line, neonatal rat ventricular myocytes (NRVM), naive rats and rats with volume overload-induced congestive heart failure (CHF) created by aorto-caval fistula. Our main findings were: (1) In embryonic rat heart cell line H9c2, Fas-mediated apoptosis was prevented by TVP1022 and propargylamine. (2) In NRVM, Fas-mediated hypertrophy was blocked by TVP1022 and propargylamine. (3) In naive rats, three weeks of treatment with propargylamine increased the Bcl-2/Bax ratio by 200% and mitochondrial PKC ζ by 125% (4) In CHF rats, after 3 weeks of treatment (1 week before surgery and 2 weeks after surgery) with TVP1022, mitochondrial PKC ζ was increased by 50%, cytosolic cytochrome C was reduced by 70% and mitochondrial Bax was reduced by 35%, compared to untreated CHF rats. (5) Importantly, TVP1022 and propargylamine completely prevented the hypertrophy as well as the reduction in fractional shortening (determined by echocardiography) in CHF rats. In conclusion, the molecular mechanisms underlying the cardioprotective efficacy of the propargylamine derivatives tested are very similar to those described for neuroprotection. Based on these findings, we propose that propargylamine derivatives should be considered as potential therapeutic agents for preventing and treating cardiovascular disorders.

*These authors share equal contributions.

Leptin resistance in diet-induced obese golden spiny mice (*Acomys russatus*)

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The golden spiny mouse is a desert-dwelling omnivore that does not store food. When food is plentiful it accumulates fat and develops diet-induced obesity, during which plasma leptin levels are significantly correlated with body fat content. The goal of the current study was to determine whether golden spiny mice are leptin resistant. We thus compared the responses of young thin golden spiny mice (ca. 10 weeks old, 42.2 ± 0.56 g, 19.7 ± 1.60 % body fat) with those of mature fat mice (93.7 ± 4.07 g, 37.8 ± 1.98 % body fat), both given exogenous leptin (mouse leptin, R&D) or saline infusion for 14 days (using Alzet osmotic minipumps model 1002), followed by 14 days of recovery.

When treated with leptin, the young golden spiny mice significantly reduced their food intake ($P < 0.05$) and did not gain weight, while saline-treated young mice did not change their food intake, and significantly gained weight ($P < 0.05$). Body mass of the leptin-treated young mice caught up with that of the saline-treated mice during the recovery period, during which no significant difference in food consumption between the two groups was found. In the mature fat golden spiny mice there was no significant effect of leptin treatment on body mass or food intake, nor was there a significant difference between the leptin-treated and saline-treated mice. We conclude that diet-induced obesity in golden spiny mice is coupled with leptin resistance, and suggest that these animals may serve as a valuable model for the study of diet-induced obesity.

The effect of extremely low frequency and amplitude magnetic fields on the cycle of cardiac myocytes

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We applied magnetic fields at frequencies 8Hz, 15Hz, 15.5Hz, 16Hz, 16.5Hz and amplitudes of the magnetic field of 16pT, 160pT, 1.6nT, 16nT, 160nT to neonatal rat cardiac myocytes in cell culture. We observed that the spontaneous activity of the myocytes changed at frequency at 16Hz and at 8Hz : the height of cytosolic calcium spikes began decreasing about 2 minutes after the radiation was applied and kept decreasing significantly during 30 minutes. About 10 minutes after radiation was discontinued the spontaneous activity resumed. Outside this range of frequencies no changes in spiking was observed. We discuss a possible theoretical explanation of this phenomenon in terms of stochastic resonance between the gating charge of the slow delay rectifier potassium channel (I_{Ks}) and the radiation. The stochastic resonance increases the open probability of the I_{Ks} channel during the plateau of the action potential in the myocyte, and thus changes its conductance in the Hodgkin-Huxley model. This, in turn, changes all currents during the cycle of the myocyte in the Luo-Rudy model. We strengthened our theoretical explanations by applying similar magnetic fields on (I_{Ks}) channels expressed on oocytes. The potassium current was increased by about 10-20% due to the magnetic exposure.

Heat acclimation-induced neuroprotection involves changes in Bcl-2 family mRNA expression and requires the phosphorylation of Akt

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Long term exposure to ambient heat (heat acclimation, HA) has been shown to be an effective means of inducing neuroprotection in a model of closed head injury (CHI). Previous observations indicated that HA mice also display a post-CHI increase in the Akt phosphorylation. Given the well recognized role of this kinase as a regulator of anti-apoptotic pathways, it was hypothesized that HA-induced neuroprotection may include an anti-apoptotic component. Post-CHI Akt phosphorylation was selectively inhibited in HA (30 days, 34±1°C) and normothermic (NT) mice in order to determine whether this process is indeed essential for the development of the neuroprotected phenotype. Mice were treated at 1h post-CHI with tricyclic nucleoside (TCN), a selective inhibitor of Akt phosphorylation and then subjected to evaluations of tissue edema formation, motor ability recovery and cognitive performance. In addition, mRNA levels of anti-apoptotic (Bcl-X_L) and pro-apoptotic (Bax and Bad) Bcl-2 factors were quantified by real-time PCR in brain segments from untreated HA and NT sham, 6h and 12h post-CHI mice. TCN treatment abolished the beneficial effects of HA on cerebral edema and object recognition test performance at 3d. Furthermore, at 7d post-CHI a deterioration in the HA group cognitive performance was observed. HA mice consistently tended to score lower on the motor ability scale and did significantly worse than NT when overall recovery over time was compared. NT recovery was unaffected by TCN. HA elicited differential effects on each of the examined Bcl-2 factors and altogether favored preservation of a constant BclXL/Bax*Bad ratio. HA mice displayed a lower basal ratio than NT, yet this ratio was maintained constant following CHI, while a robust decrease was observed between 6h and 12h post-CHI in NT. Taken together, the changes observed in Bcl-2 ratio and the necessity of Akt phosphorylation imply that anti-apoptotic effects may well be involved in HA-induced neuroprotection.

Abstracts of Posters

Poster # 1

The expression level of the voltage-dependent anion channel 1 controls life and death of the cell

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Mitochondria play a crucial role in apoptosis, with the voltage-dependent anion channel (VDAC) being an important player element. VDAC, at the outer mitochondrial membrane, serves as a controlled passage for adenine nucleotides, Ca^{2+} , and other metabolites into and out of mitochondria, and also functions as a docking site for cellular kinases, such as hexokinase.

In this study, the role of VDAC1 in regulating cell survival and death was investigated by silencing endogenous human VDAC1 (hVDAC1) expression using a short hairpin RNA (shRNA)-expressing vector, and by controlled murine VDAC1 (mVDAC1) expression. The shRNA effectively down-regulated the expression of hVDAC1 but not of mVDAC1 in human T-REx-293 cells. The stably expressing hVDAC1-shRNA-T-REx-293 cells proliferated extremely slowly, relative to untreated cells. Normal growth was, however, restored upon expression of mVDAC1 in a tetracycline-regulated manner. While low tetracycline concentrations promoted cell growth, high concentrations induced mVDAC1 over-expression, leading to cell death. hVDAC1-shRNA-T-REx cells contained low ATP and ADP levels and showed 4-fold lower ATP synthesis capacity, suggesting limited metabolite exchange between mitochondria and cytosol. hVDAC1-shRNA-T-REx cells expressing either native or E72Q-mutated mVDAC1 underwent apoptosis induced by various stimuli. Ruthenium red protected against the apoptotic effect of these stimuli in the native but not in the mutated cells, suggesting that VDAC1 regulates apoptosis independently of the apoptosis-inducing pathway, and may function as a checkpoint between cell life and death.

Poster # 2

The N-terminal of VDAC1 is required for activation of apoptosis

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Mitochondria serve a variety of functions in cell life, including ATP synthesis and Ca^{2+} signaling. In addition, mitochondria are potent integrators and coordinators of apoptosis via mediation of cytochrome c release. The mechanisms by which apoptotic initiators, including cytochrome c, are released from the mitochondria remain, however, unclear. It is well accepted that the voltage-dependent anion-selective channel (VDAC) is a component of the release pathway. VDAC supports transport of adenine nucleotides, Ca^{+2} and other metabolites across the outer mitochondrial membrane. According to proposed transmembrane topology models, VDAC1 secondary structure is comprised of a 13-strand β -barrel and a single α -helix. In this study, we addressed the role of the N-terminal α -helix of VDAC1 in apoptotic regulation. Human embryonic kidney cells, silenced for endogenous human VDAC1 by short hairpin RNA (shRNA), proliferated extremely slowly in comparison to normal cells. However, normal growth could be restored by expressing murine VDAC1. The results show that N-terminal truncated-mVDAC1 restored cell growth, mitochondrial Ca^{2+} accumulation and ATP levels, suggesting that the N-terminus is not essential for the transport activities of VDAC1. Surprisingly, $\Delta(1-26)$ mVDAC1 expressing cells were resistant to mitochondria-mediated apoptosis as induced by its overexpression or chemically by staurosporine, curcumin or As_2O_3 . These results demonstrate that the VDAC1 N-terminus is required for VDAC1-mediated apoptosis, but not essential for the normal function of VDAC1. Moreover, it appears that VDAC1 is a key player in apoptosis, regardless of the inducer and the mechanism by which it induces cell death.

Poster # 3

New fluorescent reagents specific for Ca²⁺-binding proteins

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Ca²⁺ carries information pivotal to cell life and death via its interactions with specific sites in proteins. Although numerous Ca²⁺-dependent activities are known, many proteins responsible for these cellular activities remain unidentified. In our laboratory, a novel photoreactive reagent, AzRu, was synthesized and characterized. This reagent specifically interacts, and upon UV irradiation, irreversibly binds to calcium-binding proteins, strongly inhibiting their Ca²⁺-dependent activities.

The successful synthesis of AzRu led us to synthesize fluorescent AzRu derivatives containing FITC or EITC. The fluorescent reagents FITC-Ru and EITC-Ru were purified, characterized and their specificity toward Ca²⁺-dependent proteins was demonstrated. These reagents markedly inhibited the activity of Ca²⁺-dependent proteins. Ca²⁺ accumulation in SR was inhibited with half-maximal inhibition occurring at about 15 μM and 5 μM of FITC-Ru and EITC-Ru, respectively. Ca²⁺ accumulation in mitochondria was also inhibited by the two reagents.

The fluorescence of FITC-Ru was also used to monitor its interaction with Ca²⁺-binding proteins. For example, the fluorescence of FITC-Ru was quenched upon its binding to troponin and the difference in the fluorescence intensity between free and troponin-bound FITC-Ru was used to follow FITC-Ru binding kinetics. Half-maximal binding of FITC-Ru to troponin was obtained at about 0.8 μM of FITC-Ru and maximal binding at 5 μM of the reagent.

To our knowledge, these are the first fluorescent divalent cation analogs available. Such reagents will assist in characterizing the translocation of Ca²⁺-binding proteins between different cell compartments using confocal microscopy.

Poster # 4

Interaction between prostaglandin E2 and l-cis-diltiazem, a specific blocker of cyclic nucleotide gated channels in bovine aortic endothelial cells

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Prostaglandins are known to transduce their signals via 7 transmembrane prostanoid receptors, which typically signal through coupling to G proteins and downstream second messenger molecules and protein kinase activation. Recently we have shown that cyclic nucleotides affect prostaglandins binding to bovine aortic endothelial cells independent of protein kinases. Here we show that incubation of bovine aortic endothelial cells with permeable analogs of cAMP or cGMP leads to a rapid and reversible reduction in PGE2 binding to the cells. Since cyclic nucleotides are known modulators of cyclic nucleotide gated channels, we examined the effect of a specific cyclic nucleotide gated channel blocker l-cis-diltiazem on prostaglandin E2 (PGE2) binding to bovine aortic endothelial cells. L-cis-diltiazem is shown to displace PGE2 binding to bovine aortic endothelial cells in a dose dependent manner. In addition the effect of PGE2 and l-cis-diltiazem on thapsigargin induced calcium elevation in the cells was compared. Both agents reduced in bovine aortic endothelial cells the thapsigargin induced calcium elevation by about half. PGE2 also retarded the time course of the response to thapsigargin. Simultaneous treatment of the cells with both PGE2 and l-cis-diltiazem did not yield an inhibitory effect beyond that observed with l-cis-diltiazem alone. Together our data point at the cyclic nucleotide gated channels as a feasible candidate for association with the PGE2 binding site in bovine aortic endothelial cells.

Poster # 5

Dendritic voltage-dependent sodium channels in layer 5 pyramidal neurons of the rat somatosensory cortex

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In recent years there have been a growing number of researches investigating the dendritic characters of pyramidal neurons, especially of hippocampal pyramidal neurons' dendrites due to the very well organized structure of the hippocampus. In spite of there being some interesting differences between the dendrites of hippocampal neurons and those of others neurons, the dendritic properties of cortical pyramidal neurons, in general, appear to be similar to those of hippocampal neurons. Studies from the hippocampal pyramidal neurons revealed that the properties of sodium channels appear to be quite uniform across the soma-dendritic axis. Therefore, our hypothesis is that there is a similar density of voltage-dependent sodium channels across the soma-dendritic axis in the cortical pyramidal neurons. The main goal of this research is to examine the dendritic density of voltage-dependent sodium channels at different distances from the soma in layer 5 pyramidal neurons of the rat's somatosensory cortex. In order to do so the cell-attached and outside-out configurations of the patch-clamp technique were used on five to six week old rats' brain slices. Recordings of sodium currents from somata and dendrites (up to 200 micrometers from soma) were carried out. The currents observed in both somata and dendrites were of similar amplitude and averaged at approximately 20 picoamperes, indicating that at these distances there appears to be little to no difference in voltage-dependent sodium channels' density. Future measurements will be conducted at up to 600 micrometers from soma and are presumed to affirm our initial conjectures.

Poster # 6

Constraining kinetic parameters of voltage-gated channels using a genetic algorithm

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Trans-membrane protein mechanisms such as ion-channels and their activity are at the essence of neuronal transmission. The most accurate method, so far, for determining ion-channel kinetic mechanisms is single-channel recording and analysis. Nevertheless, single-channel recordings carry several holdups and complexities, especially when dealing with voltage-gated channels. We therefore used cell-attached and nucleated patch-clamp configuration recordings to retrieve data for our analysis of voltage-gated ion-channel kinetics. The approach takes into consideration the full range of stimulation protocols used when analyzing voltage-gated ion-channels since the days of Hodgkin and Huxley. Unlike most previous analyses done protocols' results were not analyzed individually in a disjoint method, but rather as an entire set of traces from all five protocols for a simultaneous analysis. In order to fit experimental traces to several suggested kinetic models an evolutionary minimization algorithm was used. The algorithm was initially tested over simulated current traces produced using several simple Hodgkin-Huxley-like models of voltage-gated potassium and sodium channels. Currents were also produced simulating levels of noise expected from actual patch recordings. Finally, the algorithm was used for finding the kinetic parameters of several voltage-gated sodium channel models via matching its results to data recorded, in both nucleated and cell-attached configurations, from layer 5 pyramidal neurons of the rat cortex. It should be emphasized that since single-channel recording was not used the model presented here is not a biophysically accurate kinetic model. Nevertheless, the model does provide a tool for electrophysiologists in mimicking and simulating voltage-gated ion-channel kinetics on the cellular level.

Poster # 7

Constraining compartmental models using multiple voltage-recordings and evolutionary algorithms

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The last decade has witnessed a significant advancement in the ability to record membrane potential and ion channels from dendrites. Compartmental models with many non-linearly dependent parameters are used to learn the physiology of such complex neurons. However, the number of loosely constrained parameters makes it impossible to construct the desired model manually. Recently, progress has been made using automated parameter search methods, such as evolutionary algorithms. These stochastic algorithms enable the construction of a neuron compartmental model, using recorded spike trains. However, these methods are limited to somatically recorded spikes using relatively simple target functions. We have used a new fitting method based on trajectory density in a phase plane to compute a robust fitness coefficient. We exclude the time parameter by plotting the membrane potential $V(t)$ versus its first time-derivative $V'(t)$, in which the periodicity of the signal is reflected by a closed loop that can be geometrically analyzed. The plane is divided into small squares and the total number of points hitting in each is calculated. This method prevents from the algorithm to ignore the steep spikes and thus converge into a strait line. We investigated the contribution of several recording locations (soma, dendrites and axon). At each location a set of 5 currents (2 passive + 3 spike trains) was measured. We combined least square sum function for the passive currents with the trajectory density for the spiked ones. We concluded that convergence improves as more recording locations are used.

Poster # 8

Estimating robustness and parameter distribution in compartmental models of neurons

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Our understanding of the input-output function of single cells has been substantially advanced by accurate biophysically multi-compartmental models. The large number of parameters in these models has raised the necessity to use an automatic approach for finding their true values. This approach attempts to converge to a global minimum in a very high dimensional parameters' phase-plane. Due to an intrinsic noise in the neurophysiologic measurements equipment, we cannot be sure how accurate the values are that we converged to. Here we suggest that finding the parameters' distribution via Monte Carlo approach can tell us how much each parameter is sensitive to noise, and how its value distribute in real neurons. After finding the parameters' distributions, we now have a much smaller parameters-plane, which allow rigorous methods to find: 1) The sensitiveness of the model to each parameter, 2) How the quantitative variance of the data obtained can be explained by each parameter. Finally, we suggest applications, based on these methods: 1) an iterative method of dealing with the difficulty of convergence to a global minimum in a high dimensional parameters-plane. 2) Defining the amount of information obtainable when using a specific model. 3) Differentiating between similar and non-similar global minimum.

Poster # 9

Functional *in vivo* and *in vitro* evaluation of the "CritiView": A tissue viability multiparametric monitoring device

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The vitality of the tissue depends on continuous supply of oxygen, in order to maintain a positive balance between O₂ supply and O₂ demand. Decrease of O₂ supply causes a reduction in the respiratory chain activity that could be evaluated by NADH redox state. Under pathophysiological conditions such as anoxia, hypoxia, ischemia and sepsis, blood flow to vital organs (like the brain and heart) elevates on the expense of less vital organs. Since blood is the oxygen carrier - these changes are highly correlated with changes in oxygen supply to the organs and tissues. In this study, we evaluated the functionality of CritiView (CRV) – an optical-computerized device that monitors tissue viability *in vivo*. The CRV could be used in animal models as well as in patients at ICU, operation and emergency rooms. The CRV was developed by "Critisense" Ltd. In this device, various light sources (LED/Laser) excite the tissue via a bundle of optical fibers. The emitted light is transferred to the detection unit through appropriate filters. The data is then transmitted to the software for real time analysis. The parameters which are monitored include: tissue blood flow (TBF) by the LDF (laser Doppler flowmetry), tissue reflectance (correlated to blood volume), mitochondrial NADH redox state by the fluorometric technique and tissue oxyhemoglobin level using the reflectometric technique, which is based on the differences at the absorption level of oxyhemoglobin versus deoxyhemoglobin.

In order to evaluate the reliability of the CRV we did *in vitro* and *in vivo* studies. In the *in vitro* studies we compared the NADH measurements by the CRV to various NADH concentrations in solution. We found a linear correlation which confirms the reliability of NADH measurements by CRV. In the *in vivo* studies we compared the CRV to regular laboratory devices and found significant correlation under various stress situations. Since the CRV uses UVA light which may cause damage to the monitored tissue, we exposed rats and gerbils brain to several episodes of short anoxia in order to evaluate tissue integrity. We found that the UVA intensity that is used by the CRV is not dangerous and causes no damage to the tissue.

We can conclude that the CritiView is a useful device for experimental animals studied and should be applied to clinical environment although further studies are needed to verify this option.

Poster # 10

Uridine-5'-triphosphate (UTP) reduces infarct size and improves rat heart function after myocardial infarct

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We have previously found that uridine 5'-triphosphate (UTP) significantly reduced cardiomyocyte death induced by hypoxia via activating P2Y₂ receptors. To explore the effect of UTP following ischemia *in vivo* we studied four groups: sham with or without LAD ligation, injected with UTP (0.44 µg/kg iv) 30 min before left anterior descending coronary artery (LAD) ligation and UTP injection (4.4 µg/kg iv) 24 hr prior to operation. LAD occlusion caused an elevation in the heart rate (314 ± 19 bpm vs 276 ± 21 in sham animals) that was lowered when the rats were pretreated with UTP 30 minutes or 24 hours before ischemia (241 ± 48 bpm, 286 ± 17 bpm, respectively). Left ventricular end diastolic area (LVEDA), end systolic area (LVESA), fractional shortening (FS), and changes in posterior wall (PW) thickness were performed by echocardiography before and 24 hr after the operation. In addition, we measured different biochemical markers of damage and infarct size using Evans blue and TTC staining. The increase in LVEDA and LVESA of the treated animals was significantly smaller when compared to the LAD occluded rats (p<0.01). Concomitantly, FS was higher in groups pretreated with UTP 30 min or 24 hr (56 ± 14.3% and 36.7 ± 8.2%, p<0.01 respectively). The ratio of infarct size to area at risk was smaller in the UTP pretreated hearts than infarcted non treated rats (22.9 ± 6.6%, 23.1 ± 9.1%, vs 45.4 ± 7.6% respectively, p<0.001). Troponin T and ATP measurements, demonstrated reduced myocardial damage. Using Rhod-2-AM loaded cardiomyocytes, we found that UTP reduced mitochondrial calcium levels following hypoxia. In conclusion, early or late UTP preconditioning is effective, demonstrating reduced infarct size and superior myocardial function. The resulting cardioprotection following UTP treatment post ischemia demonstrates a reduction in mitochondrial calcium overload, which can explain the beneficial effect of UTP. In conclusion, this study demonstrate the potential role of UTP in the reduction of heart damage induced by myocardial infraction.

Poster # 11

Nitric oxide triggers classic ischemia preconditioning by preventing intracellular Ca^{2+} overload in cardiomyocytes

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The involvement of nitric oxide (NO) in the late phase of preconditioning is well established. However, the role of NO as a trigger or mediator of "classic preconditioning" remains to be determined. The present study was designed to investigate the role of NO in activation of Ca^{2+} extrusion mechanisms, thereby preventing intracellular calcium overload. Elevation of extracellular calcium, which generally occurs during ischemia, caused an immediate increase in intracellular calcium level ($[\text{Ca}^{2+}]_i$) and arrhythmia in newborn cardiomyocyte cultures. Treatment with the NO donor- sodium nitroprusside (SNP), decreased $[\text{Ca}^{2+}]_i$ to control level and reestablished synchronized beating of cardiomyocytes. It was shown that a decrease in extracellular Na^+ concentration, which inhibits the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, did not prevent $[\text{Ca}^{2+}]_i$ reduction by SNP. In contrast, application of thapsigargin, an inhibitor of SR Ca^{2+} -ATPase (SERCA2a), increased $[\text{Ca}^{2+}]_i$ and in its presence SNP did not reduce $[\text{Ca}^{2+}]_i$, indicating that Ca^{2+} reduction is achieved via activation of sarcoplasmic reticulum (SR). The reduction of $[\text{Ca}^{2+}]_i$ by SNP was also blocked in the presence of H-89 (a PKA inhibitor), whereas pretreatment with KN-93 (a calmodulin inhibitor) did not prevent Ca^{2+} extrusion into the SR. The effect of SNP on the reduction of $[\text{Ca}^{2+}]_i$ was prevented by ODQ (guanylyl cyclase inhibitor) and C-PTIO (NO scavenger). It was also shown that 8-pCPT-cGMP (cGMP analogue) caused a partial reduction in $[\text{Ca}^{2+}]_i$. These data suggest that SNP triggers its preconditioning effect through stimulation of soluble guanylyl cyclase and activation of protein kinase G. Taken together, our findings suggest that activation of SR Ca^{2+} -ATPase by SNP increases Ca^{2+} extrusion and prevents cytosolic Ca^{2+} overload, which might explain the protective effect of SNP in ischemia-reperfusion.

Poster # 12

The role of phosphorylated p38 mapk in the protective mechanism of adenosine receptor activation against hypoxic conditions

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Activation of either A₁ adenosine receptor (A₁R) or A₃ adenosine receptor (A₃R) elicits protection against infarction, ischemia or hypoxia. The mechanism of this protection is not fully understood. Recently it was also shown that ischemic preconditioning attenuated ischemia/reperfusion (I/R)-induced cardiac dysfunction through modulation of p38 MAPK. The purpose of this study is to investigate the involvement of p38 MAPK in the mechanism of adenosine receptor activation in cardioprotection in cardiomyocyte cultures as well as in the whole heart.

Cultured cardiomyocytes were incubated with SB203580 (a specific inhibitor for phosphorylated p38) for 15 min, then treated with CCPA or CI-IB-MECA (the agonists of A₁ and A₃ adenosine receptors, respectively) before being subjected to 90 min hypoxia. Levels of LDH released from the cells, and ATP content were measured. Phosphorylated p38 MAPK was examined using Western blot analysis on both cell culture and isolated rat hearts that were injected with CCPA or CI-IB-MECA (10 nM) 24 hours before I/R.

Results: Both A₁R and A₃R agonists reduced hypoxia-induced injury both *in vivo* and *in-vitro*. However, when SB203580 was given together with these agonists, the protection was prevented as revealed by LDH release, ATP content and mitochondrial membrane potential. It was also shown that phosphorylated p38 appeared only in heart pretreated with adenosine receptor agonists.

Conclusions: CCPA and CI-IB-MECA protect both cell culture and isolated hearts against ischemia. This protection was partially related to the increased phosphorylation of p38 MAPK before and during ischemia. It is known that phosphorylation of p38 MAPK activates intracellular signaling which protects the cytoskeleton against degradation.

Poster # 13

Lysophospholipids modulate L-type and T-type calcium channel currents in pituitary cells*

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Lysophospholipids (LPLs) are lipophilic molecules consisting of a hydrophilic head and a hydrophobic tail. It was suggested that partition of these molecules into the phospholipid bilayer alters membrane tension and thereby affects the gating of mechanosensitive ion channels. LPLs were defined by their shapes as cones, inverted-cones or cylinders. It was suggested that partitioning of cones into the outer leaflet of the phospholipid bilayer forms convex membrane structures mimicking membrane compression-or cell shrinkage. In this study we examined whether a cone shaped molecule, Lysophosphatidylcholine (LPC), mimics the previously reported hyperosmotic suppression of L-type and T-type calcium channel currents (I_L and I_T) in pituitary cells. Our main findings may be summarized as follows: **1.** Application of LPC (3-30 μ M) to the bath solution resulted in a dose dependent suppression of both I_L and I_T . **2.** This suppression of I_L and I_T was irreversible (suppression continued after washout of LPC). Full reversibility was observed only after washout of LPC with BSA (0.5 mg/ml). **3.** The effects of LPC on calcium currents were differential. The suppression of I_T was more prominent than the suppression of I_L . In addition, the suppression of I_T started after a short delay of several seconds whereas the suppression of I_L started after a long delay of 50-100 seconds. **4.** The suppression of I_L was voltage dependent with a stronger suppression at more negative potentials. **5.** The cone shaped Lysophosphatidylinositol (LPI), but not the cylinder shaped Phosphatidylcholine (PC), had similar effects as those produced by LPC on calcium currents. In summary, our results show that cone shaped LPLs, but not a cylinder shaped LPL, suppress voltage-gated calcium currents in pituitary cells. The slow onset of suppression, and the recovery which was observed only after washout with BSA, suggest that partition of LPC and LPI into the phospholipid bilayer underlies their effects. Additional experiments are needed to verify whether or not LPLs in the shape of cones mimics the hyperosmotic suppression of I_L and I_T in pituitary cells.

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Poster # 14

Reduced calcium transient in isolated cardiac myocytes depends on ATP sensitive potassium ion channel activation using low pulse, low frequency electromagnetic field

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Background: Several studies have shown a protective effect of pulsed electromagnetic field (PEMF) following acute ischemic injury in rats. Our preliminary work has shown that the ECG of rats which were exposed to PEMF was considerably altered. QRS amplitude, PR and QT intervals were decreased. It is known that activation of K_{ATP} channels causes marked shortening of the cardiac action potential and decreasing QRS amplitude. Other studies that investigated cardiac K_{ATP} channels have found that their interburst interval is 62.7 msec which is equivalent to 15.95 Hz. In the present study we hypothesized that PEMF at 15.95Hz would act as K_{ATP} channel opener and decrease Ca^{2+} ion current in vitro.

Methods: We assessed the effect of extremely low PEMF (80 nT, 15.95 Hz; for 20 minutes) on isolated cardiac myocytes obtained from 2-3 days old SD rats with (n=11) and without (n=14) K_{ATP} channel closure (2 μ M Glibenclamide) to assess changes in Ca^{2+} ion transients. Intracellular free calcium concentration ($[Ca^{2+}]_i$) was estimated from indo-1 fluorescence using the ratio method.

Results: PEMF caused reduction in Ca^{2+} transient by $57\% \pm 6\%$ compared with controls ($p=0.005$). These changes remained for 45 minutes after PEMF was terminated. In a second set of experiment PEMF reduced Ca^{2+} transient by $48\% \pm 3\%$ ($p=0.01$) and the addition of Glibenclamide under PEMF resulted in increased Ca^{2+} transient by $23\% \pm 5\%$ compared with controls ($p=0.03$). This effect lasted for 1 min and thereafter Ca^{2+} transient was further reduced by $43\% \pm 6\%$ ($p=0.01$). In a third set of experiment, Glibenclamide was first added and it resulted in widening of the Ca^{2+} transient amplitude by $30\% \pm 9\%$. Exposure to PEMF following Glibenclamide resulted in $26\% \pm 6\%$ decrease in Ca^{2+} transient compared with controls ($p=0.05$).

Conclusion: These data indicate that PEMF at a frequency of 15.95 Hz, which is equivalent to the K_{ATP} interburst interval, reduces calcium ion efflux in vitro. These data however, do not exclude the effect of PEMF on other ions/ channels that can affect Ca^{2+} transients in isolated cardiac myocytes.

Poster # 15

Exercise training *prior* to acute myocardial infarction (AMI) improves cardiac healing through alterations in heart energy metabolism

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Background: Heart metabolism is predominantly aerobic, with the majority of energy being derived from the oxidation of fatty acids. During ischemia, however, glycolytic metabolism is stimulated and contributes significantly to cardiac energy supply. Strenuous exercise also stimulates glycolysis. Following chronic training, glycolytic metabolism decreases and fat utilization is up regulated. These metabolic alterations may contribute to the cell survival during ischemia caused by acute myocardial infarction (AMI). **Our aim** was to study the effect of exercise training conducted *prior* to AMI on the activity of key enzymes involved in heart energy metabolism during left ventricular (LV) remodeling.

Methods: Male Sprague Dawley (n=28) rats underwent 3 weeks of swimming exercise training (90 min, 5 days/wk), or remained sedentary. At the end of the training/ sedentary period, all rats were subjected to AMI induced by surgical ligation of the left coronary artery and thereafter remained sedentary during a 4-week recovery period (LV remodeling period) and thereafter sacrificed. Trans-thoracic echocardiography was performed on each group at the end of the exercise/ sedentary period; 24 hours following AMI; and 4 weeks following AMI. At sacrifice, hearts were harvested and assayed for enzyme activities using standard spectrophotometric and isotope techniques.

Results: Three weeks of exercise training prior to AMI resulted in higher shortening fractions compared to the 3 wk sedentary group at 24h-post-AMI ($38.4 \pm 12.5\%$ vs. 23.68 ± 7.4 ; $p < 0.05$) and 4 weeks after AMI ($39.1 \pm 12.5\%$ vs. 22.63 ± 7.9 ; $p < 0.05$). Although hexokinase activity was higher in the trained group, the difference was not statistically significant (30 ± 3 vs. $25 \pm 3 \mu\text{mol/g dw/min}$; $p = 0.09$). However, the activities of the remaining enzymes were significantly higher in the prior-exercised group: glycogen phosphorylase (63 ± 6 vs. $40 \pm 5 \mu\text{mol/g dw/min}$; $p < 0.03$), citrate synthase (305 ± 22 vs. $216 \pm 26 \mu\text{mol/g dw/min}$; $p < 0.03$), and 3-Hydroxyacyl-CoA dehydrogenase (171 ± 26 vs. $79 \pm 8 \mu\text{mol/g dw/min}$; $p < 0.02$).

Conclusions: Three weeks of exercise training prior to AMI was sufficient to increase myocardial oxidative capacity 4 weeks following AMI, indicating improved metabolic response of the trained heart to injury. The increased capacity to oxidize carbohydrates and fats in the *prior* trained hearts may contribute to the improved cardiac function during LV remodeling.

Poster # 16

The popeye domain containing-1 (*popdc1*) gene product is required for heart recovery from ischemia-reperfusion injury in the mouse*

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The muscle-restricted *Popdc* gene family encodes highly conserved membrane proteins (*Popdc* 1-to-3) of as yet unknown function but of a well-described regulation during development and an inferred involvement in cell-cell interaction. A *Popdc1*-null mouse mutant that exhibits an apparently normal phenotype has manifested impaired skeletal muscle regeneration following injury. To test the hypothesis that *Popdc1* plays a role in cardiac injury as well, we examined the left ventricular (LV) performance of *Popdc1*-null hearts following ischemia and reperfusion (I/R) insult in an isolated heart preparation. At 30 min stabilization, hearts were subjected to 30 min global ischemia followed by 90 min reperfusion. Compared to wild type (WT), *Popdc1*-null hearts displayed inferior functional recovery from the I/R injury as summarized at 30min reperfusion (percent of baseline):

	LVDP	+dP/dT	-dP/dT	RPP
WT (n=13)	61.2±17.5	55.4±16.2	49.5±14.5	57.7±18.3
<i>Popdc1</i> -null (n=15)	42.7±8.2	43.9±10.9	39.7±10.6	40.9±8.2
<i>P</i>	0.003	0.043	0.058	0.008

LVDP=LV developed pressure; +/-dP/dT, rates of pressure development/relaxation; RPP=rate pressure product.

In line with the impaired function, the infarct size, measured at 90 min reperfusion, was significantly higher in the mutant mice, 30.0±13.9 and 14.5±6.7 percent in *Popdc1*-null versus WT (n=5/group, *p*=0.029). These functional and morphological differences, indicating increased susceptibility to I/R injury, were not associated with post-injury changes in transcript level of the three *Popdc* isoforms in either WT or *Popdc1*-null hearts. Recent evidence from our laboratory indicates that *Popdc1* may be important also for the manifestation of ischemia preconditioning.

The high conservation of *Popdc* genes and their inferred role in muscle cell injury and repair suggest that these novel proteins may have an important function in the heart.

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Poster # 17

Effects of rapamycin on normoxic and hypoxic heart cultures: role of Ca accumulation by the sarcoplasmic reticulum

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Rapamycin inhibition of cell proliferation mediates its therapeutic effects as immunosuppressant, antitumor and antifungal agent. The cytostatic effect of rapamycin on vascular smooth muscle cell proliferation has also recently been exploited in the therapeutic application of rapamycin to drug eluting stents for coronary angioplasty. For its activity, rapamycin binds to FK binding proteins (FKBP). The complex interacts with calcineurin – inhibiting the activation of the NFAT* transcription factor and with mTOR** cell cycle kinase thereby decreasing protein synthesis and cell cycle progression from G1 to S phase. A less well known mechanism affects intracellular calcium management. FKBP12/FKBP12.6 modulates the activity of ryanodine receptor, the sarcoplasmic reticulum calcium channel in skeletal/cardiac muscle respectively. Once rapamycin binds to FKBP, it dissociates it from the ryanodine receptor thereby increasing the channel open-probability. Our goal was to study the effects of rapamycin on primary heart cultures in normoxic and hypoxic conditions. Therefore, rat heart cultures were subjected to 1-30 μ M rapamycin. ⁴⁵Ca accumulation into the SR of a saponin skinned cultures was detected. Cultures were also exposed to 90 min hypoxia and reoxygenation in order to study the protective effect of rapamycin on the cardiomyocytes. Rapamycin 10 μ M significantly attenuated the proliferation of rat neonatal cardiomyocytes. Ca accumulation into the SR was decreased by rapamycin in a dose and time dependent. Rapamycin also attenuated by 40-50% LDH leakage from cardiomyocytes that were subjected to hypoxia and reoxygenation. These results suggest that rapamycin affects intracellular calcium stores and heart tolerance to hypoxic stress. The possible cardioprotective effect of rapamycin in hypoxic cardiomyocytes may be explained by decreased Ca release from the SR thereby diminishing the intracellular Ca overload via an energy deficient state.

*NFAT – nuclear factor activated T cells transcription

**mTOR - (mammalian target of rapamycin)

Poster # 18

Induction of hypertrophy in neonatal rat heart cultures and its suppression by inhibition of Poly(ADP-ribose) polymerase-1

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Background: Clinical studies have demonstrated that cardiac hypertrophy is not only an adaptational state before heart failure but is an independent risk factor for ischemia, arrhythmia and sudden death. It is therefore important to prevent the development of cardiac hypertrophy. Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that plays different roles in the cell including genome protection and most notably for our study a regulator of gene transcription by post translation modification of nuclear proteins modulating their binding to DNA. This is a highly conserved protein, activated by binding to nicked DNA and as recently discovered under a variety of physiological conditions. We have found that the chromatin-bound protein PARP-1 is activated rapidly and dose-dependently by Ca^{2+} released into the nucleoplasm in rat newborn cardiomyocytes. A rhythmical Ca^{2+} release into the nucleoplasm been observed, pending on transient elevations of Ca^{2+} in the cytoplasm and Ca^{2+} accumulation in the peri-nuclear Ca^{2+} stores. The highly condensed chromatin structure is rapidly released by poly(ADP-ribosyl)ation of linker histone H1 rendering DNA accessible to transcription. Thus, the frequency of cardiomyocyte contraction is targeted via intracellular Ca^{2+} signaling to fast and transient modifications of the chromatin by poly(ADP-ribosyl)ation. This may induce morphological changes in cardiomyocytes.

Objective: It is a central issue to understand the molecular basis underling the compensatory mechanism for mechanical adaptation in hypertrophic response. In the present study, we examined the role of poly(ADP-ribosyl)ation in induction of hypertrophy by stimulation of G-protein coupled receptors in the membrane of cultured neonatal rat cardiac myocytes.

Methods: Hypertrophy was induced in neonatal rat cardiomyocytes by treatment with AngII or adrenergic agonists. It is well known that cardiac hypertrophy is accompanied by changes in the muscle phenotype such as an expression of fetal type genes and increase in cell surface area. The effect of these agonists on both hypertrophy and poly(ADP-ribosyl)ation was examined. Changes in the surface area of the treated myocytes were quantified. Changes in PARP-1 activity during the treatments have been measured by antibody directed against ADP-ribose moieties.

Results and Conclusions: We found that poly(ADP-ribosyl)ation mediates morphological changes that characterizes hypertrophy in cardiomyocytes. After treatment with AngII we observed increased surface area of cardiac myocytes by 2 fold as compared with control. Treatment of neonatal rat cardiac myocytes with 3-AB that inhibits PARP-1 showed inhibition of AngII induced increase in cell surface area. PARP-1 activation in cardiomyocytes treated by AngII was mediated by MEK activity. PARP-1 activation mediated core histone acetylation. Suppression of PAPR-1 by targeted siRNA, suppressed Elk-1 phosphorylation and acetylation of core histones. In view of our findings indicating Ca^{2+} induced poly(ADP-ribosyl)ation, these results may associate physiological malfunctions with morphological changes in cardiomyocytes.

Poster # 19

Airsickness incidence rate during preliminary selection flights in the Israeli Air Force

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Introduction: Motion-sickness during flight, known as Air-sickness is a common condition that usually occurs during the first stages of flight training. Air-sickness may affect the qualification process of aircrew.

Methods: An Air-sickness incidence and significance mapping survey was conducted during the primary selection flights at the IAF flight school. The survey included 180 healthy young Israeli student aviators with a validated questionnaire. The student aviators reported Air-sickness symptoms after each of their first five sorties. A number of 266 sorties were performed on a Grobe-120 training aircraft, while 689 sorties were performed on a Piper Supercub aircraft.

Results: A high rate of 78.9% (142/180) among student aviators suffered from Air-sickness at least once. In 38.8% (371/955) of sorties, the student aviators suffered from Air-sickness. The highest incidence rate of Air-sickness (61% of student aviators) was found during the first sortie. In total, 15.6% of student aviators suffered from severe Air-sickness during their first flight. Particularly 12.4% of student aviators who flew the Grobe-120 suffered severe Air-sickness in comparison to just 7% of students flying on the Piper Supercub. In addition, several other issues were examined, such as: The extent of drug usage and drug efficiency, time dependency of Air-sickness symptoms appearance, symptoms durability. Another considerable issue examined was the relation between history of Motion-sickness sensitivity and Air-sickness appearance.

Conclusion: Air-sickness was very common in the first sortie during the primary selection phase in flight school. Aircraft type can increase Air-sickness incidence. Most of student aviators adapt to Air-sickness after their first flight.

Poster # 20

Striatal content of MAO-A and MAO-B following dopaminergic or dopaminergic plus serotonergic denervation: influence on response to L-dopa

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L-dopa is a powerful dopaminergic agonist to striatal neurons, but the precise way in which dopamine is formed and metabolized in the striatum following L-dopa administration is still unclear. Unilateral dopaminergic denervation of the rat striatum using 6-hydroxydopamine (6-OHDA) injected to the medial forebrain bundle is a commonly-used model to investigate the anti-parkinsonian activity of L-dopa and other dopaminergic agonists (Ungerstedt, 1971). In this model it has recently been shown that serotonergic innervation of the striatum is markedly up-regulated on the affected side (Maeda et al, 2003), and that serotonergic neurons play an important role in the decarboxylation of L-dopa to dopamine in the lesioned striatum. We have studied the composition of MAO in the striatum of rats following dopaminergic denervation with 6-OHDA and serotonergic denervation with 5,7-dihydroxytryptamine (5,7-DHT). 6-OHDA was injected to the medial forebrain bundle, and 5,7-DHT was administered intracerebroventricularly at the same operation. Three weeks later, the degree of dopaminergic lesion was examined by determination of contra-lateral turning response to apomorphine (0.05 mg/kg s.c.). Only rats showing a brisk turning response to apomorphine were used in the experiment. Much previous work has shown that such animals have greater than 95% striatal dopaminergic denervation. Rats were divided into two groups: 1- Turning response to L-dopa (6 mg/kg + 1.5 mg/kg carbidopa i.p.) was examined, one week following apomorphine test, 2- Rats were decapitated and MAO activity was determined in left striatum using radiochemical technique. Activity of MAO is expressed as percent activity of sham-operated controls. Neither MAO-A nor MAO-B activity was significantly affected by 6-OHDA lesion alone (MAO-A 94±0.05%, MAO-B 100±0.07%) or by double lesion with 6-OHDA and 5,7-DHT (MAO-A 103±0.07, MAO-B 104±0.06%). The efficiency of the serotonergic lesion was demonstrated by reduction in striatal 5-HT and 5-HIAA contents, as well as by pronounced reduction in contralateral turning response to L-dopa (one animal even demonstrated ipsilateral turning to L-dopa following the double lesion). These results indicate that most of the MAO activity in striatum is not located in either dopaminergic or serotonergic axonal varicosities.

Ungerstedt U. et al (1971) Acta Physiol Scand Suppl, 367: 69-93.

Maeda T. et al (2003) Neurosci Lett, 343: 17-20.

Poster # 21

Expression and localization of ARTS in the rat brain

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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. In many cell lines derived from peripheral tissues, ARTS is localized to the mitochondria, but upon apoptotic stimuli it translocates to the nucleus (Larisch et al, 2000). ARTS is expressed in most areas of rat brain as seen by immunohistochemistry. We have now found that in several rat brain regions, as well as in primary cultures of rat cortical and cerebellar granule neurons, ARTS is expressed in a shorter form (25 kDa) than that seen in peripheral cells (32 kDa), and lacks part of its N terminal amino acid sequence, since it binds to an antibody specific to the C-terminal but not the N-terminal sequence as shown by immunoblotting. In addition, using confocal microscopy we detected that in primary rat cortical neurons prepared from E-18 embryos and grown for 7 days IV in neurobasal medium plus B27 supplement, unlike in cell lines from peripheral tissues, ARTS is localized mainly to the nucleus but not to the mitochondria. Moreover, levels of neuronal ARTS were elevated as a result of different insults in vitro as well as in vivo. In primary rat cortical neurons, following withdrawal of B27 from the growth medium there was a strong upregulation of ARTS, especially after 48h. For an in vivo model of neuronal damage we injected 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle of adult rats. After 3 and 7 days we found an elevation of ARTS levels in substantia nigra of rats, that were injected with 6OHDA, as opposed to saline-injected controls. This data indicates that ARTS might be involved in neuronal death, but it is unclear whether its role is pro-apoptotic or otherwise, which is currently under investigation.

Larisch, S. et al (2000) Nature Cell Biology, 2:915-921

Poster # 22

Bi-directional HSP response in livestock

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Heat shock proteins (HSPs) are a subset of molecular chaperones that are involved in “house keeping” functions in the cell (protein folding, protein assembly and translocation between compartments). In addition, they work in accordance with the proteasome machinery to degrade misfolded or aggregated proteins. However, under a wide range of damaging conditions an inducible HSP response is taking place, to help cells and organisms recover and survive. We examined the HSP response of livestock to heat or protein stresses and revealed a bi-directional response: an expected elevated expression of the HSP machinery in response to heat load, and a marked decrease of HSPs as a result of low protein intake. This decrease was accompanied with a decreased expression of the proteasome machinery and an increased expression of lipolytic agents. Our results provide a new set-point for a delicate balance between protein and lipid metabolism in protein-deprived livestock. Most importantly, the results indicate a bi-directional response of HSPs to two different stressors.